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Development of an in vitro laboratory manual for nuclear medicine technology students

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Meyers, Arthur, Ph.D.

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DEVELOPMENT OF AN IN VITRO
LABORATORY MANUAL FOR
NUCLEAR MEDICINE
TECHNOLOGY
STUDENTS

by
Arthur Meyers

A dissertation submitted in partial fulfillment of the
requirement for the degree of

Doctor of Education

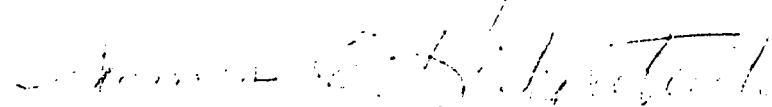
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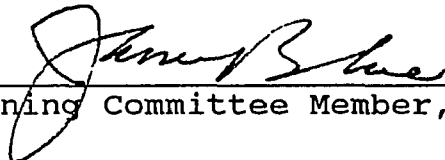
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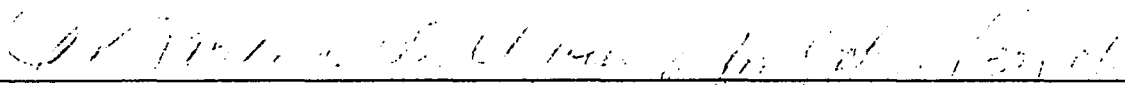
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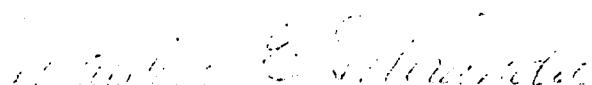
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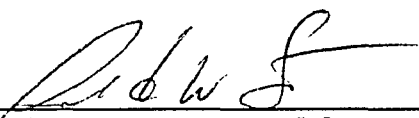
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ABSTRACT

This study evaluated existing In Vitro educational materials in qualitative and quantitative parameters that currently exist to educate potential clinicians of nationally accredited Nuclear Medicine programs.

A review of over 300 articles, texts, and manuals pertaining to In Vitro Nuclear Medicine procedures clearly demonstrated that no In Vitro laboratory manual for undergraduate students presently existed.

Every Nuclear Medicine program director in the United States was surveyed. They were asked for their overall philosophy in terms of developing an In Vitro manual and requested to evaluate the significance of 22 general principles/concepts and 34 specific laboratory testing procedures.

From the responses to the survey, an In Vitro Nuclear Medicine manual was created and appended to the study. The manual consists of lecture and study material, chapter reviews, and laboratory assignments and exercises.

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Chapter 1

THE PROBLEM

Introduction to the Problem

Health care, with all its interrelated manifestations, is America's second largest industry, and it promises to soon become number one. Many diverse allied health professionals, including those trained in Nuclear Medicine technology, are needed to meet the every-changing needs of this complex and rapidly developing segment of our economy.

To demonstrate this transition, the evolvement of all allied health professions in this century has been nothing short of phenomenal. Around 1900, one of every three people engaged in health care was a physician. By 1967 the ratio was reported to have changed to approximately one physician to ten allied health personnel. The ratio now is closer to one in twenty (Kirby, p. 88). The education of health professionals has become a major concern to physicians, administrators, and to the general public.

Historically, the education of nuclear medicine education professionals took place in a hospital or in the office of a doctor and was devoted primarily to the practical aspects of patient care; theoretical or background subjects were taught only as they were directly pertinent to practice. Under this arrangement, the cost was borne by

hospitals or physicians. In some cases, students received pay or stipends (Master Plan for Radiologic Technology Programs).

As complexity of health care increased and as nuclear medicine technologists became responsible for more sophisticated duties, hospitals and physicians recognized that colleges and universities were the appropriate sites to provide the background in science, mathematics, and communications needed for new responsibilities, frequently demanding new knowledge, inferential thinking, and decision-making. Clinical education with its patient-contact emphasis continued to be offered in hospitals and practitioners' offices. However, it has been found that many subjects directly related to professional goals of the students could often be more appropriately taught in colleges and university classrooms or laboratories as in the clinical facilities. Thus the trend in Nuclear Medicine education has moved toward universities and community colleges from the hospital setting.

Over the past 25 years development of new radiopharmaceuticals, gamma-ray cameras, advanced laboratory techniques and equipment have dramatically changed the field of nuclear medicine. Consequently, the educational requirements of those students training in the field have constantly been revised to keep pace.

Nuclear medicine technology applies the uses of tracer amounts of radioactive materials to visualize and measure regional function in living biologic systems. This non-invasive procedure has in the last two decades brought about significant improvements in basic understanding of disease processes as well as in medical diagnoses.

Throughout this tremendous growth in Nuclear Medicine, two major areas have developed within the field: In Vitro and In Vivo Nuclear Medicine.

In Vitro Nuclear Medicine may be define as that area of nuclear medicine in which the results may be expressed in a quantitative fashion. These testing procedures are performed in test tubes utilizing tracer amounts of radioactive material. In vitro laboratory procedures have made possible direct assays of many physiologic substances present in minuscule concentrations including hormonal, blood, and drug levels.

In Vivo Nuclear Medicine has become a more dominant component in the educational process of students. This aspect involves the use of gamma-ray cameras and scanners to make interpretations on a qualitative (subjective) basis.

Though all educational Nuclear Medicine programs require both In Vitro and In Vivo studies, most students do not acquire adequate training in the In Vitro laboratory areas. A recent survey by the Joint Review Committee on Accreditation of Nuclear Medicine Programs cites the need

for more emphasis on the in-vitro aspects of Nuclear Medicine Technology (Nuclear Medicine Technology Essentials Questionnaire).

They are approximately 70 accredited Nuclear Medicine Technology Programs, either at the Associate of Science, or Bachelor of Science level. Many of these programs have had problems in their recent accreditation applications due to their lack of In Vitro laboratory education.

Statement of the Problem

Although In Vitro Nuclear Medicine has been an integral part of the education of students in the field, there is at this time NO educational In Vitro laboratory manual for training students. This has been detrimental to the proper training and understanding of Nuclear Medicine's graduating students in regard to becoming qualified technologists.

There is a need to develop an In Vitro Nuclear Medicine laboratory manual for the training of students in these programs. The purpose of this research was to identify through documentation this need through a nationwide survey of Nuclear Medicine program directors and review of the literature in the field. The research resulted in the development and writing of an In Vitro laboratory manual to meet the deficiencies cited.

Questions

This research resulted in answers to the following questions:

1. Was there a need for an In Vitro laboratory manual for students in Nuclear Medicine Technology?
2. What general principles/concepts of In Vitro studies must be included in this student manual?
3. What were the types of testing methodologies and procedures that must be included in a comprehensive student In Vitro manual.
4. What aspects of In Vitro Nuclear Medicine are most commonly overlooked that need to be taught to graduating Nuclear Medicine students?

Significance of Study

Fundamentals of In Vitro Nuclear Medicine. Prior to 1960, substances present in small amounts in blood and other body fluids were exceedingly difficult to measure. Until then, chemical and bioassays of these substances, usually hormones, were somewhat crude. This led to considerable inaccuracy in the measurement of such small amounts, and results were difficult to reproduce in other laboratories. In addition, rather large samples were usually needed for

the determinations. In 1960, Yalow and Berson reported a method for the quantitative measurement of insulin based on a new technique of competitive binding assay (Rothfeld, 1985, p. 91). This was the beginning of numerous breakthroughs in the use of In Vitro laboratory techniques today.

Through the use of In Vitro Nuclear Medicine laboratory procedures, biologically active molecules present in the blood in concentrations as low as 1 part in 50 million can be accurately measured. Until recent years, the problem of measuring these proteins and sorting them out as specific entities from numerous basically similar molecules seemed virtually impossible (Chard, p. 43-45). Now the capability for measuring clinically significant substances such as hormones, drug levels, and blood components at levels of picograms or nanograms per milliliter is available to almost any In Vitro laboratory that has the appropriate equipment.

There have been numerous books, journals, and articles written describing Nuclear Medicine In Vitro's capability for precise and accurate measurement of tracers in clinical diagnosis, pharmacology, and biochemistry, and in estimable values in hematology and hormonal, metabolic, infectious, and inflammatory disorders. The impact of these Nuclear Medicine methodologies on progress in medicine should not be underestimated. In Vitro laboratory advances have greatly enhanced the field of Nuclear Medicine and its ability to

diagnose minute amount of substances in the human blood system.

Even though there is a sufficient quantity of material on In Vitro laboratory procedures in the literature, there is no definitive laboratory manual, text, or guide written to meet the needs of Nuclear Medicine Technology students. Many texts discussed in the literature review pertain to theoretical constructs, while others devoted to practical applications are written at a highly sophisticated level not intended for undergraduate students.

On October 19, 1987, letters were written to a number of highly regarded professionals in the field, requesting their professional assessment of the need for a student In Vitro laboratory manual.

One of these professionals, Sheila Rosenfeld (1987), a member of the Joint Review Committee on Accreditation of Nuclear Medicine Programs and Program Director of Nuclear Medicine at St. Louis University, replied to this letter. After discussing texts commonly used to teach In Vitro studies in Nuclear Medicine programs, Rosenfeld states, "I personally feel that there is a definite need for a student laboratory manual" (Ltr., Nov., 1987).

Wynn Harrison, Director of the Nuclear Medicine Program at Weber State University in Utah writes, "I have taught In Vitro procedures for the past four years in the Weber State College Nuclear Medicine Program, there has been no suitable

textbook or laboratory material in this area. It would be a service to the profession to have such materials" (Ltr., Dec., 1987).

Allen West, Director of the Nuclear Medicine Program at the University of Alabama Birmingham writes, "It is a shame that most programs cannot provide the necessary In Vitro laboratory procedure due to the lack of quality text or manuals to train students properly" (Ltr., Dec., 1987).

Anthony Snow, Director of a San Francisco Hospital's Nuclear Medicine Department writes, "Most students enter the work force not sufficiently trained in In Vitro Nuclear Medicine procedures, due to the lack of materials written at the undergraduate level" (Ltr., Sept., 1988).

Further telephone conversations with Henry Ernstal, Director of the Society of Nuclear Medicine,; Virginia Pappas, President of Technologist Section of the Society of Nuclear Medicine; Craig Harris, Nuclear Medicine Program Director at Duke University; and Marsha Boyd, Director of Baptist Memorial Hospital and President of the Technologist Section of the American Registry of Radiologist Technologists; all reiterated the need for a student In Vitro Nuclear Medicine laboratory manual.

A recent questionnaire sent to 1,122 registered Nuclear Medicine technologists by the Joint Review Committee on Nuclear Medicine Technology identified the fact that graduating technologists were not receiving the proper

educational requirements and did not receive sufficient experiences in In Vitro laboratory procedures.

Therefore, the purpose of this research was to survey all Accredited Nuclear Medicine Program Directors in order to gather information pertinent to:

1. Identifying the need for a student Nuclear Medicine In Vitro laboratory manual,
2. Identifying those aspects or concepts that must be included in a student manual, and
3. Identifying the types of testing methodologies and procedures that must be included in a comprehensive student In Vitro manual.

From the information gathered in the research and the content analysis of the literature, a Student Nuclear Medicine In Vitro Laboratory Manual to meet this need was developed.

Premises

The first premise is that developing an In Vitro student laboratory manual is desirable and in the best interest of Nuclear Medicine Technology education. In support of this premise, the majority of Nuclear Medicine program directors believe that a student manual is needed.

The second premise is that a student manual can be developed to meet the overall In Vitro needs of many diverse educational Nuclear Medicine programs.

The third premise is that constructing a laboratory manual was possible based on data collected. Furthermore, there was enough congruence between the directors of accredited programs in their responses that an overall consensus can be derived.

The fourth premise is that directors of accredited programs are expert enough in the field of In Vitro laboratory procedures to make intelligent decisions pertaining to its future needs.

The last premise is that a review of the literature and a content analysis of In Vitro material reveal a gap or a need for a student In Vitro manual for prospective graduating Nuclear Medicine Students.

Delimitations

The survey itself was delimited by the relatively small number of accredited Nuclear Medicine programs in the United States (approximately 70). This researcher made extensive efforts to elicit responses from all universities/colleges surveyed.

Another possible delimitation pertaining to the small number of existing Nuclear Medicine Programs is that extraneous variables may enter into the evaluation process.

These include restrictions in different universities pertaining to core requirements for graduation and the attempt of some program directors to protect their existing program and set In Vitro curriculum. There may also be an attempt by certain directors to hide their program's In Vitro laboratory weaknesses and possibly deny the need for a student manual.

Many Nuclear Medicine Program Directors may themselves have limited backgrounds and work experiences in In Vitro laboratory procedures. Others may be removed from the clinical setting by their current administrative duties and have not kept pace with the changing In Vitro laboratory field.

Finally, some universities may not employ a Nuclear Medicine program director. The dean or administrative head of that sector were asked to assume the responsibility of completing the survey. This delimitation might impact the number of questionnaires received and interpretations expressed.

Method of Research

The first step in the study was to develop a questionnaire based on content analysis of current literature which provides information the researcher desires. Secondly, the field test of the questionnaire was distributed to a small group of authorities in Nuclear

Medicine. Once the field test questionnaire was received, the survey was revised accordingly.

The final questionnaire was distributed to all accredited Nuclear Medicine Program Directors or their representatives. Follow-up contact was made until a representative sample of the survey is attained. Due to the limited numbers in the study (70), every effort was made to obtain a 100 percent response rate.

Next the questionnaire was categorized and the data analyzed based on the respondent's answers. Another content analysis was developed based on the questionnaire. This information was then utilized to:

1. Compare the assessments of Nuclear Medicine program directors with the content analysis of the current literature,
2. Categorize through general principles concepts and specific types of In Vitro testing procedures seemed most essential to students entering the field of Nuclear Medicine, and
3. Develop a student In Vitro laboratory manual based on the overall needs indicated by the review of the literature and the national survey.

Definition of Terms

AART. The American Registry of Radiologic Technologists. This association develops policies and sets standards for radiologic technologists. It also provides a written test of competence in the field of radiologist technology. This examination is nationally accepted.

Accreditation of Nuclear Medicine Program. A formal process in which a school applies for and has a site visitation of their program by an Agency that has the authority to evaluate the appropriateness of their studies.

Accuracy. Closeness to true or real value.

Advanced Radiologic Technology. Generally considered more sophisticated forms of radiology, such as nuclear medicine, C.T. scanning, nuclear magnetic resonance.

Affinity. Strength (energy) of binding to the receptor.

Analyte. The patient sample.

Antigen (Ab). A protein formed as part of an immunologic response to foreign substance.

Antigen (Ag). A substance capable of including formation of antibodies.

Antiserum. Measure of the strength of the bound Ag.

Avidity. Measure of the strength of the bound Ag.

B.S.R.T. Program. Bachelor of Science, Radiologic Technology. Usually upper-level curriculum only.

CAHEA. Committee of Allied Health Education and Accreditation.

Competencies. Specialized area in which individuals are trained in radiological sciences.

Competitive Binding Assay. A type of In Vitro testing procedure that is commonly used to analyze blood components.

Controls. May be a standard or any known concentration of testing material that serves as a comparison between successive test runs.

Cross Reactivity. Similar structured Ag that interfere with analysis of a given assay.

Equilibrium Saturation. Competition between labeled and unlabeled ligand is allowed to occur simultaneously until equilibrium is reached between the ligand and the binder.

Freud's Adjuvant. An emulsifier that enhances the immune response and allows for slow absorption into an animal's system when producing antiserum.

Hapten. A substance that is not immunogenic in itself but becomes immunogenic when complexed with another compound.

Hematocrit. Formed elements divided by whole blood.

Immunogen. A substance capable of inducing an immune response.

Immunoglobulins. A group of serum proteins (antibodies) that specifically bind the antigens.

Inflammatory Disorders. Reaction of the tissues to injury; the essential process, regardless of causative agent.

In Vitro Nuclear Medicine. Testing procedures performed in test tubes utilizing tracer amounts of radioactive material. Usually involves direct diagnostic assays of physiologic substances present in minuscule concentrations including hormonal, blood, and drug levels.

In Vivo Nuclear Medicine. The use of gamma-ray cameras and scanners to make interpretations on a qualitative basis. Commonly referred to as scans such as bone, liver, brain scans, etc.

JCAH. Joint Commission on Accreditation of Hospitals. This is the accrediting agency for all U.S. hospitals.

Joint Review Committee on Accreditation of Nuclear Medicine Programs. A committee that is granted its authority by the Committee of Allied Health Education and Accreditation, which grants accreditation to all Allied Health programs.

Laboratory Manual. A working guide to help an individual understand the application of theory into a practical nature.

Ligand. The substance that is bound.

Milliliters. 10^{-3} liters.

Modalities. Various types of technical areas in radiological sciences such as radiation therapy,

radiography, nuclear medicine, ultrasound, and C.T. scanning.

Nanograms. 10^{-9} grams.

Non-invasive Procedures. Diagnostic procedures that do not cause harm or pain to the patient receiving it.

Non-Specific Binding. When labeled Ag and Ag free serum (without Ab's) are put into the same test tubes.

Nuclear Medicine Program Director. The individual who directs, supervises, and administrates the individual Nuclear Medicine program.

Nuclear Medicine Technologist. An individual who performs diagnostic and therapeutic procedures utilizing radiopharmaceuticals.

Picograms. 10^{-12} grams.

Precision. Degree of agreement of repeated measurement of a quantity (usually expressed as coefficient of variance).

Prototype Curriculum. Course work designed to be implemented in any radiological sciences program.

Quality Control. Ensure that the results of assays are reliable and representative of true concentration.

Radioimmunoassay. Radioligand assay in which the receptor is an antibody. The use of radioactive tracers to evaluate blood components, hormonal levels, or drug levels.

Radiographer. One who is responsible for actually performing the x-ray procedure on the patient.

Radiologic Technologist. A generic term to describe any technologist that works in the field of radiology, including radiographer, nuclear medicine, ultrasound, radiation therapy, and C.T. scanning.

Rank Order. A chronological listing from top to bottom of certain items based on an assessed value.

Receptor. A substance (protein) that specifically binds a certain compound (ligand).

Sensitivity. Minimum quantity detectable.

Society of Nuclear Medicine. A national society of physicians, scientists, and technologists whose desire is to promote the field of Nuclear Medicine and maintain up-to-date material.

Specific Activity. Activity, usually expressed as $\frac{\text{uci}}{\text{g}}$ mass.

Specific Gravity. Materials' weights are being compared to that of water.

Specificity. Capacity to discriminate antigens of similar structure.

Standard. A substance that is added to certain reference tubes to serve as a yardstick for quantitation of the contents.

Standard Curve. A curve of dilutions of known amounts of substance to which the unknown may be compared.

Technical Education. Those aspects of training involving hands-on experience and less emphasis on theoretical thought-provoking ideas.

Technologist Sections. A group designed to meet the group needs of technologists of a specific discipline.

Titer. Measurement of antibody concentration--the dilution of antiserum that will bind 50 percent of added radioligand.

Organization of Study

In order to develop a strategy which ultimately results in adding to the body of knowledge in Nuclear Medicine Technology education, this study must first seriously examine the literature pertinent to the field. Secondly, develop a method of collecting relevant information related to the researcher's stated problem. Thirdly, analyze data and synthesize results in order to bring about meaningful change in Nuclear Medicine In Vitro Laboratory education.

A thorough review of the literature includes historical perspective of Nuclear Medicine education, its growth, development, recent trends, and all literature pertinent to the development of a Nuclear Medicine In Vitro Laboratory manual. It was vitally important to establish the need for this study by discussing previously related endeavors and other research which may be peripheral or corroborated to this study's eventual findings. Based on the supportive

literature findings, a well-conceived questionnaire was developed which provided research information desired.

This survey questionnaire was field-tested for reliability and validity by sending it to five established experts in the field of Nuclear Medicine, identified by the Society of Nuclear Medicine, for their input and evaluations. When returned to the researcher, questions were reconstructed to conform with the group's criticisms. Those who do not respond were contacted by follow-up.

The results were analyzed and the data used to develop an In Vitro Laboratory Manual identified as being needed in Nuclear Medicine Technology education. This laboratory manual was based on the information gathered in the national survey and currently lacking in the literature.

Chapter 2

REVIEW OF THE LITERATURE

Development of Nuclear Medicine Technology Education

This chapter provides a literature review of many important aspects within the field of Nuclear Medicine. In order to develop an appreciation for Nuclear Medicine, three major areas are discussed and analyzed. These include: Nuclear Medicine Technology Education, an overview of historical changes in Nuclear Medicine, and a content analysis of In Vitro Nuclear Medicine literature.

The first section concentrates on the development of Nuclear Medicine education. This discipline emerged through Allied Health concept which emphasizes the training, certification, and how professional practices of specialized fields developed. Other aspects contributing to Nuclear Medicine education including task analysis, credentializing, movement toward baccalaureate-level curriculums, Nuclear Medicine Associations, and advancement in technology.

The chapter continues with a fairly in-depth look at the historical development of the field of Nuclear Medicine. The discoveries and inventions that revolutionized our society's concept of diagnostic and therapeutic treatment of people using radioactive material beginning in the early

1920s to the late 1980s. Special emphasis is given to the development of In Vitro Nuclear Medicine, including radioimmunoassay principles.

The final thrust of the review of the literature centers on the content analysis of over 300 texts, articles, and manuals written on the subject of In Vitro Laboratory Procedures. The tables that were developed characterize these written works into the level of material, type of material, and whether they provide laboratory exercises.

Development of the Allied Health Concept.

For hundreds of years, medicine was practiced in conjunction with religion. During this time, many health-related decisions were based upon faith and superstition. With the Twentieth Century, there began a new era in medicine. The turn of the century marked the science age. Soon, the superstitions which served as the basis for medicine were replaced by scientifically founded facts.

The advent of new technological discoveries led to a rapid increase in the demands for health care services. One-to-one doctor-patient care became a thing of the past. These new technologies became so complex that their proper operation required specially trained personnel. As these health technologies began to develop individual autonomies, the people within the specific fields began to form their own professional societies.

The establishment of technological societies resulted in the identification of specific education requirements for each technology. This led to the process of accrediting educational institutions for each respective health field by the American Medical Association (AMA). Currently AMA has defined 23 allied health fields or professions based principally on technology (Amer. Society of Radiologic Technologists, 1972, p. 1). These include the following:

- Assistant to the Primary Care Physician
- Cytotechnologist
- Electroencephalographic Technician
- Electroencephalographic Technologist
- Histologic Technician
- Medical Assistant
- Medical Assistant in Pediatrics
- Medical Laboratory Technician (Associate Degree)
- Medical Laboratory Technician (Certificate)
- Medical Records Administration
- Medical Records Technician
- Nuclear Medicine Technologist**
- Occupational Therapist
- Operating Room Technician
- Ophthalmic Medical Assistant
- Physical Therapist
- Radiation Therapy Technologist
- Radiologic Technologist

Respiratory Therapist

Respiratory Therapy Technician

Specialist in Blood Bank Technology

Surgeon's Assistant

Each profession is autonomous in matters of training, certifying, and setting standards of professional practice.

Although the arrival of the scientific age played an important role in the development of allied health professions, there were also other factors which assisted in its development. These factors include the Allied Health Act of 1966; pressures from professional societies; increases in the number of community colleges, and their attempt to meet the needs of the community; and the establishment of schools of health-related sciences (Amer. Society of Technologists, 1972, p. 1).

Use of Task Analysis for Program Development in Allied Health, such as Nuclear Medicine Technology

Professional allied health societies evolved for a number of reasons. Some of the reasons were to prevent incompetent people from practicing, to standardize the training of personnel, to reduce the competition in the job market, and to protect technologists' positions. One major outcome of the professional societies was the establishment of registries and/or certification.

Originally, professional societies were responsible for the development and implementation of registries and/or standards of certification. As time progressed, the American Medical Association (AMA) became involved in accrediting allied health education programs. Eventually, in order for an individual to become eligible to write a specific registry or qualify for certification, allied health personnel had to graduate from a school accredited by the AMA. Currently, school accreditation is contingent upon the institution complying with the "essentials" or guidelines established by the AMA for allied health (AMA, 1978). As technology continues to advance, the list "essentials" continues to increase.

In 1970-71, the Association of Schools of Allied Health Professions and the AMA sponsored a "Study of Accreditation of Selected Health Education Programs" (JAMA, 1971, 218, p. 238-240). The objective of the study was to evaluate the current accreditation process for various allied health fields. The results of the study demonstrated that accrediting agencies, including the AMA, require a multitude of accreditation criteria. The criteria include a variety of specific courses, course credits in terms of credit hours, and hours of supervised clinical practice.

In essence, the committee defined many of these criteria as meaningless. As a result, the theory of "essentials" has been scrutinized by Ruhe and Kuhli, staff

members of the AMA. They perceive that the future criteria of allied health was stated in meaningful and measurable behavioral terms.

The World Health Organization supports the concept of defining behavioral objectives and has gone on record as stating that the failure to define goals is one reason which has prevented significant advances in the quality of health care (McBride, 1977, p. 14).

One method of defining goals is through task analysis. Task analysis involves the identification of tasks associated with a specific job. There are several approaches that may be employed to identify tasks. One approach is to have a group of "experts" in a particular field list the tasks associated with a job. A second method is to survey the population involved in performing the tasks, and allow the population to identify the relevant tasks. Probably the best method of task identification is to allow the "experts" to identify the tasks, they survey the population that performs the tasks to confirm or alter the task list established by the "experts".

The process of task analysis should include the identification of the frequency the task is performed, the amount of knowledge required to perform the task, and the importance of the task. Identification of tasks in accordance with their frequency, knowledge, and importance enables the development of an educational program. For

example, the tasks identified as requiring basic knowledge would represent the basic or elementary program course work and the tasks identified as requiring advanced knowledge would represent the advanced program course work.

The development of specific course content and unit objectives would require a further analysis of the task. The further analysis of the task would serve to identify the "steps" or "work activities" which would result in producing the identifiable task output (Bosissoneau, 1983, p. 155).

Because of the current public concern over quality health care, the state and federal governments have begun to mandate that goals and objectives be established for allied health. Some states require that allied health personnel obtain licenses in order to practice their respective specialty. This is of particular importance in Nuclear Medicine in which technologists prepare and administer radioactive materials to patients. Proponents of mandating minimum educational standards at the federal level are Senator Edward Kennedy (National Health Insurance Bill), the National Advisory Commission of Health Manpower, and the Secretary's Commission of Medical Practice.

The actions of these proponents indicate that in order for allied health personnel to have a voice in their own futures, it is important that the members of the allied health professions actively pursue the defining of their

goals and purposes in educating future individuals in their fields.

Credentialing in Nuclear Medicine Technology

Credentialing is a term used to describe how graduates of accredited Nuclear Medicine Programs become eligible to take a national registry in their chosen fields. A number of national organizations and committees helped to develop, coordinate, and implement what is today a national credentialing examination accepted throughout the country. Its brief history began only 25 years ago.

In the early 1960s, the growing importance of radioisotopes as diagnostic and therapeutic tools of radiology and a correspondent need to develop and identify individuals with the specialized skills necessary to assist physicians in the practice of Nuclear Medicine became apparent to the Board of Trustees of The American Registry of Radiologic Technologists (ARRT). Consequently, in 1962, the Board moved to begin the examination and certification of radioisotope technologists the following year. Recognizing the need for standardization of education in the new allied health specialty, the ARRT also proposed to the Board of Chancellors of the American College of Radiology (ACR) that the Council on Education of the American Medical Association be requested to consider the establishment of minimal educational standards for a one-year program for

radioisotope technologists. An interim curriculum was provided by the American Society of Radiologic Technologists (ASRT) as a basis for the first examination which was held on November 2, 1963. The 148 technologists who passed that examination were certified on December 1, 1963, to become this country's first credentialed Nuclear Medicine Technology Specialists (Lam, 1980, p.52).

The following year, the American Society of Clinical Pathologists (ASCP) in collaboration with the American Society of Medical Technologists (ASMT) implemented a program to examine and certify medical technologists in its nuclear medicine subspecialty. Subsequently, both the ASCP/ASMT and the ARRT organizations continued to separately examine and certify nuclear medicine technologists. Although there was some overlap, the ARRT generally attracted candidates who had worked and trained under radiology with emphasis on imaging procedures, whereas the ASCP/ASMT operated on a smaller scale primarily serving candidates with medical laboratory training and experience emphasizing wet laboratory procedures. However, with the advent of formalized education in Nuclear Medicine technology, these differences gradually became less pronounced.

In 1977, the ARRT and ASCP/ASMT registries cooperated as members of an ad hoc committee to draft essentials for educational programs in Nuclear Medicine technology. The

work of that committee led to the approval of the first "Essentials of an Accredited Education Program for the Nuclear Medicine Technologists" by the AMA House of Delegates in 1979. Included in the "Essentials" was a provision for a "Board of Schools" to serve as a review body for educational programs in Nuclear Medicine technology. The "Board of Schools" later became the Joint Review Committee on Educational Programs in Nuclear Medicine Technology.

As a step toward further cooperation between organizations in 1979, the ASRT Board of Directors proposed that the ARRT establish reciprocity of certification with ASCP/ASMT Board of Registry. Subsequently, the ARRT Board of Trustees authorized correspondence with other parties affected by the ASRT proposal and appointed representatives to develop a mutual exchange of information with the ASCP, ASMT, and the Society of Nuclear Medicine (Seibert, 1981, p. 346).

In March, 1981, the president of the ARRT met in Washington, DC, with representatives of the Registry of Medical Technologists (ASCP), the American Society of Medical Technologists and the Technologist Section of the Society of Nuclear Medicine to explore the possibility of forming a conjoint registry for Nuclear Medicine technology. A task group was formed "to prepare a draft of guidelines for training and certification in nuclear medicine

technology" to be considered at the annual meeting of the Society of Nuclear Medicine (Seibert, 1981, p. 52).

Later in 1981, representatives of the Joint Review Committee on Educational Programs in Nuclear Medicine Technology met with ARRT representatives at the Registry office in Minneapolis over a mutual concern with fragmentation of certification in nuclear medicine technology. Eligibility requirements, examination questions, and administrative procedures of the ARRT and ASCP/ASMT examinations were compared and discussed at length. It was agreed that it would be desirable to have a common examination in Nuclear Medicine technology. It was the consensus of those present that a conjoint committee should be established to make up future examinations using a common pool of questions.

Finally, it was concluded that the organizations should work toward a single certificate of registration in nuclear medicine technology with appropriate notations of interested parties to the certification (Seibert, 1981, p. 52).

Today, through the combined efforts of these organizations, the examination and credentialing of certified nuclear medicine technologists is accepted as proof of qualifications in all 50 states and many countries abroad.

Recent Trends in Nuclear Medicine Technology Education

The Movement Toward Baccalaureate Level Curriculum for Nuclear Medicine Programs. Nuclear medicine is generally credited with being the second largest radiological science. It has significantly changed its curricula from two-year programs to four-year baccalaureate-level degrees.

There is a little doubt that the baccalaureate degree is valuable both to an individual and to the profession to which he belongs. In a study of allied health graduates, Broski and Ballinger found that those from baccalaureate programs enjoyed higher levels of professional responsibilities, higher salaries, better geographic mobility, and greater job satisfaction than their associate degree counterparts (Broski, 1980, p. 51-485).

In that study, it was also demonstrated that baccalaureate decreed Nuclear Medicine graduates are assuming responsible positions with relatively high frequency. The positions assumed most frequently by graduates in that survey were in educational settings, administration, and in advanced or alternate imaging modalities. These findings confirm what has been revealed by a survey of Nuclear Medicine technology program directors in 1975, that there was a need for advanced training in specialized areas, administration, and educational techniques.

Growth in advanced Nuclear Medicine technology education has been affected to a large extent by the preponderance of the 24-month curriculum. In 1966, programs of two years in duration proliferated as a result of the ARRT policy requiring 24 months of formal education for examination eligibility. However, in recent years, educators have questioned whether the two-year curriculum can be the primary mode of meeting traditional as well as specialty personnel requirements in Nuclear Medicine technology. For example, in 1977, as a result of an international survey that analyzed the length of educational programs in Nuclear Medicine technology in 25 countries, Ohnysty (1978) found that basic curricula of two years in duration were in the minority, and he observed that "Based on the development of new imaging procedures, laboratory tests, variations in equipment in Nuclear Medicine and increased responsibilities of the technologists, two-year programs have proven to be inadequate to train the caliber of technologist necessary in current practice" (p, 448).

Many factors have influenced the education of Nuclear Medicine technologists to higher levels of education. In the past ten years, the shift in accredited Nuclear Medicine programs nationwide has been toward increased higher levels of educational qualifications and a reduction in the overall number of programs that provide training. Some of this reduction has been due in part to lack of adequate In Vitro

laboratory education. In 1974, the total number of accredited Nuclear Medicine programs in the United States was 177 with only 28 of those requiring two years or more to complete. In 1984, a marked reduction in the number of overall programs is noted at 110, with 70 of these programs now providing at least two years or more to complete. The trend has definitely evolved toward a higher level of education, requiring background courses that allow for a more sophisticated, career-minded Nuclear Medicine graduate. The three most important phenomena that have helped to advance educational levels higher are: Nuclear Medicine technologist associations, advancement in technology, and national accreditation agencies. It is important to examine each individually to see how they have influenced Nuclear Medicine education.

Nuclear Medicine Associations

The development of strong state Nuclear Medicine associations have helped foster higher levels of qualifications for employment in radiology and have mandated higher levels of education in certain states. Such associations have been highly correlated to states that provide highly qualified technologists, the highest level of education and generally demand the highest salaries nationwide. States such as California and Florida are excellent examples. Florida has laws regarding personnel

licensure employees that use radioactive materials and equipment. Furthermore, it has state competency examinations in many different specialty classifications-- such as radioassay, technician, technologist, and radiological administrator. The prerequisites for taking examinations include educational requirements that enhance the movement toward higher levels of sophistication of knowledge and understanding of new modalities in theoretical terms in order to be employed at that respective position.

Strong, unified state organizations have used their political clout to muster the energy to convince lawmakers that only specially-trained individuals should be allowed to use radioactive materials for diagnostic and therapeutic means. This has had the effect of elevating radiological education requirements and fostering higher levels of training and specialization.

Advances in Technology

No one can deny the massive and impressive advances in health care technology over the past few decades. Individuals that enter the medical field need to be trained on highly sophisticated, diverse computerized systems of diagnostic and therapeutic medicine. Students of Nuclear Medicine sciences are entering a dynamically growing increased specialized field including digital subtraction imaging, nuclear cardiology, radioimmunoassay, and nuclear

medicine resonance. Each of these testing procedures need specific didactic and clinical educational experiences associated with them. further these students must possess basic understanding in chemistry, biology, physics, and computers. These advances in Nuclear Medicine technology have pushed the emphasis of education to higher levels in which individuals would be required to have many preliminary courses in order to fully comprehend these new modalities.

National Accreditation Agencies

The Joint Commission on Accreditation for Allied Health programs has helped foster standards of educational proficiency. JCAH has placed great emphasis on updating educational curricula and providing students with theoretical and practical experiences that assure quality in job performance.

Ficken, a medical physician with over 15 years as a member of Nuclear Medicine programs joint review inspection teams, believes that such programs need to be placed at four-year level universities. He reasons that there is increased intellectual demands on technologists and that a university setting can provide overall better facilities, more qualified teachers, and prerequisite courses needed in order for students to fully comprehend new advances in Nuclear Medicine technology (1985). These comments and actions by JCAH have helped push the education pendulum back

toward higher educational levels for potential Nuclear Medicine technologists.

History of the Field of Nuclear Medicine

Before the Early 1920s, discoveries of Roentgen, Betquerel, Curie, and Rutherford began to revolutionize the concept of radioactivity and its biological effects and potential usefulness. But not until 1923, when Hevesy employed a natural radioisotope of lead to investigate the metabolism of lead in plants was the field of Nuclear Medicine born.

When Hevesy and Paneth used radioactive "indicators," they relied mainly on the gold-leaf electroscope. Measurement of the tracer's radioactivity provided thousandfold increases of sensitivity and accuracy over existing chemical assays, which Hevesy exploited 10 years later in following the movement of a tracer in living systems. By measuring the radiolead accumulated in various portions of the plant, he was able to quantify the uptake of lead in them. So sensitive was his gold-leaf electroscope that he could use the radiolead in quantities so minuscule as to avoid the toxic effect of lead, an important basic principle in tracer methodology. This first use of a "radioindicator" in biomedicine presaged things to come (Aebersold, 1956, p. 1027).

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The foundation and basic rationale of much of nuclear medicine stem from Hevesy's realization that the state of radioactivity of radioelements in no way affects their biochemical properties. More than anyone, Hevesy deserves the designation "grandfather of Nuclear Medicine." He visualized that a radioactive atom might be used as a "representative" tracer of stable atoms of the same element whenever and wherever it accompanied in biologic systems.

His studies in this "classical period" were limited, of course, to the few available naturally radioactive materials. These were comprised of radioisotopes of uranium, thorium, radium, polonium, bismuth, and lead-elements that were not of great biologic importance. Radium, in its transmutations into radioactive isotopes of bismuth and lead, he found most feasible for developing his tracer method of analysis (Aebersold, 1956, 1027).

Blumgart and co-workers conducted the first clinical studies with radioactive tracers in the '20s in Boston. They injected solutions of radium C (^{214}Bi) into one arm and, with a cloud chamber, detected the appearance of highly penetrating gamma rays in the other. Their aim was to study the "velocity of the circulation." In these pioneering studies, the mean normal arm-to-arm circulation time was 18 seconds, which was prolonged in patients with heart disease. Using a forerunner of the Geiger counter with platinum needle electrodes over the right atrium and left elbow, they

were able subsequently to calculate pulmonary circulation time and pulmonary blood volume and to study effects on them of various heart and lung lesions, thyroid disorders, anemia, polycythemia, and drugs. Such classical studies illustrate that minds and method were fully prepared to exploit the eventual appearance of radioactive isotopes of physiologic elements (Moss, Dalrymple, & Boyd, 1976, p. 48).

The growing understanding of the nature of the atom and of the behavior of its radiations made it a practical certainty that there would soon emerge a means to produce radioactive tracers of many elements. Among the most important was the discovery by Rutherford, in 1919, that the structure of matter could be changed by bombarding elements with alpha particles from radium.

In the 1930s, the development by Lawrence of the cyclotron made it possible to produce radioactive materials. This was the beginning of the first uses of artificial radioisotopes employed for therapeutic and diagnostic value in man.

The metabolism of phosphorus in animals and in man quickly was delineated further in a series of studies made possible by the availability of large amounts of the cyclotron-produced tracer ^{32}P . Hevesy, Chiewitz, Aten, Chaikoff, Cohn, Cook, Greenberg, Hahn, Jones, Lawrence, Perlman, and Scott were among the investigators pursuing this trail. The great efficiency with which the body

absorbed inorganic phosphorus and preferentially utilized it in hematopoietic tissues and in rapidly multiplying cells, such as those associated with malignancies, was observed. John Lawrence was the first to seize upon the therapeutic implications of these findings, and on Christmas Eve of 1936, he first used ^{32}P as phosphate in the treatment of leukemia, thus inaugurating the therapeutic employment of artificial radioisotopes (Chiewitz & Hevesy, 1935, p. 754).

In the 1940s, relative uptake of radioiodine in the thyroid and in distant metastases was being portrayed by a prototype "scanner" consisting of a shielded directionalized Geiger counter used for manual "point-by-point counting" over areas of interest. With careful and laborious charting, a map could be constructed of the distribution of uptake, and isocount lines drawn. It first occurred to Benedict Cassen, a physicist, that the scanning pattern could be refined mechanically by means of a motorized detector moving in a raster fashion over the area to be charted. The output of the detector then could be used to activate a moving pen, which could thus depict with ink dots an "image" of the relative numbers of gamma rays emanating from the area so scanned.

In the development of this important approach, several problems had to be solved. At the detector end, the Geiger counter had serious deficiencies. It was notoriously inefficient in its reactions with the penetrating gamma

photons used for external detection, and this shortcoming necessitated large doses of ^{131}I . It was necessary to find a detector more sensitive to gamma radiation to make it possible to use smaller doses of this radioisotope for diagnostic purposes (Hamilton, 1942, p. 541).

The answer came from the field of crystallography. Certain crystals of high density and transparency have the power to absorb the gamma photon and produce a flash of light called a scintillation. Fluorescent phenomena had been known for some time, and indeed, Rutherford had relied on fluorescence in many of his early studies of particles. In 1947, Kallmann devised a scintillation detector by utilizing organic crystals of naphthalene attached to the face of multiplier phototube, which had the capacity to transduce the flashes of light into amplified surges of electricity. Kallmann's first detector, crude by present standards, still was much more efficient for gamma rays than the Geiger counter tube (Anger, 1958, p. 27).

In the 1950s, Cassen developed the Gamma Scanner. Cassen's first rectilinear scanner in 1951 used inorganic calcium tungstate detector crystals, which were even much more efficient than naphthalene. Detector efficiency was improved further by Hofstadter when he activated high-density sodium iodide crystals by adding small amounts of thallium. An especial advantage of these crystals is that they may be grown from molten salt into the enormous sizes

currently being used in Nuclear Medicine. Not only do they interact highly efficiently with gamma rays to give large scintillations but the sizes of many of these light flashes are proportional to the energy of the absorbed gamma photon. Consequently, the voltage pulses triggered by the multiplier phototube may be sorted electronically to discriminate among gamma ray emissions of various energies to select only the ones of primary interest. This facilitates the localization of sources of direct emissions because one may "turn out" irrelevant off-axis, low-energy secondary photons resulting from degrading scatter of some of the primary gamma rays within the patient, improved images of the spatial distribution are the result (Anger, 1958, p. 27).

Cassen's scanning machine displayed the results as a picture by applying the amplified detector's pulse to a pen that contacted the paper by a carrying arm that moved in parallel with the directionalized scintillation detector. Response of the pen involved considerable mechanical inertia and the writing process was subject to jamming.

In the early 1960s, Hal Anger, embodying in a single person a "convergence" of nuclear physics, electronics, optics, and information handling, pursued a completely different path at the Donner Laboratory of Medical Physics of the Lawrence Berkeley Laboratory. He sought a means of utilizing gamma rays to build an image rapidly, camera-wise, in contrast with one built up over long intervals of time

with a moving scanner based on a raster principle. Gamma rays cannot be refracted with lenses, like light, or bent in magnetic fields, like charged particles and thereby "focused" (Anger, 1963, p. 56).

Besides Cassen and Anger, many other developers and manufacturers added to the body of knowledge and improved the scintillation camera.

When the camera finally caught on, and it became readily available commercially in 1964, it fairly "revolutionized the practice of clinical Nuclear Medicine." It accentuated the significance of such radioisotopes as ^{123}I and $^{99\text{m}}\text{Tc}$, whose low-particulate and gamma-ray emissions are so appropriate for camera use (Wagner, 1978, p. 121).

The development of the gamma-ray camera included multihole collimators, ever larger sodium iodide crystals, the use of larger banks of improved detector phototubes, tomographic applications, and highly sophisticated linkages with computers that has resulted in an explosion in new measurement technology.

Through this technology of gamma cameras and scanners developed in to what is referred to as In Vivo Nuclear Medicine. In the 1970s and 1980s, the ability to detect and accurately diagnose different diseases in man continued through the improvement of gamma camera.

Increases crystal diameter in gamma cameras has permitted a larger field of view in camera application; it

has also improved some aspects of scanning by allowing the geometric efficiency of the detector to be increased. With focused collimators, more emissions from a source of radioactivity can be collected on a broader crystal surface. In effect, this raises sensitivity to nuclear emissions much as a widened lens aperture increases the sensitivity to light in a camera. More emissions are collected per unit of time; thus, shorter exposures are required for detecting and recording enough events to form a statistically significant image, and the speed with which the scanning head can move on its path may be increased significantly. A crystal with a diameter of 5 inches provides roughly a threefold gain, and an 8-inch crystal an electric circuit can be made that will accept only desired pulse amplitudes--those characteristic of direct, unscattered gamma rays from the specific radioisotope used. This process is called pulse-height selection.

The result is a scintillation counter with energy specificity, or, in other words, a counter that discriminates against gamma rays from radioactive isotopes of different energy. It produces electrical pulses that can be quantified in terms of counts per second, which is important in estimating the amount of the radioactive tracer at different sites within the body (Wagner, 1978, p. 121).

Two other factors need to be discussed in relation to the growth of both In Vivo and In Vitro Nuclear Medicine in the

1970s and 1980s. They are computers and radiopharmaceuticals.

The technology of Nuclear Medicine permits direct measurements to be made of body processes in man that in the past could only be examined in experimental animals. Such study often produces large amounts of data, often in the form of quantities of radioactivity distributed in specific sites within the body, often changing over a period of time. The instrumentation utilized presents the data either as quantities that can be plotted and analyzed or as image analogues that allow the clinician to visualize the distribution of radioactive tracer materials within an organ or lesion (Natarajan, 1969, p. 823). the full diagnostic potential of nuclear data cannot be realized without computer assistance.

Today, throughout the nation there are very few Nuclear Medicine departments that do not fully utilize a dedicated Nuclear Medicine computer system. A highly trained technologist must set up the computer with proper perimeters and carefully analyze the image received in order to evaluate the lesion correctly.

In order to study the intrinsic contrast between the amount of radioactivity within a lesion as compared with that in the surrounding tissue, it is frequently necessary to use contrast enhancement to make the lesion readily visible. The technologist adjust the contrast in much the

same manner as he would with a television set. Should he adjust for high contrast, he achieves a dramatic gross differentiation between area of activity, but possible important subtleties in the blacked-out or bleached-out areas are lost to him. In short, he makes a deliberate decision to exclude certain data that the machine is capable of providing. Should he adjust for low contrast over a wide range of values, he runs the risk of overlooking diagnostically important variations that greater contrast in narrower ranges would show him. Although remarkable in capacity for spatial resolution, the human eye and brain are notoriously poor in memory and ability to quantify, precisely those attributes in which computers excel (Anger, 1983).

The second major factor in the development of Nuclear Medicine is radiopharmaceuticals. During the past 25 years, clinical applications of radioactive materials have become a major branch of medicine. The growth rate of such applications is still progressing exponentially and is now estimated at from 25 to 40 percent annually, as measured in terms of persons involved in these applications and of the industry developed to support them. Already, most hospitals contain or are developing a Nuclear Medicine facility. While nuclear procedures may never be available in every physician's office, there is scarcely a practitioner in any field whose understanding of physiology and pathophysiology

is not increases through the application of radioisotopes to the study of man (Anger, 1983).

The development of both the nuclear reactor and the cyclotron has allowed for the inexpensive production of radiopharmaceuticals for medical use.

The nuclear reactor generates power through controlled nuclear fission which introduced a new dimension into the production of artificial radioisotopes. It was appreciated that the nuclear reactions occurring in a nuclear reactor result in a high flux of neutrons that could be used for the generation of radioisotopes. Almost any material could be lowered into the reactor and subjected to neutron bombardment, producing "neutron-excess" radioisotopes (MacRae, 1974, p. 497)).

The cyclotron utilized the concepts developed at Lawrence Laboratories of particle acceleration to provide radionuclides. In cyclotron, the particles are repeatedly accelerated through intermediate voltages to achieve high energy. Protons are commonly used as a bombarding material. It has the advantage over a nuclear reactor in providing a wide variety of radionuclides. Another advantage is that it is capable of producing certain useful radionuclides, preliminary short lived, that are not produced in significant quantities in nuclear reactors.

Development of In Vitro Nuclear Medicine

Nuclear Medicine's capacity for precise and accurate measurement of tracers has made possible a variety of laboratory assays of great importance in clinical diagnosis, pharmacology, biochemistry, and many other fields. The studies of blood ferrokinetics, volume determinations, and vitamin B-12 absorption have been of inestimable value in hematology. Other radioassays have sharpened diagnosis in a host of hormonal, metabolic, infectious and inflammatory disorders. All of these testing procedures that utilize tracer amount of radioactive material in test tube sampling analysis have become commonly referred to as either In Vitro Nuclear Medicine or Radioimmunoassay.

Radioimmunoassay is a term that describes the use of radioactive tracers to assay or analysis of an unknown quantity. Radioimmunoassay (RIA) came into being not by directed design but as a fallout from what might be considered an unrelated study. Prompted by the suggestion of I. Arthur Mirsky that maturity-onset diabetes might not be due to an absolute deficiency of insulin secretion but rather to its abnormally rapid degradation by an enzyme which Mirsky called insulinase (Thorell, 1984, p. 4), Solomon A. Berson attempted to study the metabolism of ^{131}I -insulin following intravenous administration to diabetic and non-diabetic subjects (Thorell, 1984, p. 4). He observed a slower rate of disappearance of the ^{131}I -insulin from the plasma of insulin-treated subjects than from the plasma of

untreated subjects. He postulated that the slower disappearance was a consequence of the binding of labeled insulin to antibodies that had developed in response to treatment with foreign proteins--i.e., animal insulins.

The use of radioisotopic techniques for studying the primary reaction of antigen with antibody and analyzing soluble antigen-antibody complexes initiated a revolution in theoretical immunology, and it is now generally appreciated that peptides as small as vasopressin and oxytocin are antigenic in some species and the equilibrium constants for the antigen-antibody reaction can be as great as 10^{14} L/mol. (Rothfeld, 1985, p. 14).

Radioimmunoassay Principle

The principle of RIA is quite simple. The concentration of the unknown, unlabeled antigen is obtained by comparing its inhibitory effect on the binding of radioactively labeled antigen to specific antibody with the inhibitory effect of known standards. It should be appreciated that there is no requirement for standards and unknowns to be identical chemically or to have identical biologic behavior. A properly validated assay is one in which the concentration in the unknown sample is independent of the dilution at which it is assayed. As described later, some assays have proven to be properly validated due to lack

of immunologic identity between standards and the sample whose concentration is to be determined.

The RIA principle need not be limited to immune systems but has been extended to other systems in which, in place of the specific antibody, there is a specific reactor of binding substance. Herbert and associates (Herbert, et al, 1959, p. 433; 1960, p. 160) first demonstrated the applicability of competitive radioassay to the measurement of vitamin B₁₂ in a liver receptor assay using ⁶⁰Co-vitamin B₁₂ and intrinsic factor as the binding substance. However, it remained for Rothenberg (1961, p. 45) laboratory and Ekins (1960, p. 453) to develop assays for serum vitamin B₁₂ using this principle. Ekins and later Murphy (1964, p. 679) employed thyroxine binding globulin as the specific reactor for the measurement of serum thyroxine.

In the 1950s, when bioassay procedures were in the forefront, the first presentation of the potential of hormonal measurements by RIA went virtually unnoticed (Yalow & Berson, 1960, p. 1157). Somewhat more interest was generated by the demonstration of the practical application of RIA to the measurement of plasma insulin in man. It became evident that the sensitivity and simplicity of RIA permitted ready assay of hundreds of plasma samples, each as small as a fraction of a milliliter. It made possible measurement not only of single blood samples (as had been performed on occasion with In Vivo bioassay) but also of

multiple samples, thus permitting study of dynamic alterations in circulating insulin levels in response to physiologic stimuli.

In the 1960s, results of numerous studies were consistent with the assumption that immunologic activity reflected biologic activity and that the behavior of endogenous hormone in plasma resembled that of exogenous purified hormone added to plasma. Thus, plasma ACTH paralleled plasma cortisol throughout the day, responded appropriately to a variety of stressful stimuli, was elevated in patients with adrenal insufficiency not receiving steroids and was suppressed by steroid therapy, etc (Yalow & Berson, 1960, p. 1157). There were many similar studies with other peptide hormones measured by RIA.

RIA was initially employed primarily for the assay of peptide hormones since its exquisite sensitivity was required to permit their measurement in plasma in the unstimulated state in which the concentrations may range from 10^{-13} M to 10^{-10} M. However, the specificity and simplicity of RIA methodology soon led to its application to the measurement of non-peptidal hormones as well. Chopra (1972) first described RIAs for tyrosine (p. 938) and triiodothyronine (T_3). The sensitivity and specificity of the RIA for the thronines and the ability to measure thyrotropin (TSH) (Chopra, p. 938) have made possible a nationwide screening program for neonatal hypothyroidism, a

disease that must be treated shortly after birth to avoid irreversible mental retardation (Chopra, p. 938).

By 1969, a sensitive solid-phase RIA for plasma estradiol-17 beta had been described. Soon thereafter the number of applications of RIA to the measurement of steroid hormones grow very rapidly. Within five years, in a review of the field, Abraham (1969) indicated that RIA had been applied to the measurement of every known hormonal steroid and to many other steroids without known biological activity (p. 866).

RIA in Pharmacology

Although RIA appeared to belong almost exclusively to endocrinology during the first decade after its description, the rediscovery of the work of Landsteiner (1945) on production of antibodies by low molecular weight haptens coupled to proteins led to the realization that RIA could be adapted to the measurement of drugs. At the end of that decade Oliver, et al (1968), published a classic paper describing the measurement of digitoxin in human serum by RIA (p. 1035). At present, commercial kits for the measurement of the cardiac glycosides are routinely employed in hundreds of laboratories. A particularly important application in toxicology is the assay in plasma of the concentration of antibiotics for which there is a very narrow range between efficacy and toxicity. Microbiological

assays for these drugs are time consuming and expensive. The introduction of the RIA for gentamicin (Oliver, 1968, p. 1035). led the way, and commercial kits soon became available for assay of many antibiotics. RIAs for the host of other drugs are now available; many are in simple kit form.

RIA in Infectious Diseases

The first application of RIA to the field of infectious disease began with the development of an assay for hepatitis B antigen, then known as Australia antigen (Purcell, Holland, Walsh, et al, 1969, p. 383).

Over the past few years the incidence of acquired immunodeficiency syndrome (AIDS) due to infection with a tretovirus has skyrocketed, and the incidence rate is increasing yearly. Although an enzyme-linked immunoassay for detection of antibody to this virus contributes to the safety of blood used for transfusion, a test for the virus itself is urgently required. It is evident that there are infected persons without antibody, either because it is too early in the course of infection for antibody to have developed or because compromise of the immune system may decrease antibody production. It is not unreasonable to hope that an RIA for the virus will be developed that will permit improved safety of bank blood.

It can be predicted that RIA and related techniques will be applied increasingly to the detection of active infectious disease. Straus, et al (1981), have reported an RIA for a tuberculo-protein that can make the diagnosis of active tuberculosis more rapidly and in a much less expensive, simpler, and far safer fashion than with the use of classic microbacteriologic methodology (p. 3214). Even were this technology applicable only to the diagnosis of tuberculosis, it would remain a major breakthrough. However, it should serve as a model for the development of assays for proteins associated with other slow-growing organisms, the diagnosis of which may be difficult with standard microbiologic methodology.

Future of RIA

This review of the development of a small sampling of applications of RIA emphasizes the fact that in science new truths become evident when new tools become available. For more than a quarter century, RIA has been an important tool, fruitfully employed in diverse areas of biomedical investigation and clinical medicine. Can we predict what the relative usage of radioactive and non-radioactive labels in immunologic methods for diagnosis (immunodiagnos-tics) will be in the future? A Wall Street investment concern has attempted such a prediction (Dunne, 1981). They have estimated that in 1980 the market segment for RIA in

immunodiagnosics was \$332 million, for non-radioisotopic therapeutic drug monitoring \$43 million, and for all other non-radioisotopic immunoassays \$28 million; in 1990, they predict markets of \$785 million, \$625 million, and \$838 million respectively for these three methodologies. Thus, from a commercial point of view RIA usage is expected to more than double during the decade of the 1980s, but its fraction of the total immunodiagnosics market is expected to drop from over 80 percent to about 35 percent. Why? First of all, many of the new applications simply do not require the exquisite sensitivity of RIA. For instance, in therapeutic drug monitoring the concentrations being determined are generally a million-fold higher than the concentrations of peptide hormones. Furthermore, assays for drugs generally need not be quantitative over a multiple hundredfold range. It is often necessary only to determine whether the concentration is high enough to be efficacious and not so high as to be toxic. In the field of infectious diseases, where immunodiagnosis will play a major role, the choice between radioisotopic and non-radioisotopic methodology again would depend on the sensitivity required; in the presence of massive infection, the massive infection the sensitivity of RIA is not likely to be necessary. However, in a number of instances, those promoting non-radioisotopic labels are not necessarily suggesting that these have any technical advantage, but rather that

regulatory procedures and fear of radiation at any level would make radioisotopic label less desirable. Nonetheless, RIA is likely to remain the method of choice for what has been its first application, the measurement of peptide hormones in plasma and other body fluids, since their concentrations are well below $10^{-10}M$, and the sensitivity of RIA is essential (Dunne, 1981).

RIA and related methodologies have opened new vistas in science and medicine. RIA is now used in thousands of laboratories around the world to measure hundreds of substances of biologic interest. Even now, a quarter century after the introduction of RIA, there remain many additional fields that can be explored with its help.

Review of In Vitro Literature

A selective review of the nuclear medicine In Vitro literature was performed on over 300 articles, text books, and manuals. This researcher examined the literature and categorized the materials into a content analysis chart. The five areas were: Level of Material, Type of Material, Possibility of Providing Laboratory Exercises, General Principles, Concepts Discussed, and Specific Testing Procedures Mentioned.

The following is presented as a content analysis chart and a summary of resultant values obtained.

Content Analysis of In Vitro Nuclear Medicine

Literature. Column indexes and abbreviation conventions:

Column 1, Bibliography

Column 2, Level of Material

under = undergraduate

grad = graduate

tech = technical

Column 3, Form of Material

Column 4, Laboratory Exercises Provided

Column 5, Concepts Discussed

Column 6, Specific Testing Procedures

# 1	# 2	# 3	# 4	# 5	# 6
Berson, S.A., and Yalow, R.S., editors: Method in investigative and diagnostic endocrinology, vol. 2A and 2B, Amsterdam, 1973, North-Holland Publishing Co.	tech text				endocrine tests
Cameron, E.H. D., Hiller, S.G., and Griffiths, K.: Steroid immunoassay, Cardiff, U.K. 1985, Alpha Omega Publishing Co.	tech arti- cle			basic principles	steroid testing
Diczfalusy, E., editor: Immunoassay of Gonadotrophins. Karolinska Symposia No.1, Acta Endocrinol. (eds) 142(suppl.): 1979	tech arti- cle			basic reactions	gonadotropins
Hayes, R.L., Goswitz, F.A., and Pearson Murphy, B.E., editors: Radioisotopes in medicine; in vitro studies. A.E.C. Symposium Series 13, Oak Ridge, Tenn., 1978: U.S. Atomic Energy Commission	grad arti- cle			radiolabeling; basic principles/chemical reactions	
International Atomic Energy Agency: In vitro procedures with radioisotopes in medicine, Vienna, 1970, I.A.E.A.; International Atomic Energy Agency: radioimmunoassay and related procedures in medicine, Vienna, 1984, I.A.E.A	un- der	text	exercises of basic concepts	basic procedures	
Jaffe, B.M., and Behrman, H.R., editors: Methods of hormone radioimmunoassay, New York, 1984, Academic Press Inc.	tech text			basic principles; concepts	
Kirkham, K.E., and Hunter, W.M., editors: Radioimmunoassay methods, Edinburgh, 1971, Churchill Livingstone.	tech text			methodology, equipment kit; evaluations	
Luft, R., and Yalow, R.S., editors: Radioimmunoassay; methodology and application in physiology and in clinical studies, Stuttgart, 1974, Georg Thieme Verlag;	grad text			many principles; concepts	types of tests
Odel, W.D., and Daughaday, W.H., editors: Principles of competitive protein-binding assays, Philadelphia, 1971, J.B. Lippincott Co.	tech text			basic principles	
Pastermak, C.A., editor: Radioimmunoassay in clinical biochemistry, London, 1975: Heyden & Son Ltd.	grad text			basic concepts	bio-chemistry
Ranson, J.P.: Practical competitive binding assay methods, St. Louis, 1986, The C.V. Mosby Co.	tech text			many principles; concepts	numerous tests
Rothfeld, B., editor: Nuclear medicine in vitro, Philadelphia, 1984, J.B. Lippincott Co.	grad text			many principles; concepts	numerous tests
Sonksen P.H., editor: Radioimmunoassay and saturation analysis, Br. Med. Bull. 30(1): 1974.	tech arti- cle			separation technique	
Bassiri, R.M., and Utiger, R.D.: The preparation and specificity of antibody to thyrotropin releasing hormone, Endocrinology 90:722, 1972.	tech arti- cle			preparation, calibration	thyropin releasing hormone
Berson, S.A., and Yalow, R.S.: Kinetics of reaction between insulin and binding antibody, J. Clin. Invest. 36:873, 1987.	grad arti- cle			reactions	insulin

Berson, S.A., and Yalow, R.S.: Immunoassay of protein hormones; dependence of sensitivity of assay on energy of antigen-antibody reaction. In Pineus, G., Thimann, K.V., and Astwood, E.B., editors: The hormones, vol.IV, II D, New York, London, 1964, Academic Press, Inc., pp. 567-572	tech:arti- cle	basic Ag - Ab reactions	
Berson, S.A., Yalow, R.S., Bauman, A., Rothchild, M.A., and Newerly, K.: Insulin- ¹³¹ I metabolism in human subjects; demonstration of insulin binding globulin in the circulation of insulin treated subjects, J. Clin. Invest. 35:170, 1986	grad:arti- cle	basic reactions	insulin
Bolton, A.E., and Hunter, W.M.: The labeling of proteins to high specific radioactivities by conjugation to a ¹²⁵ I-containing acylating agent, Biochem. J. 133:529, 1973.	tech:arti- cle	radiolabeling	
Catt, K., Niall, H.D., and Tregear, G.W.: Solid phase radioimmunoassay of human growth hormone, Biochem. J. 100:31, 1986	tech:arti- cle	separation technique	
Catt, K., and Tregear, G.W.: Solid phase radioimmunoassay in antibody-coated tubes, Science 158:1570, 1967	un- der cle	separation technique	
Chard, T., Kitau, M.S., and Landon, J.: The development of a radioimmunoassay for oxytocin; radioiodination, antibody production and separation techniques, J. Endocrinol. 46:296, 1980	grad:arti- cle	radiolabeling separation technique	oxytocin
den Hollander, F.C., and Schuurs, A.H.: Discussion. In Kirkham, K.E., and Hunter, W.M., editors: Radioimmunoassay methods, Edinburgh, 1971, Churchill Livingstone, p. 419	tech:text	methodology, basic principles	
Desbuquois, B., and Aurbach, G.D.: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays, J. Clin. Endocrinol. Metab. 33:732, 1971.	tech:arti- cle	separation technique reaction concept	peptide hormones
Ekins, R.P.: The estimation of thyroxine in human plasma by an electrophoretic technique, Clin. Chim. Acta 5:463, 1980.	tech:arti- cle	separation technique; calculation results	T-4 test
Ekins, R.P.: Theoretical aspects of saturation analysis. In International Atomic Energy Agency: In vitro procedures with radioisotopes in medicine, Vienna, 1980, I.A.E.A., p.325.	tech:man- ual	exercise for use of separat- ion tech- nique	varied basic principles/ separation technique
Ekins, R.P.: Radioimmunoassay design. In Pasternak, C.A., editor: radioimmunoassay in clinical biochemistry, London, 1975, Heyden & Son Ltd.	grad:text	interactions for reactions in chemical levels	
Ekins, R.P., Newman, G.B., and O'Riordan, J.L.H.: Theoretical aspects of saturation and radioimmunoassays. In Hayes, R.L., Goswitz, F.A., and Pearson Murphy, B.E., editors: Radioisotopes in medicine; In vitro studies, A.E.C. Symposium Series 13, Oak Ridge, Tenn., 1978, U.S. Atomic Energy Commission, p.59.	tech:arti- cle	separation techniques	

Feinstein, A.R., Bethard, W.F., and McCarthy, J.D.: A new method, using radio-iron, for determining the iron binding capacity of human serum, J.Lab. Clin. Med. 42:907, 1953.	very tech	arti- cle	radiolabeling / binding	Fe- testing
Feldman, H., and Rodbard, D: Mathematical theory of radioimmunoassay. In Odell, W.D., and Daughaday, W.H., editors: Principles of competitive protein binding assays, Philadelphia, 1971, J.B. Lippincott Co., pp. 158	grad	arti- cle	calculation analysis of testing	
Froehan, L.A., Reichlin, H., and Sokal, J.E.: Immunologic and biologic properties of antibodies to glucagonserum albumin polymer, Endocrinology 87:1055, 1980	tech	arti- cle	properties of RIA reactions	glucagon serum testing
Greenwood, F.C., Hunter, W.M., and Glover, J.S.: The preparation of ¹³¹ I labelled human growth hormone of high specific activity, Biochem. J. 89:114, 1963	tech	arti- cle	preparation, labeling	HGH testing
Hales, C.N., and Randle, P.S.: Immunoassay of insulin with insulin-antibody precipitate, Biochem. J. 88:137, 1963.	grad	arti- cle	basic react- ions, pro- perties	insulin
Hamolsky, M.H., Stein, M., and Freedberg, A.S.: The thyroid hormone-plasma protein complex in man. II. A new in-vitro method for study of uptake of labelled hormonal compounds by human erythrocytes, J. Clin. Endocrinol.Metab.17:33, 1987	grad	arti- cle	basic reactions	thyroid
Hossein, G., Ryan, R.J., Mayberry, W.E., and Hockert, T.: Radioimmunoassay for triiodothyronine (T ₃). I. Affinity and specificity of the antibody for T ₃ , J. Endocrinol. 33:509, 1971.	tech	arti- cle	basic concepts	T ₃
Hughes-Jones, M.C.: Nature of the reaction between antigen and antibody, Br.Med. Bull. 19:171, 1963.	un- der	arti- cle	basic react- ion Ag-Ab	
Erlanger, B.F., Borek, G., Beiser, S.M., and Lieberman, S.: Steroid-protein conjugates of bovine serum albumin with testosterone and with cortisone, J. Biol. Chem 228:713, 1967.	tech	arti- cle	preparation characterist- ics	albumin, testoste- rone, & cortisone
Karush, F.: Immunologic specificity and molecular structure, Adv. Immunol. 2:1, 1962	tech	arti- cle	basic structures	
McFarlane, A.S.: Efficient trace-labelling of proteins with iodine, Nature 182:53, 1958.	tech	arti- cle	labelling	proteins
Miles, L.E.M., and Hales, C.N.: Labelled antibodies and immunological assay systems, Nature 219:186, 1968.	tech	arti- cle	labelling	
Orskov, H.: Wick-chromatography for the immunoassay of insulin, Scand. J. Clin. Lab. Invest. 20:297, 1967.	tech	arti- cle	separation technique	
Rodbard, D.: Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays, Clin. Chem. 20:1255, 1984.	tech	arti- cle	quality con- trol in e- quipment	

Rodbard, D., and Cooper, J.A.: A model for the prediction of confidence limits in radioimmunoassays and competitive protein binding assays. In International Atomic Energy Agency: In vitro procedures with radioisotopes in medicine, Vienna, 1970, I.A.E.A., P. 659.	grad arti- cle		quality control	
Roitt, I.M., and Torrigiani, G.: Identification and estimation of undergraded thyroglobulin in human serum, <i>Endocrinology</i> 81:421, 1987.	tech arti- cle		reactions, concepts	thyroglo- bulin
Rosa, V., Pennisi, F., Bianchi, R., Federighi, G., and Donato, L.: Chemical and biological effects of iodination on human albumin, <i>Biophys. Acta</i> 133:486, 1987.	very arti- tech cle		chemical & biological concepts; radiolabeling	
Rosselin, G., Assan, R., Yalow, R.S., and Berson, S.A.: Separation of antibody bound and unbound peptide hormones labelled with iodine-131 by talcum powder and precipitated silica, <i>Nature</i> 212:355, 1986.	tech arti- cle		separation technique	peptide hormones
Roth, J.: Peptide hormone binding to receptors; a review of direct studies in vitro, <i>Metabolism</i> 22:1059, 1973.	tech arti- cle		binding of receptors	peptide hormones
Thorell, J.I.: Use of immunosorbent in the preparation of "antigen-free" plasma; an adequate diluent for standards in radioimmunoassay, <i>Clin. Chim. Acta</i> 22: 579, 1986.	tech arti- cle		preparation of Ag-Ab	
Thorell, J.I., and Holmstrom, B.: Production of antisera against highly purified human follicle-stimulating hormone, luteinizing hormone and thyroid stimulating hormone, <i>J. Endocrinol.</i> 70:355, 1976.	grad arti- cle	produc- tion of Ab's	separation technique	TSH, LSH
Wide, L., and Porath, J.: Radioimmunoassay with the use of Sephadex-coupled antibodies, <i>Biochim. Biophys. Acta</i> 130:257, 1986.	tech arti- cle			
Yalow, R.S.: Radioimmunoassay; practices and pitfalls, <i>Circ. Res.</i> 32(suppl. 1): 116, 1973.	un- arti- der cle		basic concepts	
Yalow, R.S., and Berson, S.A.: General principles of radioimmunoassay. In Hayes, R.L., Goswitz, F.A., and Pearson Murphy, B.E., editors: <i>Radioisotopes in medicine; in vitro studies</i> , AEC Symposium Series 13, Oak Ridge, Tenn., 1968, U.S. Atomic Energy Commission, p. 7.	un- arti- der cle		varied concepts & principles	
Yalow, R.S., and Berson, S.A.: Special problems in the radioimmunoassay of small peptides, In <i>Protein and polypeptide hormones</i> , vol. I, 1968, Excerpta Medica Foundation, p. 71.	tech arti- cle		binding properties	peptides
Yalow, R.S., and Berson, S.A.: Review paper; general aspects of radioimmunoassay procedures. In International Atomic Agency: In vitro procedures with radioisotopes in medicine, Vienna, 1970, I.A.E.A., p. 455.	un- arti- der cle		general con- cepts; prin- ciples	
Alexander, M.M., and Jennings, J.F.: Radioimmunoassay of serum triiodothyronine on small, reusable, Sephadex columns, <i>Clin. Chem.</i> 20:1353, 1974.	tech arti- cle		calibration, preparation	T-3
Braverman, L.E., Vagenakis, A.G., Foster, A.E., and others: Evaluation of a simplified technique for the specific measurement of serum thyroxine concentration, <i>J. Clin. Endocrinol. Metab.</i> 32:497, 1981.	tech arti- cle		reactions, basic techniques	T-4

Brown, B.L., and Ekins, R.P.: A specific saturation assay technique for serum triiodothyronine. In International Atomic Energy Agency: In-vitro procedures with radioisotopes in medicine, Vienna, 1980., I.A.E.A., p. 569.	tech arti- cle	separation technique	T ₃
Burger, A., Suter, P., Nicod, P., and others: Reduced active thyroid hormone levels in acute illness, Lancet 1:653, 1976.	tech arti- cle	basic reactions	thyroid
Burman, K.D., Wright, F.D., Earll, J.M., and Wartofsky, L.: Evaluation of a rapid and simple technique for the radioimmunoassay of triiodothyronine(T ₃), J. Nucl. Med. 16:622, 1975.	tech arti- cle	evaluation of techniques	T ₃
Challand, G.S., Ratcliffe, W.A., and Ratcliffe, J.G.: Semi-automated radio-immunoassays for total serum thyroxine and triiodothyronine, Clin.Chim. Acta 60:25, 1975.	tech arti- cle	quality control	T ₃ , T ₄
Chopra, I.J.: A radioimmunoassay for measurement of thyroxine in unextracted serum, J. Clin. Endocrinol. Metab. 34:938, 1972.	tech arti- cle	calculation of results	T ₄
Chopra, I.J., and Crandall, B.F.: Thyroid hormones and TSH in amniotic fluid, N. Engl. J. Med. 293:740, 1975.	tech arti- cle		thyroid, TSH
Chopra, I.J., Ho, R.S., and Lam, R.: An improved radioimmunoassay of triiodothyronine in serum; its application to clinical and physiological studies, J. Lab. Clin. Med. 80:729, 1972.	tech arti- cle	evaluation of techniques	T ₃ , T ₄
Chopra, I.J., Solomon, D.H., and Beall, G.M.: Radioimmunoassay for measurement triiodothyronine in human serum. J. Clin. Invest. 50:2033, 1971.	tech arti- cle	calculations of results	T ₃
Clark, F., and Horn, D.B.: Assessment of thyroid function by the combined use of the serum protein-bound iodide and resin uptake of ¹³¹ I-triiodothyronine J.Clin. Endocrinol. Metab. 35:39, 1985.	tech arti- cle	radiolabeling	T ₃
Kanagasabapathy, A.S., Wellby, M.L.: The use of merthiolate for TBG-blocking in the radioimmunoassay of triiodothyronine, Clin. Chim. Acta 55:267, 1974.	tech arti- cle	reaction techniques	TBG
Larsen, P.R.: Direct immunoassay of triiodothyronine in human serum, J. Clin. invest. 51: 1939, 1982.	tech arti- cle	calculation of results	T ₃
Mayberry, W.E., Bilstad, J.M, and Sizemore, G.W.: Radioimmunoassay for human thyrotropin, Ann. Intern. Med. 74:471, 1981.	grad arti- cle	calculation of results	thyropin
Michell, J.L., Harden, A.B., and O'Rourke, M.F.: The in-vitro resin sponge uptake of triiodothyronine- ¹³¹ I from serum in thyroid disease and pregnancy, J. Clin. Endocrinol. Metab. 20:1474, 1980.	grad arti- cle	separation technique	T ₃
Mincey, E.K., Thorson, S.C., Brown, J.L., and others: A new parameter of thyroid function, the effective thyroxine ratio, J.Nucl. Med. 13:165, 1982.	tech arti- cle	calculations of results	thyroid functions
Mitsuma, T., Colucci, J., Shenkman, L., and others: Rapid simultaneous radio-immunoassay for triiodothyronine and thyroxine in unextracted serum, Biochem. Biophys. Res. Commun. 46:2107, 1982.	tech arti- cle	basic reactions	T ₃

Mitsuma, T., Gershengorn, M., Colucci, J., and others: Radioimmunoassay of triiodothyronine in unextracted human serum, <i>J.Clin.Endocrinol.Metab.</i> 33:364, 1981.	tech arti- cle	basic reactions	T ₃
Murphy, B.E.P., and Pattee, C.J.: Determination of thyroxine utilizing the property of protein-binding, <i>J.Clin. Endocrinol. Metab.</i> 24:187, 1984.	tech arti- cle	calculation of results	protein testing
Nauman, J.A., Nauman, A., and Werner, S.C.: Total and free triiodothyronine in human serum, <i>J. Clin. Invest.</i> 46:1346, 1987.	tech arti- cle	calculation of results	free T ₃
Nelson, J.C., Johnson, D.E., and Odell, W.D.: Serum TSH levels and thyroidal response to TSH stimulation in patients with thyroid disease, <i>Ann. Intern. Med.</i> 76:47, 1982.	tech arti- cle	basic con- cepts of reaction	TSH
Sekadde, C.B., Slaunwhite, R.W., and Aeltour, T.: Rapid radioimmunoassay of triiodothyronine, <i>Clin. Chem.</i> 19:1016, 1983.	tech arti- cle	basic con- cepts of reaction	T ₃
Seth, J., Rutherford, F.J., and McKenzie, I.: Solid phase radioimmunoassay of thyroxine in untreated serum, <i>Clin. Chem.</i> 21(10):1406, 1975.	tech arti- cle	separation technique	T ₄
Solomon, D.H., Bennotti, J., and others: A nomenclature for tests of thyroid hormones in serum; report of a committee of the Americal Thyroid Association, <i>J. Clin. Endocrinol. Metab.</i> 34:884, 1972.	un- arti- der cle	basic reactions	thyroid
Sterling, K., Bellabarba, D., Newman, E.S., and others; Determination of triiodothyronine concentration in human serum, <i>J. Clin. Invest.</i> 48:1150, 1979.	tech arti- cle	calculation of results	T ₃
Sterling, K., Bernner, M.A., and others: The significance of triiodothyronine (T ₃) in maintenance of euthyroid status after treatment of hyperthyroidism, <i>J. Clin. Endocrinol. Metab.</i> 33:729, 1971.	tech arti- cle	quality control	T ₃
Sterling, K., and Milch, P.O.: Thermal inactivation of thyroxine-binding globulin for direct radioimmunoassay of thyidothyronine in serum, <i>J. Clin.Endocrinol.</i> 38:866, 1974.	tech arti- cle	basic react- ions kit evaluation	T ₄
Sterling, K., Refellof, S., and others: T ₃ thyrotoxicosis; thyrotoxicosis due to elevated serum triiodothyronine levels, <i>J.A.M.A.</i> 213:571, 1980.	tech arti- cle	basic react- ions kit evaluation	T ₃
Vaitukaitis, J.L., Ross, G.T., Reichert, L.E., Jr., and others: Immunologic basis for within and between species cross-reactivity of luteinizing hormone, <i>Endocrinology</i> 91:1337, 1972.	grad arti- cle	cross react- ivity; basic concepts	LSH testing
Weeke, J., and Orskoy, H.: Ultrasensitive radioimmunoassay for direct determination of free triiodothyronine concentration in serum, <i>Scand. J. Clin. Lab. Invest.</i> 35:237, 1975.	tech arti- cle	calculations of results	T ₃
Wenzel, K.W., and Meinhold, H.: Radioimmunoassay for T ₃ and T ₄ in unextracted sera. In International Atomic Energy Agency: Radioimmunoassay and related procedures in medicine, Vienna, 1984, I.A.E.A., p.127.	tech arti- cle	basic reactions	T ₃ T ₄

Werner, S.C., Acebedo, G., and Radichevich, I.: Rapid radioimmunoassay for T ₄ and T ₃ in the same sample of human serum, <i>J. Clin. Endocrinol. Metab.</i> 38(3): 493, 1974.	tech arti- cle	basic reactions	T ₃ T ₄
Archibald, E.L., Nincey, E.K., and Morrison, R.T.: Estimation of serum proteins by competitive protein binding assay, <i>Clin. Biochem.</i> 5:232, 1982.	tech arti- cle	calculations of results	proteins
Barakat, R.M., and Ekins, R.P.: Assay of vitamin B ₁₂ in blood; a simple method, <i>Lancet</i> 2:25, 1961.	tech arti- cle	basic techniques	vitamin B ₁₂
Boguslaski, R.C., and Rathjen, C.P.: A column radioassay for the quantification of vitamin B ₁₂ , <i>Clin. Chim. Acta</i> 62:349, 1975.	tech arti- cle	separation technique, quality control	vitamin B ₁₂
Carnel, R., and Colman, C.A., Jr.: Radioassay for serum vitamin B ₁₂ with the use of saliva as the vitamin B ₁₂ binder, <i>J. Lab. Clin. Med.</i> 74:967, 1979.	grad arti- cle	techniques of RIA	vitamin B ₁₂
Fisher, J.W., Thompson, J.F., and Espada, J.: A radioimmunoassay for human urinary erythropoietin, <i>Isr. J. Med. Sci.</i> 7:873, 1981.	tech arti- cle	preparation, labeling	RBC testing
Frenkel, E.P., McCall, M.S., and White, J.D.: Recognition and resolution of errors in the radioisotopic assay of serum vitamin B ₁₂ , <i>Am. J. Clin. Pathol.</i> 53:891, 1980.	tech arti- cle	evolution of calculations	B ₁₂
Rothenberg, S.P.: Assay of serum vitamin B ₁₂ concentration using Co ⁵⁷ , B ₁₂ , and intrinsic factor, <i>Proc. Soc. Exp. Biol.</i> 108:45, 1981.	tech arti- cle	labeling preparations	B ₁₂
Herbert, V., Wasserman, L.R., Frank, O., Pasher, I., and Baker, H.: Values of fasting serum "folic acid" levels, <i>Fed. Proc.</i> 18:246, 1979.	tech arti- cle	basic concepts	folic acids
Rothenberg, S.P., DaCosta, M., and Lawson, J.: The determination of erythrocyte folate concentration using a two-phase ligand-binding radioassay, <i>Blood</i> 43: 437, 1984.	tech arti- cle	labeling, separation technique	folates
Rothenberg, S.P., DaCosta, M., and Rosenberg, Z.: A radioassay for serum folate; use of a two-phase sequential-incubation, ligand-binding system, <i>N. Engl. J. Med.</i> 286:1335, 1972.	tech arti- cle	labeling, separation technique	folates
International Atomic Energy Agency: Standardization and quality control. In <i>Radioimmunoassay and Related Procedures in Medicine (Berlin Symposium, 1977)</i> , Vol. 2, pp 3-167. Vienna, International Atomic Energy Agency, 1978.	tech arti- cle	quality control	
Van De Miel, D.F., Goedemans, W.T., De Vries, J.A., and Molding, M.G.: Radioimmunoassay of vitamin B ₁₂ using a polyurethane sponge and competitive protein binding assay using <i>Lactobacillus leichmannii</i> . In <i>International Atomic Energy Agency: Radioimmunoassay and related procedures in medicine, Vol. 2</i> , Vienna, 1974, I.E.A.E., p.185	tech text 	separation technique, basic concepts	B ₁₂ , proteins
Rodbard D: Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. <i>Clin Chem</i> 20: 1255-1270, 1984.	grad arti- cle	quality control	

Radbard D: Statistical aspects of radioimmunoassays. In Odell W. Daughaday WH (eds): Principles of Competitive Protein-Binding Assays, pp 204-259. Philadelphia, J B Lippincott, 1971.	un- der	text	varied basic concepts	
Rodbard D, Rayford PL, Ross GT: Statistical quality control of radioimmunoassays. In McArthur JW, Colton T(eds): Statistics in Endocrinology, pp 411-429. Cambridge, MA, MIT Press, 1987.	grad	text	quality control	
Ekins RP, Sufi S, Malan PG: An "intelligent" approach to radioimmunoassay sample counting employing a microprocessor-controlled sample counter. In Radioimmunoarray and Related Procedures in Medicine (Berlin Symposium, 1977), Vol.1, pp 437-455. Vienna, International Atomic Energy Agency, 1978.	tech	arti- cle	basic principles	
Finney DJ, Phillips P: The form and estimation of a variance function, with particular reference to radioimmunoassay. Applied Statistics 26: 312-320, 1977.	grad	text	calculations of results	
Feldman HA, Rodbard D: Mathematical theory of radioimmunoassay. In Odell W, Daughaday WH(eds): Principles of Competitive Protein-Binding Assays, pp 158-203. Philadelphia, JB Lippincott, 1981.	grad	text	calculation of results	
Rodbard D, Bridson W, Rayford PL: Rapid calculation of radioimmunoassay results. J Lab Clin Med 74:770-781, 1979.	grad	arti- cle	calculation of results	
Rodbard D, Frazier GR: Statistical analysis of radioligand assay data. In O'Malley BW, Hardman JG (eds): Hormone Action. Part B, Peptide Hormones. Methods in Enzymology, Vol.37, pp. 3-22, New York, Academic Press, 1975.	grad	arti- cle	calculation of results	
Acton FS: Analysis of Straight-Line Data. New York, Dover, 1986.	grad	arti- cle	calculation of results	
Bliss CI: Dose-response curves for radioimmunoassays. In McArthur JW, Colton T (eds): Statistics in Endocrinology, pp. 431-447. Cambridge, MA, MIT Press, 1987.	grad	arti- cle	calculation of results	
Hales CN, Randle PJ: Immunoassay of insulin with insulin-antibody precipitate. Biochem J 88; 137-146, 1983.	tech	arti- cle	basic princi- ples, con- cepts	insulin testing
Murphy BEP: Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. J Clin Endocrinol Metab 27:973-990, 1987.	tech	arti- cle	basic con- cepts, appli- cations cal- culations	steroid testing
Leclercq R, Taljedal IB, Wold S: Evaluation of radioisotope data in steroid assays based on competitive protein binding. Clin Chim Acta 36: 257-259, 1982.	tech	arti- cle	calculations of results	steroid testing
Marbach P, Gotz U, Veteau JP et al: RIA analysis by means of non-linearized response functions. In Radioimmunoassay and Related Procedure in Medicine (Berlin Symposium, 1977), Vol.1, pp.383-397. Vienna, International Atomic Energy Agency, 1978.	tech	farti- cle	calculations of results	
Brown GM, Boshans RL, Schalch DS: Computer calculation of radioimmunoassays. Comput Biomed Res 3:212-217, 1980.	grad	text	calculations of results	

Weaver CK, Cargille CM: Radioimmunoassay: A Simplified procedure for dose interpolation. J Lab Clin Med 77:661-664, 1981.	grad:arti- cle	calculations of results	
Marshner I, Erhardt F, Scriba PC: Calculation of the radioimmunoassay standard curve by spline function. In Radioimmunoassay and Related Procedures in Clinical Medicine and Research (Istanbul Symposium, 1973), pp 111-122. Vienna, International Atomic Energy Agency, 1984.	grad:arti- cle	calculations of results	
Malan PG, Cox MG, Long EMR et al: A multibinding site model-based curve-fitting program for the computation of RIA data. In Radioimmunoassay and Related Procedures in Medicine (Berlin Symposium, 1977), Vol.1, pp. 425-436. Vienna, International Atomic Energy Agency, 1978.	grad:text	exercises: of fitt- ing stan- dard curves	calculations of results
Malan PG, Cox MG, Long EMR et al: Curve-fitting to radioimmunoassay standard curves: Spline and multiple binding site models. Ann Clin Biochem 15:132-134, 1978.	tech:arti- cle	calculations of results	
Malan PG, Cox MG, Long EMR et al: Development in curve-fitting procedures for radioimmunoassay data using a multiple binding site model. In Siemaszko F(ed): Computing in Clinical Laboratories, pp. 238-290. New York, John Wiley & Sons, 1978.	tech:arti- cle	calculations of results	
Rodbard D, Munson PJ, Delean A: Improved curve-fitting, parallelism testing, characterization of sensitivity and specificity, validation, and optimization for radioligand assays. In Radioimmunoassay and Related Procedures in Medicine (Berlin Symposium, 1977), Vol.1, pp 469-504. Vienna, International Atomic Energy Agency, 1978.	tech:arti- cle	basic react- ion, con- cepts, calculations	
Rodbard D, Feldman HA: Theory of proteinligand interaction. In O'Malley BW, Hardman JG (eds): Hormone Action. Part A, Steroid Hormones. Methods in Enzymatology, Vol.36, pp. 3-16. New York, Academic Press, 1975.	tech:text	varied concepts, principles	
Delean A, Munson PJ, Rodbard D: Multi-subsite receptors for multivalent ligands: Application to drugs, hormones, and neurotransmitters. Mol Pharmacol 15:60-70, 1979.	grad:text	basic react- ions, equ- libria	drugs, hormones
Fletcher JE: A generalized approach to equilibrium models. J Phys Chem 81:2374-2378, 1977.	grad:arti- cle	calculations of results	
Rodbard D, Bertino RE: Theory of radioimmunoassays and hormone-receptor interactions: Simulation of antibody divalency, cooperativity, and allosteric effects. In O'Malley BW, Means AR (eds): Receptors for Reproductive Hormones. Advances in Experimental Medicine and Biology, Vol.36, pp.327-341. New York, Plenum, 1983.	very:text tech:	calculation of results, basic reactions	
Fletcher JE, Ashbrook JD, Spector AA: Computer analysis of drug-protein binding data. Ann NY Acad Sci 226:69-81, 1983.	tech:arti- cle	calculations of results	drug testing
Delean A, Stadel JM, Lefkowitz RJ: A ternary complex model explains the agonist-specific binding properties of the adenylyate cyclase-coupled beta-adrenergic receptor. J Biol Chem 255: 7108-7117, 1980.	grad:arti- cle	calculations of results	

Bruni C, Germani A, Koch G et al: Derivation of antibody distribution from experimental binding data. J Theor Biol 61:143-170, 1976.	grad arti- cle	calculations of results, basic equat- ion concepts	
Perlstein MT: Evaluation of commercial RIA kits. I: Recovery. Ligan Quartely 1, NO. 4:4, 1978.	tech arti- cle	evaluations, calculations, kit evalua- tion	
Chard T: An Introduction to Radioimmunoassay and Related Techniques, pp 431-433. Amsterdam, Elsevier/North-Holland Biomedical Press, 1978.	un- text der	numerous ba- sic concepts	numerous tests
Perlstein MT: Evaluation of commercial RIA kits. II: Parallelism effects of protein matrices and mathematical treatment of data in validating an assay. Ligand Quartely 2, No. 1:6, 1979.	tech arti- cle	kit evaluation	
Grasbeck, R, and Saris NE: Establishment and use of normal values. Scand J Clin Lab Invest (Suppl 110) 24:62, 1979.	tech arti- cle	calculations of results	
Sunderman FW, Jr: Current concepts of "normal values," "reference values" and "discrimination values" in clinical chemistry. Clin Chem 21:1873, 1985.	tech arti- cle	calculations for results	
Carmel R: Artifactual radioassay results due to serum contamination by intravenous radioisotope administration. Am J Clin Pathol 70: 364-367, 1978.	tech arti- cle	pt. sampling preparation	
Witherspoon L, Spuler SE, Garcia MM et al: Effects of contaminant radioactivity on results of ¹²⁵ I-radioligand assay. Clin Chem 25:1975, 1979.	tech arti- cle	pt. sampling preparation	
Nickoloff EL: Radioisotopes as interfering substances in radioimmunoassays. Ligand Quartely 2, No. 3:22, 1979.	tech arti- cle	interfering substances, reactions	
Read CH, Stone DB: An immunological assay for minute amounts of human pituitary growth hormone. AMA J Dis Child 96:538, 1958.	tech arti- cle	basic reactions, principles	PTH testing
Berson SA, Yalow RS: The use of K ⁴² or P ³² erythrocytes and I ¹³¹ -tagged human serum albumin in simultaneous blood volume determinations, J Clin Invest 31: 575, 1982.	tech arti- cle	basic reactions, principles	blood volumes
Berson SA, Yalow RS, Schreiber SS et al: Tracer experiments with I ¹³¹ -labelled human serum albumin: Distribution and degradation studies. J Clin Invest 32: 746, 1983.	tech arti- cle	labelling techniques in labeling	
Roth J, Glick SM, Yalow RS et al: Secretion of human growth hormone: Physiologic and experimental modification. Metabolism 12:477, 1963.	tech arti- cle	basic physiology	HGH testing
Glick SM, Roth J, Yalow RS et al: Immunoassay of human growth hormone in plasma. Nature 199:784, 1973.	tech arti- cle	Basic reac- tions princi- ples	HGH testing

Lau KS, Gottlieb C, Wasserman LB et al: Measurement of serum vitamin B ₁₂ level using radioisotope dilution and coated charcoal. Blood 26:202, 1985.	tech arti- cle	separation technique calculations, basic reactions	vitamin B ₁₂
Rothenberg SP: Assay of serum vitamin B ₁₂ concentration using Co ⁵⁷ -B ₁₂ and intrinsic factor. Proc Soc Exp Biol Med 108:45, 1961.	tech arti- cle	basic reactions	vitamin B ₁₂
Rothenberg SP: A radioenzymatic assay for folic acid. Nature 206:1154, 1985			
Barakat RM, Ekins RP: Assay of vitamin B ₁₂ in blood-A simple method. Lancet 2:25, 1981.	tech arti- cle	basic reactions	T ₄
Murphy BEP, Pattee CJ: The determination of thyroxine utilizing the property of protein-binding. J Clin Endocrinol Metab 24:187, 1984.	tech arti- cle	calculation of results	T ₄
Rodbard D, Rayford PL, Cooper JA et al: Statistical quality control of radioimmunoassays, J Clin Endocrinol Metab 28:1412, 1978.	grad arti- cle	quality cont- rol, calcu- lations	
Furuyama S, Mayer DM, Nugent CA: A radioimmunoassay for plasma testosterone: Steroids 16:415, 1980.	grad arti- cle	basic princi- ples of reactions	testos- terone testing
Parker CW: Nature of immunological responses and antigen-antibody interaction. In Odell WD, Daughaday WH (eds): Principles of Competitive Protein-Binding Assays, P 25. Philadelphia, J B Lippincott, 1971.	tech text	interactions, reactions	
Spector S, Parker CW: Morphine: Radioimmunoassay. Science 168:1347, 1970.	tech arti- cle	interactions, reactions	morphine testing
Murphy BEP: The application of the property of protein-binding to the assay of minute quantities of hormones and other substances. Nature 201:679, 1984.	tech arti- cle	basic reactions, properties	hormone testing
Mikhail G, Wu CH, Ferin M et al: Radioimmunoassay of plasma estrone and estradiol. Steroids 13:731, 1979.	tech arti- cle	basic react- ion equations	estrone testing
Odell WD, Abaraham GE, Skowsky WR et al: Production of antiserum for radioimmunoassay. In Odell WD, Daughaday WH (eds): Principles of Competitive Protein-Binding Assays, P 57. Philadelphia, J B Lippincott, 1981.	tech text	production of radiolabels	
Hunter WM: Radioimmunoassay. In Weir DM (ed): Handbook of experimental immunology, p 608. Oxford, Blackwell Scientific Publications, 1967.	tech man- ual	experi- ments in basic immuno- ology	 concepts, principles
Galskov A: Radioimmunochemical corticotropin determination. Acta Endocrinol 69, (Suppl) 162:5, 1982.	grad arti- cle	calculations, basic reactions	cortico- tropin

Berson SA, Yalow RS: State of human growth hormone in plasma and changes in stored solution of pituitary growth hormone. <i>J Biol Chem</i> 241:5745, 1986.	tech arti- cle	quality control, calculations	HGH
Landon J, Livanou T, Greenwood FC: The preparation and immunological properties of ¹³¹ I-labeled adrenocorticotrophin. <i>Biochem J</i> 105: 1075, 1987.	grad arti- cle	preparations of reactions	adreno- cortico- trophin
Springwell PH: An unreactive tyrosine residue in insulin and the exclusive iodination of the A chain. <i>Nature</i> 101:1372, 1981.	grad arti- cle	preparations of reactions	
Butt WR: The iodination of follicle-stimulating and other hormones for radioimmunoassay. <i>J Endocrinol</i> 55:453, 1972.	tech arti- cle	radiolabeling	FSH
Marchalonis JJ: An enzymic method for the trace iodination of immunoglobulins and other proteins. <i>Biochem J</i> 113:299, 1979.	tech arti- cle	radiolabeling	proteins
Bolton AE, Hunter WM: The labelling of proteins to high specific radioactivities by conjugation to a ¹²⁵ I-containing acylating agent. <i>Biochem J</i> 133:529, 1973.	tech arti- cle	radiolabeling	proteins
Miles CN, Randle PJ: The preparation and properties of purified ¹²⁵ I-labelled antibodies to insulin. <i>Biochem J</i> 108:611, 1978.	tech arti- cle	preparation, basic concepts	insulin
Donini S, D'Alessio I, Donini P: Radioimmunoassay of human chorionic gonadotropin (HCG) and human luteinizing hormone (LH) using insoluble antibodies. In Rosenberg E: <i>Gonadotropins 1968</i> , p 263, Los Altos, CA, Geron X, 1978.	grad arti- cle	preparation, basic con- cepts, cal- culations	HCG, LH
Paul WE, Odell WD: Radiation inactivation of the immunological and biological activities of human chorionic gonadotropin. <i>Nature</i> 203:979, 1974.	tech arti- cle	calculations	gonado- tropin
Odell WD, Ross GT, Rayford PL: Radioimmunoassay for human luteinizing hormone. <i>Metabolism</i> 15:287, 1986.	tech arti- cle	preparation of materials	LH
Glick SM, Roth J, Lonergan ET: Survival of endogenous human growth hormone in plasma. <i>J Clin Endocrinol Metab</i> 24:501, 1978.	tech arti- cle	calculation of results	HGH
Midgley AR, Jr: Radioimmunoassay: A method for human chorionic gonadotropin and human luteinizing hormone. <i>Endocrinology</i> 79:10, 1986.	tech arti- cle	calculation of results	LH, gona- dotropin
Haber E, Koerner T, Page LB et al: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. <i>J Clin Endocrinol Metab</i> 29:1349, 1979.	grad arti- cle	preparation, measurements	angioten- sin
Wilkinson, R, Luetscher JA, Nokes GW: Iodination of angiotensin I for use in Radioimmunoassay. <i>J Clin Endocrinol Metab</i> 33:545, 1971.	grad arti- cle	preparation, measurements	
Yalow RS: Radioiodination of peptide hormones: Procedures and problems. In Odell WD, Daughaday WH (eds): <i>Principles of Competitive Protein-Binding Assays</i> , p 298. Philadelphia, JB Lippincott, 1971.	un- der	basic principles, concepts	

Read CH, Bryan GT: Immunological studies of human growth hormone. Ciba Found Colloq Endocrinol 13:68, 1980.	tech arti- cle	reactions, HGH elements involved
Roth J, Klein LA, Petersen NJ: Vasopressin antibodies and a sensitive radio-immunoassay. J Clin Invest 45:1064, 1986.	tech arti- cle	calibrations, elements
Jaffe BM, Newton WT, McGuigan JE: The effect of Carriers on the production of antibodies to the gastrin tetrapeptide. Immunochemistry 7:715, 1980.	tech arti- cle	production of radiolabels
Ullman EF, Schwarzberg M, Rubenstein KE: Fluorescent excitation transfer immuno-assay. A general method for determination of antigens. J Biol Chem 251:4172, 1976.	grad arti- cle	fluorescent excitation analysis
Pratt JJ, Woldring MG, Villerius L: Chemiluminescence-Linked Immunoassay. J Immunol Methods 21:179-184, 1978.	grad arti- cle	chemilumines- cence analysis
Carrico RJ, Christner JE, Boguslaski RC et al: A method for monitoring specific binding reactions with cofactor labeled ligands. Ana Biochem 72:271-282, 1986	tech arti- cle	basic reactions, concepts
Rubenstein KE, Schneider RS, Ullman EF: "Homogeneous" enzyme immunoassay. A new immunochemical technique. Biochem Biophys Res Commun 47:846-851, 1982.	grad arti- cle	enzyme analysis
Engvall E, Perlmann P: Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 8:871-874, 1971.	grad arti- cle	enzyme analysis
Van Weeman BK, Schuurs AHM: Immunoassay using antigen enzyme conjugates. FEBS Lett 15:232-236, 1981.	grad arti- cle	enzyme analysis
Burd JF, Wong RC, Feeney JE et al: Homogeneous reactant-labeled fluorescent immunoassay for therapeutic drugs exemplified by gentamicin determination in human serum. Clin Chem 23:1402-1408, 1977.	grad arti- cle	fluorescent analysis
Finley PR, Williams RJ, Lichti DA: Evaluation of a new homogeneous enzyme inhibitor immunoassay of serum thyroxine with use of a bichromatic analyzer. Clin Chem 26:1723-1726, 1980.	grad arti- cle	enzyme analysis
Dandliker WB, Feigen GA: Quantification of the antigen-antibody reaction by the polarization of fluorescence. Biochem Biophys Res Commun 5:299-304, 1981.	grad arti- cle	fluorescence analysis
Engvall E, Jonsson K, Perlmann P: Enzyme-linked immunosorbent assay. II. Quantitative assay of protein antigen immunoglobulin G, by means of enzyme-labeled antigen and antibody-coated tubes. Biochem Biophys Acta 251:427-423, 1981.	grad arti- cle	enzyme analysis
Engvall E, Perlmann P: Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled antiimmunoglobulin in antigen-coated tubes. J Immunol 109:129-135, 1972.	grad arti- cle	enzyme analysis

Maiolini R, Masseyeff R: A sandwich method of enzymeimmunoassay. I. Application to rat and human alpha-fetoprotein. J. Immunol Methods 8:223-234, 1975.	tech arti- cle	separation technique, enzyme analysis	
Belanger L, Sylvestre C, Dufour D: Enzyme-linked immunoassay for alpha-fetoprotein by competitive and sandwich procedures. Clin Chim Acta 48:15-18, 1983.	tech arti- cle	separation technique, enzyme analysis	
Maiolini R, Ferrua B, Masseyeff R: Enzymeimmunoassay of human alpha-fetoprotein. J Immunol Methods 6:353-362, 1975.	tech arti- cle	separation technique, enzyme analysis	alpha- feto- protein
Eisen HN, Siskind GM: Variation in affinities of antibodies during the immune response. Biochemistry 3:996-1008, 1964.	grad arti- cle	basic reactions, concepts	
Avrameas S, Ternynck T, Guesdon J-L: Coupling of enzymes to antibodies and antigens. Scand J Immunol (Supp 7) 8:7, 1978.	grad arti- cle	enzyme analysis	
Kitagawa T, Aikawa T: Enzyme coupled immunoassay of insulin using a novel coupling reagent. J Biochem 79:233-236, 1976.	tech arti- cle	enzyme analysis	insulin
Kato K, Hamaguchi Y, Fukui H et al: Enzyme-linked immunoassay. I. Novel method for synthesis of the insulin-beta-D-galactosidase conjugate and its applicability for insulin assay. J Biochem 78:235-237, 1985.	tech arti- cle	enzyme analysis	insulin
Kabakoff D: In Maggio ET (ed): Enzyme-immunoassay. Boca Raton, FL, CRC Press, 1980.	tech text	enzyme analysis	insulin
Clark B, Engvall E: Enzyme-linked immunosorbent assay (ELISA): Theoretical and practical aspects. In Maggio ET (ed): Enzyme Immunoassay, pp 167-180. Boca Raton, FL, CRC Press, 1980.	tech text	enzyme ana- lysis, prac- tical appli.	
Oellerich M: Enzyme immunoassays in clinical chemistry: Present status and trends. J Clin Chem Clin Biochem 18:197-208, 1980.	tech arti- cle	enzyme analysis	
Bakeman S: Enzyme immunoassays. Laboratory Management pp 21-29, August 1980.	tech arti- cle	enzyme analysis	
Wisdom GB: Enzyme-immunoassay. Clin Chem 22:1243-1255, 1986.	tech arti- cle	enzyme analysis	
Scharpe SL, Cooreman WM, Bonne WJ et al: Quantitative enzyme immunoassay: Current status. Clin Chem 22:733-738, 1976.	tech arti- cle	enzyme analysis	
Voller A: Heterogeneous enzyme-immunoassays and their applications. In Maggio ET (ed): Enzyme-Immunoassay, pp 181-196. Boca Raton, FL, CRC Press, 1980.	tech arti- cle	calculations, enzyme analysis	

Ishikawa E, Kato K: Ultrasensitive enzyme immunoassay. Scand J Immunol 8:43-55, 1978.	tech arti- cle	calculations, enzyme analysis	
Balancharad GC, Gardner R: Two immunofluorescent methods compared with a radial immunodiffusion method for measurement of serum immunoglobulins. Clin Chem 24:808-814, 1978.	tech arti- cle	fluorescent analysis	
Ullman E, Khanna P: Fluorescence excitation transfer immunoassay. In Colovick SP, Kaplan NO (eds): Methods in Enzymology, Vol. 74, pp 28-60. New York, Academic Press, 1981.	tech arti- cle	fluorescent analysis	
Rubenstein KE: Homogeneous enzyme immunoassay today. Scand J Immunol 8:57-62, 1978.	tech arti- cle	enzyme analysis	
Gibbons I, Skold C, Rowley GL et al: Homogeneous enzyme immunoassay for proteins employing beta-galactosidase. Anal Biochem 102:167, 1980.	tech arti- cle	enzyme analysis	proteins
Chang J, Gotcher S, Gushaw JB: Homogeneous enzyme immunoassay for theophylline in serum and plasma. Clin Chem 1981.	tech arti- cle	enzyme analysis	
Svendsen R, Sorenson B: The concentration of unconjugated estrone and 17 beta-oestradiol in plasma during pregnancy. Acta Endocrinol 47:237, 1984.	very arti- tech cle	concentration reactions	estrone
Miswender GD, Midgley AR, Jr: Hapten-Radioimmunoassay for steroid hormones. In: Peron FG, Caldwell BV (eds): Immunologic Methods in Steroid Determination. New York, Appleton-Century-Crofts, 1980.	tech arti- cle	basic concepts, reactions	steroid
Paradisi R et al: Radioimmunoassay of three estrogens and three androgens in the same plasma sample after extraction and chromatographic separation. Acta Endocrinol 94:229, 1980.	tech arti- cle	basic concepts, reactions	steroids
Stanczyk FZ, Miyakawa I, Gobeismann U: Direct radioimmunoassay of urinary estrogen and pregnanediol glucuronides during the menstrual cycle. Am J Obstet Gynecol 137:443, 1980.	tech arti- cle	basic concepts, reactions	estrogen
Judd HL: Ovarian function throughout life. In Abraham GE (ed): Radioimmunoassay systems in Clinical Endocrinology. New York, Marcel Decker, 1981.	grad text	advanced reaction chemistry.	
Kundu N, Grand M: Radioimmunoassay of 15 alpha-hydroxyestriol (estratetrol) in pregnancy serum. Steroids 27:785, 1976.	grad arti- cle	reactions, concepts	estratetrol
Soares JR, Zimmermann E, Gross S: Direct Radioimmunoassay of 16-glucosiduronate metabolites of estriol in human plasma and urine. FEBS Lett 61:263, 1986.	tech arti- cle	reactions, concepts	estratetrol
Scanlon MF, Ress-Smith B, Hall R: Thyroid-stimulating hormone: Neuroregulation and clinical application. Parts 1 and 2. Clin Sci Mol Med 55:1, 129, 1978.	tech arti- cle	reactions, concepts	TSH
Aoki N, Hashimoto A, Yamamoto T: Increased serum thyrotropin after administration of metoclopramide in man. IRCS Med Sci 4:542, 1976.	tech arti- cle	reactions, concepts	thyrotropin

Collu R: Abnormal pituitary hormone response to thyrotropin-releasing hormone: An index of central nervous system dysfunction. In Tolis G et al (eds): Clinical Neuroendocrinology: A Pathophysiological Approach. New York, Raven Press, 1979.	grad;text	concepts or reactions, chemistry	pituitary hormone, TRH
Krugman LG, Hershman JM, Chopra IJ et al: Patterns of recovery of the hypothalamic-pituitary-thyroid axis in patients taken off chronic thyroid therapy. J Clin Endocrinol Metab 41:70, 1985.	grad;arti-cle	concepts or reactions, chemistry	pituitary thyroid
Sanchez-Franco F, Carcicedo GL, Martin-Zurro A et al: Transient lack of thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH) in treated hyperthyroid patients with normal or low serum thyroxine (T ₄) and triiodothyronine (T ₃). Clin endocrinol Metab 38:1098, 1984.	grad;arti-cle	concepts or reactions, chemistry	TSH, TRH, T ₃
Harada A, Hershman JM, Reed AW et al: Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. J Clin Endocrinol Metab 48:793, 1979.	grad;arti-cle	concepts or reactions, chemistry	TSH, T ₃ , T ₄
Refsum HE, Stromme SB: Serum thyroxine, triiodothyronine and thyroid-stimulating hormone after prolonged heavy exercise. Scand J Clin Lab Invest 39:455, 1979.	grad;arti-cle	concepts or reactions, chemistry	TSH, T ₃ , T ₄
Vagenakis AG: Regulation of TSH secretion. In Tolis G et al (eds): Clinical Endocrinology: A Pathophysiological Approach. New York, Raven Press, 1979.	grad;arti-cle	concepts or reactions, chemistry	TSH
Vinik AI, Kalk WJ, McLean H et al: Fasting blunts the TSH response to synthetic thyrotropin-releasing hormone (TRH). J Clin Endocrinol Metab 40:509, 1975.	grad;arti-cle	concepts or reactions, chemistry	TSH, TRH
Lemarchand-Beraud T: Radioimmunoassay of thyroid-stimulating hormone (TSH) in plasma. In Breuer H, Hamel D, Kruskemper HL (eds): Methods of Hormone Analysis. New York, John Wiley & Sons, 1976.	grad;text	concepts or reactions, chemistry	TSH
Patel YC-Berger HG, Hudson B: Radioimmunoassay of serum thyrotropin: Sensitivity and specificity. J Clin Endocrinol Metab 33:768, 1971.	grad;arti-cle	concepts or reactions, chemistry	thyrotropin
Perkary AE, Hershman JM, Parlow AF: A sensitive and precise radioimmunoassay for human thyroid-stimulating hormone. J Clin Endocrinol Metab 41:676, 1975.	grad;arti-cle	concepts or reactions, chemistry	TSH
Utiger R: Thyrotropin. In Jaffe BM, Behram HR (eds): Methods of Hormone Radioimmunoassay, 2nd ed. New York, Academic Press, 1979.	grad;arti-cle	concepts or reactions, chemistry	general hormone analysis
Chen W, Heminger L, Maxon HR et al: Non-specific binding as a source of error in thyrotropin radioimmunoassay with polyethylene glycol as separating agent. Clin chem 26:487, 1980.	tech;arti-cle	calculations of results, separation technique	thyrotropin

Burger HG, Patel VC: TSH and TRH: Their physiological regulation and the clinical applications of TRH. In Martini L, Besser GM (eds): Clinical Neuroendocrinology, New York, Academic Press, 1977.	grad arti- 	concepts, principles, reactions in chemistry	TRH
Snyder PJ, Muzyka R, Johnson J et al: Thyrotropin-releasing hormone provokes abnormal follicle-stimulating hormone (FSH) and luteinizing hormone responses in men who have pituitary adenomas and FSH hypersecretion. J Clin Endocrinol Metab 51:744, 1980.	grad arti- cle 	concepts, principles, reactions in chemistry	FSH, TRH
Baker SB: Determination of protein-bound iodine. J Biol Chem 173:715-724, 1948.	grad arti- cle 	radiolabeling	
Korcek L, Tabachnick M: Thyroxine-protein interactions. Interaction of thyroxine and triiodothyronine with human thyroxine binding globulin. J Biol Chem 251:3558-3562, 1976.	grad arti- cle 	concepts, principles, reactions, binding	T ₄
Sakurada T, Rudolph M, Fang SL et al: Evidence that triiodothyronine and reverse triiodothyronine are sequentially deiodinated in man. J Clin Endocrinol Metab 46:916-922, 1978.	grad arti- cle 		T ₃
Pangaro L, Burman KD, Wartofsky L et al: Radioimmunoassay for 3,5-diiiodothyronine and evidence for dependence on conversion from 3,5,3'-triiodothyronine. J Clin Endocrinol Metab 50:1075-1081, 1980.	grad arti- cle 	radiolabeling reactions, principles	T ₃ T ₄
Ekins RP: The estimation of thyroxine in human plasma by an electrophoretic technique. Clin Chim Acta 5:453-459, 1960.	grad arti- cle 	general concepts, separation technique	T ₄
Murphy BEP: Application of the property of protein-binding to the assay of minute quantities of hormones and other substances. Nature 201:679-682, 1984.	grad arti- cle 	general principles, concepts	protein
Chopra IJ, Solomon DH, Ho RS: A radioimmunoassay of thyroxine. J Clin Endocrinol Metab 33:865-868, 1981.	tech arti- cle 	general principles, concepts	T ₄
Chopra IJ: A radioimmunoassay for measurement of thyroxine in unextracted serum. J Clin Endocrinol Metab 34:938-947, 1972.	tech arti- cle 	calculation of results	T ₄
Mitsuma T, Colucci J, Shenkman L et al: Rapid simultaneous radioimmunoassay for triiodothyronine and thyroxine in unextracted serum. Biochem Biophys Res Commun 46:2107-2113, 1982.	grad arti- cle 	calculation of results	T ₄
Ginsberg J, Segal D, Ehrlich RM et al: Inappropriate triiodothyronine (T ₃) and thyroxine (T ₄) radioimmunoassay levels secondary to circulating thyroid hormone autoantibodies. Clin Endocrinol (Oxf) 8:133-139, 1977.	tech arti- cle 	calculation of results	T ₃ T ₄
Karlsson FA, Wibell L, Wide L: Hypothyroidism due to thyroid-hormone binding antibodies. N Engl J Med 296:1146-1148, 1977.	grad arti- cle 	general concepts, principles	thyroid

Kochupilla N, Yalow RS: Preparation, purification, and stability of high specific activity ^{125}I -labelled thyronines. <i>Endocrinology</i> 102:128-135, 1978.	grad:arti- cle	radiolabeling: thyroid separation technique,
Ratcliffe WA, Challand GS, Ratcliffe JB: A critical evaluation of separation methods in radioimmunoassays for total triiodothyronine and thyroxine in unextracted human serum. <i>Ann Clin Biochem</i> 11:224-229, 1974.	grad:arti- cle	calculation of results thyroid
Witherspoon LR, Shuler SE, Garcia MM et al: Effects of contaminant radioactivity on results of ^{125}I -radioligand assay. <i>Clin Chem</i> 25:1975-1977, 1979.	grad:arti- cle	radiolabeling:
Larsen PR: Direct immunoassay of triiodothyronine in human serum. <i>J Clin Invest</i> 51:1939-1949, 1972.	tech:arti- cle	radiolabeling: T_3
Chopra IJ, Ho RS, Lam R: An improved radioimmunoassay of triiodothyronine in serum: Its application to clinical and physiological studies. <i>J Lab Clin Med</i> 80:729-739, 1982.	tech:arti- cle	radiolabeling: T_3
Fang VS, Refetoff S: Radioimmunoassay for serum triiodothyronine: Evaluation of simple techniques to control interference from binding proteins. <i>Clin Chem</i> 20:1150-1154, 1974.	tech:arti- cle	radiolabeling: T_3
Sharib H, Ryan RJ, Mayberry WE: Triiodothyronine (T_3) radioimmunoassay: A critical evaluation. <i>Mayo Clin Proc</i> 47:934-937, 1982.	tech:arti- cle	radiolabeling: T_3
Fisher DA, Sack J, Oddie TH et al: Serum T_4 , TBG, T_3 , uptake, T_3 , reverse T_3 , and TSH concentrations in children 1 to 15 years of age. <i>J Clin Endocrinol Metab</i> 45:191-198, 1977.	tech:arti- cle	reactions, general principles T_3 , T_4
Chopra IJ: A radioimmunoassay for measurement of 3,3',5'-triiodo-L-thyronine reverse (T_3). <i>J Clin Invest</i> 54:583-592, 1974.	tech:arti- cle	reactions, general principles T_3
Nicod P, Burger A, Staehell V et al: A radioimmunoassay for 3,3',3'-triiodo-L-thyronine in unextracted serum: Method and clinical results. <i>N Clin Endocrinol Metab</i> 42:823-829, 1976.	tech:arti- cle	reactions, generag principles T_4
Sterling K, Brenner MA: Free thyroxine in human serum. Simplified measurement with the aid of magnesium precipitation. <i>J Clin Invest</i> 45:153-163, 1966.	tech:arti- cle	calculation of results T_4
Clark F, Horn DB: Assessment of thyroid function by the combined use of serum protein-bound iodine and resin uptake of ^{131}I -triiodothyronine. <i>J Clin Endocrinol Metab</i> 25:39-45, 1985.	tech:arti- cle	binding, reactions, general concepts T_3 , T_4
Chopra IJ, Van Herle AJ, Chua Teco GN et al: Serum free thyroxine in throidal and nonthyroidal illnesses: A comparison of measurements by radioimmunoassay, equilibrium dialysis, and free thyroxine index. <i>J Clin Endocrinol Metab</i> 51:135-143, 1980.	tech:arti- cle	calculations of results, basic reaction principles T_4

Elkins RP, Ellis SM: The radioimmunoassay of free thyroid hormones in serum. In Robbins J, Braverman LE (eds): Thyroid Research, pp 597-600. Amsterdam, Excerpta Medica, 1975.	tech arti- cle 	calculations T ₄ of results, basic reaction principles
Bayer MF, McDougall IR: Radioimmunoassay of free thyroxine in serum: Comparison with clinical findings and results of conventional thyroid-function tests. Clin Chem 26:1186-1192. 1980.	tech arti- cle 	calculations T ₄ of results, basic reaction principles
Rothenberg SP: Assay of serum vitamin B ₁₂ concentration using Co ⁵⁷ -B ₁₂ and intrinsic factor. Proc Soc Exp Biol Med 1981; 108: 45.	tech arti- cle 	reactions, vitamin iodination B ₁₂ analysis
Berson SA, Yalow RS: Radioimmunoassay of ACTH in plasma. J Clin Invest 1978; 47:2725.	tech arti- cle 	basic ACTH reaction
Berson SA, Yalow RS: Insulin in blood and insulin antibodies. Am J Med 1986; 40:676.	tech arti- cle 	basic insulin reaction
Chopra IJ: A radioimmunoassay for measurement of thyroxine in unextracted serum. J Clin Endocrinol Metab 1982; 34: 938	tech arti- cle 	basic T ₄ reaction
Utiger RD: Radioimmunoassay of human plasma thyrotropin. J Clin Invest 1985; 44:1277.	tech arti- cle 	basic thyrotro- reaction pin
Abraham GE: Preliminary communications; solid-phase radioimmunoassay of estradiol-17 beta. J Clin Endocrinol Metab 1979;29:866.	grad arti- cle 	separation estradiol techniq
Abraham Ge: Radioimmunoassay of steroids in biological materials, in radioimmunoassay and Related Procedures in Medicine, Vol. II. IAEA, Vienna, 1984, pp 3-28	grad arti- cle 	basic chemi- steroids cal proper- ties, reactions
Dunne RC Jr, Daniels A: Immunodiagnosics: A Technology in transition. New York, Martin Simpson Research Associates, 1981.	tech arti- cle 	basic chemi- cal proper- ties, reactions
Rodbard, David: Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Nat. Inst. Child Health Hum. Dev., Natl. Inst. Health, Bethesda, Md., USA. Clin Chem. (Winston-Salem, N.C.): 20(10), 1255-70, 1980.	grad arti- cle 	quality con- trol, calcu- lations of results
Ekins, R.P.: Basic principles and theory [of saturation assay]. Inst. Nucl. Med. Middlesex Hosp. Med. Sch. London, Engl. Brit. Med. Bull., 30(1), 3-11, 1983	tech arti- cle 	basic princi- ples, con- cepts, calcu- lation of re- sults, sepa- ration technique

Witherspoon, Lynn R.: The evaluation of commercial radioimmunoassay kits. Dep. Nucl. Med., Ochsner Med. Inst. New Orleans, LA., USA. Contin. Educ. Lect. (Soc. Nucl. Med., Southeast. Chapter) 15, pp 15., 1986.	tech arti- cle	kit evaluation	
Chen, I-Wen; commercially available fully automated systems for radioligand assay. Part I Overview. Coll. Med., Univ. Cincinnati, Cincinnati, OH, USA. Ligand Rev., 2(2), pp 46-50, 1984.	tech arti- cle	quality control on equipment	
Chen, I-Wen: Commercially available fully automated systems for radioligand assay. Part 2. Performance characteristics. Coll. Med., Univ. Cincinnati, Cincinnati, OH, USA. Ligand Rev., 2(3), pp 46-48, 1981.	tech arti- cle	quality cont- rol on equip- ment, calcu- lation of results	
Hunter, WM: Radioimmunoassay and saturation analysis. Preparation and assessment of radioactive tracers. Br Med Bull pp 18-23, Jan. 1984.	tech arti- cle	separation technique, calculation of results	
Woodhead, J.S., Addison, G.M., Hales, C.N.: Immunoradiometric assay and related techniques. Dep. Med. Biochem., Welsh Natl. Sch. Med. Cardiff, Wales, Brit. Med. Bull., 30(1), pp 44-48, 1983.	tech arti- cle	calculation of results, technique assessment	
Diamond BA, Yelton DE, Scharff MD.: Monoclonal antibodies. A new technique for producing serologic reagents. N Engl J Med. 304(22): 1344-9. May 23, 1983.	tech arti- cle	reactions, basic concepts	monoclo- nal anti- bodies
Witherspoon, Lynn R., Shuler, Stanton E., Garcia, M.M., Zollinger, L.A.: Assessment of serum myoglobin as a marker for acute myocardial infarction. Ochsner Med. Inst. New Orleans, LA., USA. J. Nucl. Med., 20(2), pp 115-19, 1986	tech arti- cle	reaction assessment	myoglo- bin
Wide, L.: Clinical significance of measurement of reaginic (IgE) antibody by RAST (radioallergosorbent technique). Dep. Clin. Chim., Univ. Hosp. Uppsalla, Swed. Clin Allergy, 3, Suppl., pp 583-95, 1985.	tech arti- cle	calculation of results preparations	IgE
Smith, Roy G., Sestili, Mary Ann.: Methods for ligand-receptor assays in clinical chemistry. Obstet. Gynecol., Baylor Coll. Med. Houston, TX 77030, USA. Clin. Chem. (Winston-Salem, N.C.), 26(5), pp 543-50, 1981.	tech arti- cle	basic prepa- ration tech- nique, basic principles, concepts	
Schall, Roy F., Jr, Teneso, Hal J.: Alternatives to radioimmunoassay: labels and methods. Organon Diagnostics, El Monte, CA 91734, USA. Clin Chem. (Winston-Salem, N.C.), 27(7), pp 1157-64, 1983.	tech arti- cle	radiolabeling preparation	
Di Bella, Francis P, Arnaud, Claude D.: In vitro assays for parathyroid hormone, calcitonin, and vitamin D. Upjohn Co. Kalamazoo, Mich., USA. Nucl. Med: Endocrinol., pp 228-64. Edited by Rothfeld, Benjamin. Lippincott: Philadelphia, PA., 1980	tech text	general con- cepts, ele- ments, react- ions	PTH, cal- citonin, vitamin B ₁₂

Catt, Kevin, Rothfeld, Benjamin: Renin and Aldosterone. Endocrinol. Reprod. Res. Branch, NIH, Bethesda, MD., USA. Nucl. Med.: Endocrinol., pp 353-64. Edited by Rothfeld, Benjamin. Lippincott: Philadelphia, PA, 1986.	tech;text	general concepts, elements, reactions	Renin & aldosterone
Chopra, Inder J.: Radioimmunoassay of iodothyronines. Sch. Med., Univ. California, Los Angeles, CA, USA. Clin. Biochem. Anal., 5 (Handb. Radioimmunoassay), pp 679-703., 1983.	tech;text	general concepts, elements, reactions	T ₄
Nakamura, Yasuko, Copra, Inder J., Solomon, David H. Cent. Health Sci., Univ. California Los Angeles, CA, USA. J. Nucl. Med., 18(11), pp 1112-15, 1984.	tech;text	general concepts, elements, reactions	T ₄
Ruoslahti, Erkki., Seppala, Markku.: Radioimmunoassay of alpha-fetoprotein. Dep Immunol, City of Hope Natl Med Cent, Duarte, CA, USA. Clin Biochem Anal., 5(Handb. Radioimmunoassay), pp 543-69, 1985.	tech;text	general concepts, elements, reactions	alpha-fetoprotein
Bangham, D.R., Cotes, P. Mary: [Assay] standardization and standards. Natl Inst Biol Stand Control, London, Engl. Brit Med Bull, 30(1), pp 12-17, 1986	un-der	general concepts, standards	
Bovers, George N., Jr., Burnett, Robert W., McComb, Robert B.: Preparation and use of human serum control materials for monitoring precision in clinical chemistry. Clin Chem Lab, Hartford Hosp. Hartford, Conn. USA. Clin Chem (Winston-Salem, N.C.), 21(12), pp 1830-6, 1981.	tech;text	preparation of reaction, kit evaluation	
Goldenberg, D.M., Neville, A.M., Carter, A.C., Go, V.L.W., Holyoke, E.D., Isselbacher, K.J., Schein, P.S., Schwartz, M.: CEA (carcinoembryonic antigen): its role as a marker in the management of cancer. Med Cent, Univ Kentucky, Lexington, KY 40536, USA. J. Cancer Res Clin Oncol., 101(3), pp 239-42, 1978.	tech;article	preparation of reaction, kit evaluation	CEA
Carcinoembryonic antigen: its role as a marker in the management of cancer. National Institutes of Health, USA, Br Med J., 282(6261), pp 373-5, 1976.	tech;article	preparation of reaction, kit evaluation	CEA
Cleeland, R., Christenson, J., Usategui-Gomez, M., Heveran, J.: Detection of Drugs of abuse by radioimmunoassay: a summary of published data and some new information. Res Div Hoffmann-La Roche, Nutley, NJ, USA. Clin Chem (Winston-Salem, N.C.), 22(6), pp 712-25, 1985.	tech;article	basic concepts, preparation, reactions	drug testing
Walters, G.O., Jacobs, A., Worwood, M., Trevett, D., Thomson, W.: Iron absorption in normal subjects and patients with idiopathic hemochromatosis. Relation with serum ferritin concentration. Dep Haematol Welsh Natl Sch Med, Cardiff, Wales, Gut, 16(3), pp 188-92, 1985.	tech;article	preparation, basic technique	Fe
Maitra, Shyamal K., Yoshikawa, Thomas T, Guze, Lucien B., Schotz, Michael C.: Determination of aminoglycoside antibiotics in biological fluids: a review. Dep Med Univ, California, Los Angeles, CA 90024, USA. Clin chem (Winston-Salem, N.C.), 25(8), pp 1361-7, 1985.	grad;article	preparation, basic technique	aminoglycoside

Playfair, J.H.L., Hurn, B.A.L., Schulster, Dennis.: Production of antibodies and binding reagents. Dep Immunol Middlesex Hosp Med Sch, London, Engl. Brit Med Bull., 30(1), pp 24-31, 1983.	tech:arti- cle	production of antibodies, radiolabeling
Ratcliffe, J.G.: Separation techniques in saturation analysis. Dep Pathol Biochem., R. Infirm. Glasgow, Scot. Brit Med Bull., 30(1), pp 32-7, 1980.	tech:arti- cle	separation technique
Hunter, W.M.: Preparation and assessment of radioactive tracers. Radioimmunoassay Team, Med Res Counc. Edinburgh, Scot. Brit Med Bull., 30(1), pp 18-23, 1981.	tech:arti- cle	preparation, calculation of results
Rayford, Phillip L., Miller, Thomas A., Thompson, James C.: Secretin, Cholecystokin and newer gastrointestinal hormones. Second of two parts. Dep Surg Univ. Texas, Galveston, TX, USA. N Engl J Med, 294(21), pp 1157-64, 1980.	grad:arti- cle	preparation, gastroin- testinal of reaction hormones
Lock, John Paul, Schalch, Don S.: Anterior pituitary: gonadotropic hormones. Worcester Diabetes Metab Assoc Inc. Worcester, Mass., USA. Nucl Med: Endocrinol., pp 159-82, Edited by: Rothfeld, Benjamin, Lippincott: Philadelphia, PA, 1986.	grad:arti- cle	preparation, gonado- tropic of reaction hormones
Hsu, Tah-Hsiung., Yang, Shirley S.L.: Thyroid function tests, Johns Hopkins Univ Sch Med. Baltimore, MD., USA. Nucl Med: Endocrinol., pp 34-54. Edited by: Rothfeld, Benjamin. Lippincott: Philadelphia, PA, 1982.	tech:arti- cle	preparation, thyroid basic concept of reaction
Albert, Solomon N. Blood volume and extracellular fluid volume. 2nd ed. Charles C. Thomas, 1981.	grad:text	preparation, blood components
Chard, T. Laboratory techniques in biochemistry and molecular biology. North Holland Publishing Co., 1981.	tech:man- ual	biochem- ical, molecular biology technique
Copeland, B.E. Quality control manual. Revised ed. American Society of Clinical Pathologists, 1983.	tech:text	quality control
Freeman, Leonard M.; Blaufax, M. Donald, Eds. Radioimmunoassay. Grune and Stratton, 1985.	tech:text	varied aspect principles testing procedure
Moss, A.J.; Dalrymple, Glenn V.; Boyd, Charles M. Practical radioimmunoassay. C.V. Mosby Co., 1986.	un- der	varied aspect principles testing procedure
Ranson, John P. Practical competitive binding assay methods. C.V. Mosby Co., 1986.	un- der	varied aspect principles testing procedure
Rothfeld, Benjamin, ed. Nuclear medicine in vitro. 2nd ed. J.B. Lippincott, 1983	tech:text	varied aspect principles testing procedure
Thorell, Jan I.; Larson, Steven M. Radioimmunoassay and related techniques. C.V. Mosby Co., 1978.	tech:text	varied aspect principles testing procedure

Thorell, Jan I., ed. Radioimmunoassay design and quality control. Pergamon Press, 1983.	tech;text		varied aspects	testing principles & procedure
Bach JF: Introduction. In immunology, Bach JF, ed. New York, John Wiley & Sons, 1978, pp 1-11.	grad;article		basic reactions,	chemistry
Dressle, RL, Spicer JA: Laboratory science. In Nuclear Medicine Technology and Techniques, Bernier DR, Langan JK, Wells LD, eds. St Louis, The CV Mosby Co, pp 131-161, 1981.	tech;manual	basic blood component	basic concepts,	principles simple testing procedure
Demers LM, Derck DD: Centria system 2. The Ligand Review 2(3):50-57, 1980.	tech;article		basic concepts,	principles
National Committee for Clinical Laboratory Standards, Guidelines for Standards Assess the Quality of Radioimmune Assay Systems, 1976.	tech;article		quality control, calculation of results	
Rodbard D: Data processing for radioimmunoassays: An overview in Clinical Immunochimistry, pp 477-494, 1978	grad;text		basic concepts	reactions chemistry
Chopra IJ: Radioimmunoassay of iodothyronines. In Handbook of Radioimmunoassay, Abraham GE, ed. New York, Marcel Dekker, Inc, 1977, pp 679-703.	tech;text		numerous concepts,	testing principles procedures
Ruoslahti E, Seppala M: Radioimmunoassay of alpha-Fetoprotein. Ibid., pp 543-567.	tech;article		basic principles,	alpha-fetoprotein
Bowers GN, Burnett RW, McComb RB: Preparation and use of human serum control materials for monitoring precision in clinical chemistry. Clin Chem 21:1830-1836, 1985.	tech;article		preparation	
Carcinoembryonic antigen: Its role as a marker in the management of cancer. A NIH Consensus Development Conference. Ann Intern Med 94:407-407, 1981.	tech;text		preparation, reactions	CEA basic concepts
Hoofnagle JH: Serologic markers of Hepatitis B virus infection. In Annual Review of Medicine: Selected Topics in the Clinical Sciences, Vol. 32, Greger WP, Coggins CH, Hancock EM, eds. Palo Alto CA, Annual Reviews Inc, 1981, pp 1-11	grad;article		preparation, reactions	basic concepts

Diamond BA, Yelton DE, Scharff MD: Monoclonal antibodies. N Engl J Med 304:1344-1349, 1981.	grad arti- cle	preparation, reactions basic concepts	monoclo- nal anti- bodies
Viatukaitis JL: Specific human chorionic gonadotropin assay. In Methods of Hormone Radioimmunoassay, Jaffe BM, Behrman HR, eds. New York, Academic Press, 1979, pp 817-829.	tech arti- cle	preparation, reactions basic concepts	HCG testing
Playfair JHL, Hurn BAL, Schulster D: Production of antibodies and binding reagents. Br Med Bulletin 30:24-31, 1984.	tech arti- cle	radiolabeling	
Ratcliffe JG: Separation techniques in saturation analysis. Br Med Bulletin 30:32-37, 1984.	tech arti- cle	separation technique	
Saar N, Bachmann AM, Gordon RD: Radioenzymic measurement of norepinephrine, epinephrine, and dopamine: stability, enzymic activity, and sensitivity. Clin Chem 27:626-628, 1981.	tech arti- cle	basic reaction, calculation of results	norepine- phrine, epineph- rine
Schall RF Jr, Tenoso HJ: Alternatives to radioimmunoassay: labels and methods, Clin Chem 27:1157-1164, 1981.	tech arti- cle	basic concept principles	
Smith RG, Sestili MA: Methods for ligandreceptor assays in clinical chemistry. Clin Chem 26:543-550, 1980.	tech arti- cle	basic concept principles	
Witherspoon LR, Shuler SE, Genre CF, et al: Two radioimmunoassays compared with isoenzyme electrophoresis for the detection of serum creatine kinase-MB in acute myocardial infarction. J Nucl Med 23:136-142, 1982.	tech arti- cle	basic concept principles	creatin kinase

Summary of Resultant

Level of Material. In order to evaluate the need for a student laboratory In Vitro manual, it was necessary to examine the literature content and categorize in terms of (NOTE: The numbers following each section relate to the total frequency of occurrence for that topic mentioned within the literature:

Undergraduate Level: Understandable to beginning students in field or general public (13).

Technical Level: Written for individual in field (206).

Graduate Level: Highly sophisticated; for advanced educational-level individuals (91).

Type of Material. This is the form the material was written in, either an article, text, or manual.

Article: Categorized as in a journal or presented as a paper in a symposium (262).

Textbooks: Larger volumes covering numerous area and topics of In Vitro material (48).

Manuals: Written as a guide to students to work through problems and exercises (4).

Providing Laboratory Exercises. Of the over 300 types of In Vitro literatures examined only seven times were any exercises included. The types of exercises were: basic RIA, concepts, separation techniques, calculation of results,

labeling, basic reactions, basic immunology, and blood components sampling procedures.

General Principles and Concepts Discussed. The literature was categorized in 11 overall principles and concepts, each different type were placed into one of these areas:

Basic Principles/Concepts: This included materials that discussed elements, standards, structures, basic TIA properties and methodologies and typical RIA examples (62).

Basic Reactions: Concepts included concentrations, components of RIA, equations, chemical properties, physiology, interfering substances (65).

Radiolabeling: Labeling of radioactive materials, production and purification of labeled isotopes (29).

Basic Procedures: Techniques, evaluation of techniques, applications, calibrations (13).

Kit Evaluation: Assessment of basic manufacturer RIA testing kits (12).

Separation Techniques: Separation of bond-free (26).

Calculations of Results: Measurement, assessment, evaluations (54).

Preparation: Patient sampling, binding properties, receptors, Ag-Ab preparation (18).

Quality Control: RIA laboratories, equipment, kits (19).

Alternative Immuno Blood Testing: Enzyme, luminescence, fluorescent excitation testing (24).

Specific Testing Procedures. A review of the In Vitro literature reveals numerous types of testing procedures discussed. The following is a list in descending order of the most commonly mentioned testing procedures (NOTE: Numbers correspond to frequency distribution of test procedures mentioned within literature):

thyroid T₃, T₄ - (56)
blood volumes - (18)
schilling testing - (15)
vitamin B₁₂ - (13)
red blood cell survival - (11)
WBC labeling - (10)
peptide (protein hormones) - (10)
insulin - (9)
iron (Fe) recovery - (8)
drug testing - (8)
TSH - (7)
thyrotropin - (7)
CEA - (7)
estradiol, estrone - (7)
folic acid - (6)
alpha-feto protein - (6)

3 - 5

cortisol

testosterone
FSH
HCG
LSH
PTH
creatine kinase
renin, aldosterone
aminoglycoside
ACTH
gonadotropin
steroid testing
IGE
angiotension
monoclonal antibodies
norepinephrine
TBG
LH
digoxin

In summary, this chapter attempts to review those aspects most influential on the development of In Vitro Nuclear Medicine education. This review of the literature exposes the reader to many reasons for the development of Nuclear Medicare Technology education, the historical changes influencing the field of Nuclear Medicine and finally the tabulated content analysis of over 300 articles, texts, and manuals written within In Vitro Nuclear Medicine.

This content analysis of the review of In Vitro literature is used as the basis for the development of the nationwide survey sent to all Nuclear Medicine program directors. The general principles/concepts, testing procedures and laboratory exercises cited in the literature are evaluated by the program director as to their relative importance in the educational process of nuclear medicine students.

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Chapter 3

METHODOLOGY

Introduction

Chapter III is concerned with the methods and procedures that were used in this study. Information was provided regarding the selection and description of the subjects, and description of the development and administration of the instruments.

Setting and Subjects

Based on the content analysis of the Nuclear Medicine In Vitro Laboratory literature a questionnaire was developed. Before any nationally accepted survey could be sent out to all Nuclear Medicine program directors the validity and reliability of the questionnaire had to be established.

Five authorities in the field of Nuclear Medicine technology were identified to evaluate the initial surveys contents. A letter was sent to the Joint Review Committee on Accreditation of Nuclear Medicine Program and the Society of Nuclear Medicine asking these groups to choose individuals they deem as experts in the field. From the suggested list of individuals, five were selected at random and received by mail the researcher's initial survey.

The final sampling population consisted of all Accredited Nuclear Medicine Technology Program Directors in the United States (approximately 70). A list of accredited programs were supplied by the Joint Committee on Education in Radiologic Technology (1988 listings).

Directors of each accredited degree program were selected not so much for their educational and professional experience, but because they may have the most dominant voice in the actual implementation and future use of this In Vitro Student Manual into their curriculums.

Since there may not be a Nuclear Medicine program director for a given university, the person directly responsible for the program (dean, chairman, etc.) were asked to complete the questionnaire. Their title was designated on the form. No differences were analyzed between various titles as long as they have direct responsibility for the Nuclear Medicine technology program.

The following Procedural Chart outlines the basic sequential steps undertaken that ultimately lead to the development of an In Vitro laboratory manual.

PROCEDURAL CHART

Content analysis of literature is used to develop basic questionnaire on the needs of In vitro Nuclear Medicine procedures.



Letter to Joint Review Committee on Accreditation of Nuclear Medicine Program and Society of Nuclear Medicine asking them to choose individuals they deem as experts in In Vitro Laboratory procedures to evaluate questionnaire.



Five experts randomly selected from list provided.



Questionnaire was revised and necessary changes made based on criticisms of experts.



Survey is sent to all Nuclear Medicine Programs Directors nationwide (approximately 70 schools).



Data is collected and analyzed.



In Vitro Laboratory Manual is written to meet education needs cited.



Manual is sent to experts for their critique and comments about how well manual will meet needs in Nuclear Medicine In Vitro Laboratory.

Procedure

A letter was developed and sent to the Joint Review Committee on Accreditation of Nuclear Medicine Programs and the Society of Nuclear Medicine asking them to identify experts in the field of Nuclear Medicine education. A random selection of five names were used to validate the researcher's initial questionnaire.

The five individuals chosen randomly were: Wynn Harrison, Director of Nuclear Medicine, Weber State University, Utah; John Hamlett, Director of Nuclear Medicine, Temple University; K. McEnerney, Director of Nuclear Medicine, California State University, Berkeley; Sheila Rosenfield, Director of Nuclear Medicine, St. Louis University; John Rollins, Director of Nuclear Medicine, University of Alabama, Birmingham.

A cover letter and questionnaire was mailed to these Nuclear Medicine Technology authorities in the field of Nuclear Medicine technology. The cover letter explained the

purpose of the study and the importance of their input. They were requested to respond by a certain date. Telephone calls followed to three experts who did not return a questionnaire asking or reminding them for their response. A second letter and questionnaire was sent with appropriate follow-up contact made until all five authorities did in fact complete and return the questionnaire.

From the five authorities responses an overall review and necessary changes were made to adjust to the criticisms of these individuals. The final survey was sent to all Nuclear Medicine Program Directors from the University of Nevada, Las Vegas with a self-addressed, stamped envelope for easy return. In hopes of increasing participation the participants were asked whether they wished to have the results of the research sent to them.

Again, due to the relatively small number of accredited Nuclear Medicine programs, any director that did not respond was contacted by phone and reminded to please make every effort to respond to the survey. A second cover letter and questionnaire was sent to those who had not responded. Every attempt was made to receive an overall response rate of 70 percent or better.

From all Nuclear Medicine Program Directors that did respond the data was analyzed and a In Vitro Laboratory Manual was developed by this researcher.

Finally a cover letter and the In Vitro Laboratory Manual developed was sent to two of the original five experts for their overall evaluation and commentary.

Instrumentation

Each Nuclear Medicine program director (or person responsible for program) throughout the United States was asked to complete a questionnaire for this survey research. This instrument was designed to develop a nationally accepted In Vitro Student Laboratory Manual based on data collected.

The survey was first field tested by five authorities in the field of Nuclear Medicine education, identified by either the CAHEA or the Society of Nuclear Medicine. The surveys were returned to this researcher and questions were reconstructed to conform with the overall groups' criticisms.

The follow-up questionnaire was designed to have program directors evaluate the appropriateness of general principles, concepts and specific testing procedures essential to a quality Nuclear Medicine Student In Vitro Laboratory Manual. A cover letter sent to all those surveyed explaining the purpose of developing an In Vitro Laboratory Manual for their curricula.

The survey first asked the respondent to identify the school's name, their name and their title. Initially four

yes/no type questions are asked of the respondent. These relate to the individuals philosophical perspective of the projected In Vitro Laboratory Manual.

The questionnaire is then broken down into two major sections. The first section includes a series of statements dealing with components of basic principles and theories to be discussed within the laboratory manual. A list of twenty-two (22) general principles and concepts are provided to be evaluated for their relative importance in In Vitro Nuclear Medicine education.

The second section relates the testing procedures which need to be part of a successful Nuclear Medicine In Vitro Laboratory Manual. A list of 34 specific testing procedures associated with In Vitro testing were supplied. These lists for both sections were derived from the content analysis in the In Vitro literature review.

In both section I and section II the participants were asked to evaluate the significance or importance of general principles, concepts and specific tests listed by each selecting the appropriate option described.

1. Must be part of: should be thoroughly discussed or reviewed.
2. Must be part of: does not need thorough discussion.
3. Should be part of: some aspects are vital to In Vitro education.

4. Could be part of: nice to know.
5. Is irrelevant.

The survey respondents are then asked to please add general principles, concepts or specific testing procedures they feel have been omitted that are essential to an In Vitro Laboratory Manual of prescribed lists.

This chapter examined the overall methodology and process which was undertaken for this research. Discussion included the setting for the investigation and the subjects utilized. A procedural chart attempted to simplify the sequential steps. An indepth look at procedure followed, along with a comprehensive analysis of the questionnaire utilized in the national survey.

Chapter 4

COLLECTION AND ANALYSIS OF DATA

Introduction

This chapter will discuss how the research was collected and analyzed. The entire population of accredited Nuclear Medicine programs in the United States were surveyed for this study. The questionnaire was sent from the University of Nevada, Las Vegas, with return envelopes. Based on the 74 percent response rate, the average values for all questions and statements were tabulated and analyzed.

The sampled population consisted of all Nuclear Medicine Program Directors in the United States (approximately 70). This group was selected because they have the strongest influence on the development and future implementation of an In vitro Student Manual into their curricula. Therefore, any valid study of future changes regarding In vitro education must include the perceptions of these individuals.

The survey was designed to have the Program Directors assess the need in the development of an In vitro Student Manual. Then if such a need exists, evaluate the appropriateness of particular general principles, concepts or specific type of tests in an In vitro Student Manual. The questionnaire was mailed out from the University of

Nevada at Las Vegas with a cover letter explaining the purpose of the survey. Telephone calls followed, and follow-up surveys to those directors that did not initially respond, asking or reminding them of the importance of their input of the 70 Nuclear Medicine programs surveyed nationwide. Fifty-Two responded to the questionnaire. One of the schools reported back that they had dropped their Nuclear Medicine program. Therefore, the actual number of completed surveys were 51 out of a possible population of 69; this represents 74% of the total Nuclear Medicine programs in existence in the United States. The study achieved its goal of at least seventy percent (70%) response rate.

The large overall collection of questionnaires clearly demonstrates the meaningfulness and importance to the Nuclear Medicine educational community the need for an In vitro Laboratory Manual to be developed.

The Nuclear Medicine Program Directors surveyed were first presented with four (4) yes/no type questions and asked to please circle the answer that best represents their philosophy in terms of the projected In vitro Laboratory Manual. The overall raw data results follow each question.

1. Do current texts, workbooks or laboratory manuals meet the needs of your nuclear medicine students with respect to In vitro procedures?

<u>Yes</u>	<u>No</u>
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9	41
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Eighty-two percent (82%) of these surveyed felt that the current literature do not meet the needs of the Nuclear Medicine students. This fact seems to support the review of the literature which reveals no laboratory manual was currently available to educators that provided a thorough examination of In vitro procedures in Nuclear Medicine.

2. Do you believe an In vitro Nuclear Medicine Undergraduate Level Manual is needed to properly train your students?

<u>Yes</u>	<u>No</u>
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43	6
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In response to this question eighty-eight percent (88%) of the Nuclear Medicine educators felt that a manual was needed.

3. Does a laboratory In vitro Manual need to be written to meet this need?

<u>Yes</u>	<u>No</u>
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40	8
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The overwhelming response was affirmative by the various educators surveyed. Eighty-three percent (83%) felt strongly enough to indicate that a laboratory manual needed to be written.

4. Should such a manual provide both a discussion of theoretical constructs and laboratory exercises for students?

<u>Yes</u>	<u>No</u>
41	9

In this question the surveyor was attempting to see if the Nuclear Medicine educators felt that a manual needed to in fact integrate the theory and practical work concepts. Again, the overwhelming number of participants were convinced, with a response rate of 85 percent that an In vitro Manual was needed both theoretical constructs and laboratory exercises.

The next two sections of the survey asked the Nuclear Medicine experts to evaluate the importance of various basic principles, theories and testing procedures which need to be a part of any successful In vitro Nuclear Medicine Educational Manual. Each respondent was asked to place a numerical value on each item listed in both sections.

The numerical values were as follows:

1. Must be part of: should be thoroughly discussed or reviewed.
2. Must be part of: does not need thorough discussion.
3. Should be part of: some aspects are vital to In vitro education.
4. Could be part of: nice to know.

5. Is irrelevant.

Section I included statements dealing with components of basic principles and theories. The average results of all participants follows each item.

Elements of R.I.A. antibody-antigen reaction.	<u>1.3</u>
Growth development, historical changes in In vitro Laboratory Technology.	<u>3.2</u>
Properties and methodologies of typical R.I.A. tests.	<u>1.4</u>
Components of R.I.A. equations of equilibrium, chemical properties, physiology.	<u>1.2</u>
Basic blood components and interfering substances.	<u>1.3</u>
Separation of free from bound techniques.	<u>1.6</u>
Calculations of results, assessment and evaluation of resultant values.	<u>1.6</u>
Evaluation of different techniques and kits.	<u>2.8</u>
Quality control on instrumentations, equipment in laboratory.	<u>1.5</u>
Typical R.I.A. testing procedures.	<u>1.3</u>
Labeling of radioactive material.	<u>2.6</u>
Production and purification of labeled isotopes.	<u>2.7</u>
Preparation of patient samples, blood and urine.	<u>2.2</u>
Gamma well counters and liquid scintillation.	<u>1.3</u>
Alternative immune testing procedures, enzyme, luminescence, gas spectroscopy.	<u>1.7</u>
Proper intravenous injection techniques.	<u>1.9</u>
Statistics and computer data.	<u>1.5</u>
Presentations in R.I.A.	
Blood volumes.	<u>1.9</u>
W.B.C. labeling.	<u>2.1</u>

R.B.C. labeling.	<u>1.4</u>
B-12 absorption testing, Schilling testing.	<u>1.7</u>
Iron recovery.	<u>2.8</u>

All basic principles and concepts described were classified by evaluators as (3.2) or numerically lower. This would seem to indicate that every principle and theory listed needed to be at least some part of the In vitro Manual.

Of the 22 statements and numerical values that followed three (3) categories were developed to evaluate the extent that these items would appear in the Nuclear Medicine Laboratory Manual.

1.6 or lower - thoroughly discussed and reviewed.

2.2 - 1.7 - discussed, but not in great detail.

3.5 - 2.3 - needs to be part of, briefly analyzed and discussed.

This scale was developed and utilized because almost all general principles/concepts listed were considered by educators as at least in the range of should be part of In Vitro education. The educators were asked to please add any principle or theory not included in this list. Only one (1) item was listed on more than 5 returned surveys. This was the concept of dilution principles listed 12 separate times. It was therefore included into the laboratory manual overall concepts discussion and analysis.

Section II of the survey asks the Nuclear Medicine Program Directors to evaluate the appropriateness of different types of testing procedures. Using the same scale utilized in section I, the experts selected numerical values (1-5) for each item on the list of 34 testing procedures provided.

The final total averaged results for the national survey for section II was as follows:

Thyroid	1.2	Angiotensin	3.4
Blood Volume	1.4	Drug Testing	1.4
Iron Recovery	1.8	I.G.E.	2.8
Cortisol	3.0	Monoclonal Antibodies	2.1
Testosterone	2.1	Norepinephrine	4.5
Estradiol, estrogen	2.1	Digoxin	2.6
CEA	1.6	LH	2.2
Peptide	4.2	TBG's	1.4
RBC Survival	1.6	Folic Acid	1.6
LSH	1.9	Alpha-feto Protein	1.9
Insulin	3.8	HCG	2.6
TSH	1.4	Vitamin B-12	1.5
PTH	2.9	Schilling Test	1.6
WBC Labeling	1.5	Thyrotropin	4.2
Renin, aldosterone	2.7	Creatinine	3.4
ACTH	3.6	Aminoglycoside	4.2
		Gonadotropin	3.4

Again, the respondents were asked to add any other testing procedures that could enhance Nuclear Medicine In vitro education.

The following testing procedures were named as important enough to be part of the developed laboratory manual.

Hepatitis Testing

Gastrin

Myoglobin

Calcitonin

Gentamicin

Phenytoin

Theophylline

Of these responses only hepatitis testing appeared on more than three (3) separate surveys. It was added to seven different survey responses. This the researcher felt was significant and is thoroughly discussed in the laboratory manual. The other tests are only briefly reviewed.

Summary of Resultant Values of 51 Nuclear Programs

Nationwide The following basic principles, concept or testing procedures were ranked (1.6 or lower) and are thoroughly discussed and reviewed in the developed Nuclear Medicine In vitro Manual.

Elements of R.I.A., antibody-antigen reaction.

Components of R.I.A. equations of equilibrium, chemical properties, physiology.

Basic blood components and interfering substances.

Separation of free from bound techniques.

Calculations of results, assessment and evaluation of resultant values.

Quality control on instrumentations, equipment in laboratory.

Typical R.I.A. testing procedures.

Properties and methodologies of typical R.I.A. tests.

Proper intravenous injection techniques.

Statistics and computer data presentation in R.I.A.

Blood volumes.

W.B.C. labeling.

R.B.C. survival.

Gamma well counters and liquid scintillation.

Vitamin B-12.

Schillings.

CEA.

Thyroid.

Blood volumes.

TSH.

Drug Testing.

RBC survival.

TBG's.

Folic Acid.

WBC labeling.

* Hepatitis testing.

* Dilution principles.

(* Added to list by respondents.)

The following basic principles, concepts or testing procedures were ranked between (2.2 - 1.7) and are discussed, but not in great detail:

Evaluation of different techniques and kits.

Labeling of radioactive material.

Production and purification of labeled isotopes.

Preparation of patient samples - blood or urine.

Alternative immune testing procedures, enzyme, luminescence, gas spectroscopy.

Monoclonal antibodies.

LH.

Alpha-feto protein.

Iron recovery.

Estradiol, estrogen.

Testosterone.

FSH.

LSH.

The following basic principles, concepts or testing procedures were ranked between (2.3 - 3.5) and are briefly discussed and analyzed in the In vitro Laboratory Manual.

Growth, development, historical changes in In vitro Laboratory Technology.

I.G.E.

Digoxin.

Cortisol.

HCG.

PTH.

Creatinine.

Renin, Aldosterone.

Angiotensin.

* Gastrin.

* Myoglobin.

* Calcitonin.

* Gentamicin.

* Phenytoin.

* Theophylline.

(*These testing procedures were added by the Nuclear Medicine Program Directors surveyed. None of these tests appeared on more than three (3) separate respondent surveys.)

Three categories were developed based on the overall average values assessed to each general principle/concept or testing procedure by the Nuclear Medicine program directors. Items ranked (1.6 or lower) are thoroughly discussed and reviewed in the Nuclear Medicine student In Vitro manual. This section included 27 properties. The next category included principles/concepts or tests which ranked between 1.7 to 2.2 and were discussed in the In Vitro manual, but

not in great detail. This section encompassed 13 principles/concepts or testing procedures. The final category incorporated basic principles/concepts or testing procedures ranked 2.3 to 3.5 and was briefly analyzed in the In Vitro manual. This group included 14 such items. Items with a numerical value greater than 3.6 was not included in the manual.

Chapter 5
SUMMARY, CONCLUSIONS, RECOMMENDATIONS,
AND RECOMMENDATIONS FOR FURTHER STUDY

This chapter briefly reviews the problem, summarize the results, and identify the conclusion based on the research. The major emphasis focuses on examining how well the four initial questions raised in Chapter 1 were answered by the research data. Then suggest alternative ways of solving the problems raised and provide recommendations for further research.

Restatement of the Problem

The purpose of this study was to examine the necessity for development of an In Vitro student laboratory manual to be used by accredited nuclear medicine technology programs. A review of the literature reveals through a content analysis that such a manual is not in existence.

A questionnaire was developed and distributed to all accredited nuclear medicine program directors nationwide. The survey elicited responses pertaining to general principles, concepts, and specific testing procedures they deemed essential to an educationally sound In Vitro laboratory manual for graduating students.

Summary

Based on the responses from the questionnaire, this section discusses the overall findings of the study. The initial questions posed which the basis of all the research was formulated is discussed, and analyzed.

The research attempted to answer four initial questions posed by the researcher.

1. Is there a need for an In Vitro laboratory manual for students in nuclear medicine technology?

First, through an extensive content analysis of In Vitro nuclear medicine literature it was clear that no such manual existed. There is no undergraduate level of material that provides laboratory exercises, conceptual discussion, and analysis of specific testing procedures vital to educating successful nuclear medicine technology students.

Secondly, 82 percent of the nuclear medicine program directors responding to the national survey felt that current texts, workbooks, or laboratory manuals did not meet the needs of their nuclear medicine students with respect to In Vitro procedures. Furthermore, 88 percent of the nuclear medicine educators felt that a manual was needed.

2. What general principles/concepts of In Vitro studies must be included in this student manual?

Through the extensive content analysis of the In Vitro literature, 22 general principles/concepts were cited. From these lists the national survey asked nuclear medicine

program directors to assess the importance of each item and add to this list whatever was in their view missing.

3. Identify the types of testing methodologies and procedures that must be included in a comprehensive student In Vitro manual.

Again, through the content analysis of the literature and the national survey 34 predominant testing procedures merged as important enough to be part of a comprehensive nuclear medicine In Vitro laboratory manual.

4. What aspects of In Vitro nuclear medicine are most commonly overlooked that need to be taught to graduating nuclear medicine students?

This question, though not specifically asked of the national survey respondents, can be deduced from the data collected. A number of general principles, concepts and testing methodologies not mentioned in the literature were sighted by nuclear medicine program directors as essential to this developed manual.

Conclusion

All of the data collected from both the review of the nuclear medicine literature and the national survey clearly indicates a need for development and future implementation of an In Vitro laboratory manual in nuclear medicine programs nationwide. This researcher has analyzed this data by developing and writing a student In Vitro manual at the

undergraduate level to meet this need. This laboratory In Vitro manual consists of nine chapters discussing general principles, concepts and specific testing procedures performed in nuclear medicine. The manual also provides 12 laboratory exercises for students to have hands-on experiences.

This laboratory manual will be made available to CAHEA, the Society of Nuclear Medicine, and nuclear medicine programs nationwide. The information gathered from this study can also be used by program directors in assessing their current In Vitro curricula and identify deficiencies.

One limitation that must be cited, is that due to time constraints and a tremendous amount of nuclear medicine materials, certain aspects may not have received the full extent of discussion they warranted.

Based on the data collected from this study further research in this area and other such texts, manuals need to be developed. Clearly there is a tremendous need not being met by the current writings in the field of nuclear medicine education. Future research may wish to utilize this research data and emphasis in greater detail general principles/concepts or specific testing procedures. Others may desire to devote their energies on laboratory exercises for students. It is quite obvious that much more can be researched and developed to foster nuclear medicine In Vitro education to a higher level.

BIBLIOGRAPHY

- Abraham, G.E. (1969). Preliminary communication; solid-phase radioimmunoassay of estradiol-17 beta. Journal of Clin Endocrinol Metab. (29) 866.
- Abraham, G.E. (1979). Preliminary communications; solid-phase radioimmunoassay of estradiol-17 beta. Journal of Clin. Endocrinol Metab., (29) 866.
- Abraham, G.E. (1984). Radioimmunoassay of steroids in biological materials, in radioimmunassay and related procedures in medicine, Vol. II. IAEA, Vienna, p. 3-28.
- Action FS: Analysis of straight-Line data. New York: Dover.
- Aebersold, P.C. (1956). The development of nuclear medicine. American Journal Roentgenol, Radium the Nuclear Medicine, (75) 1027.
- Albert, S.N. (1981). Blood volume and extracellular fluid volume, 2nd ed. Thomas.
- Allied Medical Education Directory, 1978. American Medical Association. Chicago: AMA.
- Anger, H.O. (1958). Scintillation camera. Review of Science Instrumentation, (21) 27.
- Anger, H.O. (1963). Gamma-ray and positron scintillation camera. Nucleonics, (21) 10.
- Anger, H.D. (1983). Radioisotope cameras, vol. 1. G. L. Hine, Ed. New York: Academic Press.
- Aoki, N.; Hashimoto, A.; & Yamamoto, T. (1976). Increased serum thyrotropin after administration of metoclopramide in man. IRCS Med. Sci., (4) 542.
- Archibald, E.L.; Mincey, E.K.; & Morrison, R.T. (1982). Estimation of serum proteins by competitive protein binding assay. Clin. Biochem., (5) 232.
- Bach, J.F. (1978, Ed.). Immunology, New York: Wiley, P. 1-11.
- Baker, S.B. (1948). Determination of protein-bound jodine. Journal of Biol. Chem., (173) 715-24.

- Bakerman, S. (198). Enzyme immunoassays. Laboratory Management, p. 21-9.
- Balanchard, G.C. & Gardner, R. (1978). Two immunofluorescent methods compared with a radial immunodiffusion method for measurement of serum immunoglobulins. Clin. Chem., (24) 808-14.
- Bangham, D.R. & Cotes, P.M. (1986). [Assay] standardization and standards. Brit. Medical Bulletin, 30(1) 12-7.
- Barakat, R.M. & Ekins, R.P. (1961). Assay of vitamin B₁₂ in blood; a simple method. Lancet, (2) 25.
- Bassiri, R.M. & Utiger, R.D. (1972). The preparation and specificity of antibody to thyrotropin releasing hormone. Endocrinology, 90, p. 722.
- Bayer, M.F. & McDougall, I.R. (1980) Radioimmunoassay of free thyroxine in serum: Comparison with clinical findings and results of conventional thyroid-function tests. Clin. chem., (26) 1186-92.
- Belanger, L.; Sylvestre, C.; & Dufour, D. (1983). Enzyme-linked immunoassay for alpha-fetoprotein by competitive and sandwich procedures. Clin. Chem. Acta., (48) 15-8.
- Berson, S.A. & Yalow, R.S. (1964). Immunoassay of protein hormones; dependence of sensitivity of assay on energy of antigen-antibody reaction. In Pineus, G., Thimann, K.V., & Astwood, E.B., editors. The hormones, Vol 4, 2. Newyork: Academic Press, pp. 567-72.
- Berson, S.A. & Yalow, R.S. (1973, Eds.). Method in investigative and diagnostic endocrinology, Vol. 2A & 2B. Amersterdam, Holland: North-Holland.
- Berson, S.A. & Yalow, R.S. (1978). Radioimmunoassay of ACTH in plasma. Journal of Clin. Invest., (47) 2725.
- Berson, S.A. & Yalow, R.S. (1982). The use of K⁴² or P³² erythrocytes and I¹³¹-tagged human serum albumin in simultaneous blood volume determinations. Journal of Clin. Invest., (31) 575.
- Berson & Yalow, R.S. (1986). Insulin in blood and insulin antibodies. American Journal of Medicine, (40) 676.
- Berson, S.A. & Yalow, R.S. (1986). State of human growth hormone in plasma and changes in stored solution of

- pituitary growth hormone. Journal of Biol. Chem., (241) 5745-6.
- Berson, S.A. & Yalow, R.S. (1987). Kinetics of reaction between insulin and binding antibody. Journal of Clin. Invest., 36, p. 873.
- Berson, S.A.; Yalow, R.S.; Bauman, A.; Rothchild, M.A.; & Newerly, K. (1986). Insulin-I¹³¹ metabolism in human subjects; demonstration of insulin binding globulin in the circulation of insulin treated subjects. Journal of Clin. Invest., 35, p. 170.
- Berson, S.A.; Yalow, R.S.; Schreiber, S.S.; et al. (1983). Tracer experiments with I¹³¹-labelled human serum albumin: Distribution and degradation studies. Journal of Clin. Invest., (32) 746.
- Bliss, CC.I. (1987). Dose-response curves for radioimmunoassays. In J.W. McArthur and T. Colton (eds.), Statistics in Endocrinology, Cambridge, MA: MIT Press, p 3-22.
- Boguslaski, R.C. & Rathjen, C.P. (1975). A column radioassay for the quantification of vitamin B₁₂. Clin. Chem. Acta, (62) 349.
- Bolton, A.E. & Hunter, W.M. (1973). The labeling of proteins to high specific radioactivities by conjugation to a ¹²⁵I-containing acylating agent. Biochem. Journal, 133, p. 529.
- Bosissoneau, R. (1983). Importance of continuing education. Radiologic Technology, (49) 2, p. 155.
- Bowers, G.N., Jr.; Burnett, R.W.; & McComb, R.B. (1985). Preparation and use of human serum control materials for monitoring precision in clinical chemistry. Clin. Chem., 21(12) 1830-6.
- Braverman, L.E.; Vagenakis, A.G.; Foster, A.E.; & others (1981). Evaluation of a simplified technique for the specific measurement of serum thyroxine concentration. J. Clin. Endocrinol. Metab., 32, p. 497.
- Broski, D.C. & Ballinger, P.W. (1980). Paper chase: Does academic degree make a difference? Radiologic Technology, (51) 485.
- Brown, B.L. & Elkins, R.P. (1980). A specific saturation assay technique for serum triiodothyronine. In International Atomic Energy Agency: In-Vitro procedures

with radioisotopes in medicine, Vienna, I.A.E.A., p. 569.

- Brown, G.M.; Boshans, R.L.; & Schalch, D.S. (1980). Computer calculation of radioimmunoassays. Comput. Biomed. Res., (3) 212-17.
- Bruni, C.; Germani, A.; Koch, G.; et al. (1976). Derivation of antibody distribution from experimental binding data. Journal of Theor. Biol., (61) 143-70.
- Burd, J.F.; Wong, R.C.; Feeney, J.E.; et al. (1977). Homogeneous reactant-labeled fluorescent immunoassay for therapeutic drugs exemplified by gentamicin determination in human serum. Journal of Clin. Chem., (23) 1402-8.
- Burger, H.G. & Patel, Y.C. (1977). TSH and TRH: Their physiological regulation and the clinical applications of TRH. In L. Martini & G.M. Besser (eds.) Clinical Neuroendocrinology. New York: Academic.
- Burger, A.; Suter, P.; Nicod, P.; et al. (1976). Reduced active thyroid hormone levels in acute illness. Lancet, p. 653.
- Burman, K.D.; Wright, F.D.; Earll, J.M.; & Wartofsky, L. (1975). Evaluation of a rapid and simple technique for the radioimmunoassay of triiodothyronine (T₃). Journal of Nuclear Medicine, (16) 622.
- Butt, W.R. (1972). The iodination of follicle-stimulating and other hormones for radioimmunoassay. Journal of Endocrinol., (55) 453.
- Cameron, E. H.; Hiller, S.G.; & Griffiths, K. (1985). Steroid immunoassay. Cardiff, U.K.: Alpha Omega.
- Carcinoembryonic antigen: Its role as a marker in the management of cancer. Brit. Medical Journal, 282(6261) 373-5, 1976.
- Carcinoembryonic antigen: Its role as a marker in the management of cancer. Ann. Internal Medicine, (94) 407, 1981.
- Carmel, R. (1978). Artifactual radioassay results due to serum contamination by intravenous radioisotope administration. American Journal of Clin. Pathol., (70) 364-67.

- Carmel, R. & Coltman, C.A., Jr. (1979). Radioassay for serum vitamin B₁₂ binder. Journal of Lab. Clin. Med., (74) 967.
- Carrico, R.J.; Christner, J.E.; Boguslaski, R.C.; et al. (1986). A method for monitoring specific binding reactions with cofactor labeled ligands. Ana. Biochem., (72) 271-82.
- Catt, K.; Niall, H.D.; & Tregear, G.W. (1986). Solid phase radioimmunoassay of human growth hormone. Biochem. Journal, 100, p. 31.
- Catt, K. & Rothfeld, B. (1986). Renin and aldosterone. Endocrinol. Reprod. Res. Branch. NIH. Bethesda, MD., USA. Nucl. Med.: Endocrinol., p. 353-64.
- Catt, K. & Tregear, G.W. (1967). Solid phase radioimmunoassay in antibody-coated tubes. Science, 158, p. 1570.
- Challand, G.S.; Ratcliffe, W.A.; & Ratcliffe, J.G. (1975). Semi-automated radioimmunoassays for total serum thyroxine and triiodothyronine. Clin. Chem. Acta, (60) 25.
- Chang, J.; Gotcher, S.; & Gushaw, J.B. (1981). Homogeneous enzyme immunoassay for theophylline in serum and plasma. Clin. Chem.
- Chard, T. (1978). An Introduction to radioimmunoassay and related techniques. Amsterdam: Biomedical Press, p. 43-45; 431-433.
- Chard, T. (1981). Laboratory techniques in biochemistry and molecular biology. North Holland Publ.
- Chard, T.; Kitau, M.S.; & Landon, J. (1980). The development of a radioimmunoassay for oxytocin; radioiodination, antibody production and separation techniques. Journal of Endocrinol, 46, p. 296.
- Chen, I. (1981). Commercially available fully automated systems for radioligand assay. Part 2. Performance characteristics. Coll. Med., Univ. Cincinnati, OH, USA. Ligand Review, 2(3) 46-8.
- Chen, I. (1984). Commercially available fully automated systems for radioligand assay, Part I overview. Coll. Med., Univ. Cincinnati, OH, USA. Ligand Review, 2(2), p. 46-50.

- Chen, W.; Heminger, L.; Maxon, H.R.; et al. (1980). Non-specific binding as a source of error in thyrotropin radioimmunoassay with polyethylene glycol as separating agent. Clin. Chem., (26) 487.
- Chiewitz, O. & Hevesy (1935). Radioactive indicators in the study of phosphorus metabolism. Nature, (136) 754.
- Chopra, I.J. (1972). A radioimmunoassay for measurement of thyroxine in unextracted serum. Journal of CLIN Endocrinol Metab (34) 938.
- Chopra, I.J. (1974). A radioimmunoassay for measurement of 3,3',5'-triiodo-L-thyronine reverse (T₃). Journal of Clin. Invest., (54) 583-92.
- Chopra, I.J. (1977). Radioimmunoassay of iodothyronines. In G.E. Abraham, Ed., Handbook of radioimmunoassay. New York: Dekker, p. 679-703.
- Chopra, I.J. (1982) A radioimmunoassay for measurement of thyroxine in unextracted serum. Journal of Clin. Endocrinol. Metab., (34) 938.
- Chopra, I.J. (1983). Radioimmunoassay of iodothyronines. Sch. Med., Univ California, Los Angeles, CA, USA. Clin. Biochem. Anal, 5 (Handbook Radioimmunoassay), p. 679-703.
- Chopra, I.J. & Crandall, B.F. (1975). Thyroid hormones and TSH in amniotic fluid. New England Journal of Medicine. (293) 740.
- Chopra, I.J.; Ho, R.S.; & Lam, R. (1972). An improved radioimmunoassay of triiodothyronine in serum; its application to clinical and physiological studies. Journal of Lab. Clin. Medicine. (80) 729.
- Chopra, I.J.; Solomon, D.H.; & Ho, R.S. (1981). A radioimmunoassay of thyroxine. Journal of Clin. Endocrinol Metab., (33) 865-8.
- Chopra, I.J.; Solomon, D.H.; & Beall, G.N. (1971). Radioimmunoassay for measurement triiodothyronine in human serum. Journal of Clin. Invest. (50) 2033.
- Chopra, I.J.; Van Herle, A.J.; Teco, G.N.; et al. (1980). Serum free thyroxine in throidal and nonthyroidal illnesses: A comparison of measurements by radioimmunoassay, equilibrium dialysis, and free thyroxine index. Journal of Clin. Endocrinol Metab., (51) 135-43.

- Clark, B. & Engvall, E. (1980). Enzyme-linked immunosorbent assay (ELISA): Theoretical and practical aspects. In E.T. Maggie (ed.) Enzyme Immunoassay. Boca Raton, FL: CRC Press, p. 167-80.
- Clark, F. & Horn, D.B. (1985). Assessment of thyroid function by the combined use of the serum protein-bound iodide and resin uptake of ¹³¹I-triiodothyronine. Journal of Clin. Endocrinol. Metab. (35) 39.
- Cleeland, R.; Christenson, J.; Usategui-Gomez, M.; & Heveran, J. (1985). Detection of drugs of abuse by radioimmunoassay: A summary of published data and some new information. Clin. Chem., 22(6) 712-25.
- Collu, R. (1979). Abnormal pituitary hormone response to thyrotropin-releasing hormone: An index of central nervous system dysfunction. In G. Tolis, et al. (eds.) Clinical neuroendocrinology: A pathophysiological approach. New York: Raven.
- Copeland, B.E. (1983). Quality control manual, revised ed. American Society of Clinical Pathologists.
- Dandliker, W.B. & Feigen, G.A. (1981). Quantification of the antigen-antibody reaction by the polarization of fluorescence. Biochem. Biophys. Res. Commun., (5) 299-304.
- Delean, A.; Munson, P.J.; & Rodbard, D. (1979). Multi-subsite receptors for multivalent ligands: Application to drugs, hormones, and neurotransmitters. Mol Pharmacol. (15) 60-70.
- Delean, A.; Stadel, J.M.; & Lefkowitz, R.J. (1980). A ternary complex model explains the agonist-specific binding properties of the adenylyl cyclase-coupled beta-adrenergic receptor. Journal of Biol. Chem., (255) 7108-7117.
- Demers, L.M. & Derck, D.D. (1980). Centria system 2. Ligand Review, 2(3) 50-7.
- den Hollander, F.C. & Schuurs, A.H. (1971). Discussion. In Kirkham, K.E. & Hunter, W.M., editors, Radioimmunoassay methods. Edinburgh: Churchill Livingstone, p. 419.
- Desbuquois, B. & Aurbach, G.D. (1971). Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. Journal of Clin. Endocrinol. Metab., 33, p. 732.

- Diamond, B.A.; Yelton, D.E.; & Scharff, M.D. (1983).
Monoclonal antibodies. A new technique for producing
serologic reagents. New England Journal of Medicine,
304(22) 1344-9.
- Di Bella, F.P. & Arnaud, C.D. (1980). In vitro assays for
parathyroid hormone, calcitonin, and vitamin D. Upjohn
Co., Kalamazoo, MI, USA. Nucl. Med. Endocrinol, p. 228-
64.
- Diczfalusy, E. (1979, Ed.). Immunoassay of gonadotrophins.
Karolinska Syposia No. 1, Acta Endocrinol. (eds) 142.
- Donini, S.; D'Alessio, I.; & Donini, P. (1978).
Radioimmunoassay of human chorionic gonadotropin (HCG)
and human luteinizing hormone (LH) using insoluble
antibodies. In E. Rosenberg (ed.): Gonadotropins 1968.
Los Altos, CA: Geron X, p. 263.
- Dressle, R.L. & Spicer, J.A. (1981). Laboratory science. In
D.R. Bernier; Langan, J.K.; and Wells, L.D.; eds.,
Nuclear medicine technology and techniques. St. Louis,
MO: CV Mosby, p. 131-61.
- Dunne, R.C., Jr. & Daniels, A. (1981). Immunodiagnosics: A
technology in transition. New York: Simpson Research
Assoc.
- Eisen, H.N. & Siskind, S.W. (1964). Variation in affinities
of antibodies during the immune response. Biochemistry,
(3) 996-1008.
- Ekins, R.P. (1960). The estimation of thyroxine in human
plasma by an electrophoretic technique. Clin. Chem.
Acta, (5) 453.
- Ekins, R.P. (1975). Radioimmunoassay design. In Pasternak,
C.A., editor, Radioimmunoassay in clinical
biochemistry. London: Heyden & Son.
- Ekins, R.P. (1980). The estimation of thyroxine in human
plasma by an electrophoretic technique. Clin. Chem.
Acta, 5, p. 463.
- Ekins, R.P. (1980). Theoretical aspects of saturation
analysis. In International Atomic Energy Agency: In
vitro procedures with radioisotopes in medicine,
Vienna, I.A.E.A., p. 325.

- Ekins, R. P. (1983). Basic principles and theory (of saturation assay). Inst. Nucl. Med. Middlesex Hosp. Med. Sch., London, England. Brit. Med. Bull., (1), 3-11
- Ekins, R.P. & Ellis, S.M. (1975). The radioimmunoassay of free thyroid hormones in serum. In J. Robbins and L.E. Braverman (eds.) Thyroid research, Amsterdam: Excerpta Medica, p. 597-600.
- Ekins, R.P.; Newman, G.B.; & O'Riordan, J.L.H. (1978). Theoretical aspects of saturation and radioimmunoassays. In Hayes, R.L.; Goswitz, F.A., & Pearson-Murphy, B.E., editors: Radioisotopes in medicine; In vitro studies, A.E.C. Symposium Series 13, Oak Ridge, Tenn., U.S. Atomic Energy Commission, p. 59.
- Ekins, R.P.; Sufi, S. & Malan; P.G. (1978). An "intelligent" approach to radioimmunoassay sample counting employing a microprocessor-controlled sample counter. In Radioimmunoarray and Related Procedures in Medicine (Berlin Symposium, 1977), Vol. 1, p. 437-455. Vienna, International Atomic Energy Agency.
- Engvall, E.; Jonsson, K.; & Perlmann, P. (1981). Enzyme-linked immunosorbent assay. II. Quantitative assay of protein antigen Immunoglobulin G, by means of enzyme-labeled antigen and antibody-coated tubes. Biochem. Biophys. Acta., (251) 423-7.
- Engvall, E. & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA): Quantitative assay of immunoglobulin G. Immunochemistry, (8) 871-4.
- Engvall, E. & Perlmann, P. (1972). Enzyme-lined immunosorbent assay, ELOSA. III. Quantitation of specific antibodies by enzyme-labeled antiimmunoglobulin in antigen-coated tubes. Journal of Immunology, (109) 129-35.
- Erlanger, B.F.; Borek, G.; Beiser, S.M.; & Lieberman, S. (1987). Steroid-protein conjugates of bovine serum albumin with testosterone and with corisone. J. Biol. Chem., 228, p. 713.
- Fang, V.S. & Refetoff, S. (1974). Radioimmunoassay for serum triiodothyronine: Evaluation of simple techniques to control interference from binding proteins. Clin. Chem., (20) 1150-4.
- Feinstein, A.R.; Bethard, W.F.; & McCarthy, J.D. (1953). A new method, using radioiron, for determining the iron

- binding capacity of human serum. J. Lab. Clin. Med., 42, p. 907.
- Feldman, H. & Rodbard, D. (1981). Mathematical theory of radioimmunoassay. In Odell, W.D., & Daughaday, W.H., Eds, Principles of competitive protein binding assays. Philadelphia: Lippincott, p. 158-203.
- Ficken, D. (Dec., 1985). Nuclear medicine program JCAH review committee, UNLV.
- Finley, P.R.; Williams, R.J.; & Lichti, D.A. (1980). Evaluation of a new homogeneous enzyme inhibitor immunoassay of serum thyroxine with use of a bichromatic analyzer. Clin. Chem., (26) 1723-6.
- Finney, D.J. & Phillips, P. (1977). The form and estimation of variance function, with particular reference to radioimmunoassay. Applied Statistics, (26), 312-20.
- First fifty years (1922-1972): The American registry of radiologic technologists. American Society of Radiologic Technologists, 1972.
- Fisher, D.A.; Sack, J.; Oddie, T.H.; et al. Serum T4, TBG T3, uptake, T3, reverse T3, and TSH concentrations in children 1 to 125 years of age. Journal of Clin. Endocrinol Metab., (45) 191-8.
- Fisher, J.W.; Thompson, J.F.; & Espada, J. (1981). A radioimmunoassay for human urinary erythropoietin. Isr. Journal of Med. Sci., (7) 873.
- Fletcher, J.E. (1977) A generalized approach to equilibrium models. Journal of Phys. Chem., (81) 2374-78.
- Fletcher, J.E.; Ashbrook, J.D.; & Spector, A.A. (1983). Computer analysis of drug-protein binding data. Ann NY Acad. Sci., (226) 69-81.
- Freeman, L.M. & Blaufax, M.D. (1985, Eds.). Radioimmunoassay. Grune & Stratton.
- Frenkel, E.P.; McCall, M.S.; & White, J.D. (1980). Recognition and resolution of errors in the radioisotopic assay of serum vitamin B₁₂. American Journal of Clin. Pathol., (53) 891.
- Frohman, L.A.; Reichlin, H.; & Sokal, J.E. (1980). Immunologic and biologic properties of antibodies to glucagonserum albumin polymer. Endocrinology, 87, p. 1055.

- Furuyama, S.; Mayer, D.M.; & Nugent, C.A. (1980). A radioimmunoassay for plasma testosterone. Steroids, (16) 415.
- Galskov, A. (1982). Radioimmunochemical corticotropin determination. Acta Endocrinol, (69), (Suppl.), 162, 5.
- Gharib, H.; Ryan, R.J.; & Mayberry, W.E. (1982). Triiodothyronine (T3) radioimmunoassay: A critical evaluation. Mayo Clin. Proc., (47) 934-7.
- Gibbons, I.; Skold, C.; Rowley, G.L.; et al. (1980). Homogeneous enzyme immunoassay for proteins employing beta-galactosidase. Anal. Biochem., (102) 167.
- Ginsberg, J.; Segal, D.; Ehrlich, R.M.; et al. (1977). Inappropriate triiodothyronine (T₃) and thyroxine (T₄) radioimmunoassay levels secondary to circulating thyroid hormone autoantibodies. Clin. Endocrinol, (8) 133-9.
- Glick, S.M.; Roth, J.; & Lonergan, E.T. (1978). Survival of endogenous human growth hormone in plasma. Journal of Clin. endocrinol. Metab., (24) 501.
- Glick, S.M.; Roth, J.; Yalow, R.S.; et al. (1973). Immunoassay of human growth hormone in plasma. Nature, (199) 784.
- Goldenberg, D.M.; Neville, A.M.; Carter, A.C.; Go, V.L.W.; Holyoke, E.D.; Isselbacher, K.J.; Schein, P.S.; & Schwartz, M. (1978). CEA (carcinoembryonic antigen): its role as a marker in the management of cancer. Journal of Cancer Res. Clin. Oncol., 101(3) 239-42.
- Grasbeck, R. & Saris, N.E. (1979). Establishment and use of normal values. Scand. Journal of Clin. Lab. Invest., (Suppl. 110), (24) 62.
- Greenwood, F.C.; Hunter, W.M.; & Glover, J.S. (1963). The preparation of ¹³¹I labeled human growth hormone of high specific activity. Biochem. Journal, 89, p. 114.
- Haber, E.; Koerner, T.; Page, L.B.; et al. (1979). Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. Journal of Clin. Endocrinol Metab., (29) 1349.

- Hales, C.N. & Randle, P.S. (1983). Immunoassay of insulin with insulin-antibody precipitate. Biochem. Journal, 88, p. 137-46.
- Hamilton, J.G. (1942). The use of radioactive tracers in biology and medicine. Radiology, (39) 541.
- Hamolsky, M.W.; Stein, M.; & Freedberg, A.S. (1987). The thyroid hormone-plasma protein complex in man. II. A new in-vitro method for study of uptake of labelled hormonal compounds by human erythrocytes. J. Clin. Endocrinol. Metab., 17, p. 33.
- Harada, A.; Hershman, J.M.; Reed, A.W.; et al. (1979). Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. Journal of clin. Endocrinol Metab., (48) 793.
- Harrison, W. (Dec., 1987). Ltr. from Program Director of Nuclear Medicine, Weber State Univ.
- Hayes, R. L.; Goswitz, F.A.; & Pearson-Murphy, B.E. (1978, Eds.). Radioisotopes in medicine; in vitro studies. A.E.C. Symposium Series 13, Oak Ridge, Tenn., U.S. Atomic Energy Commission.
- Herbert, V. (1959). Studies on the role of intrinsic factor in vitamin B₁₂ absorption, transport, and storage. American Journal of Clinical Nutrition, (7) 433.
- Herbert, V.; Castro, Z.; & Wasserman, L.R. (1960). Stoichiometry relation between liver-receptor, intrinsic factor and vitamin B₁₂. Proc Soc. Exp. Biol. Med., (104) 160.
- Herbert, V.; Wasserman, L.R.; Frank, O.; Pasher, I.; & Baker, H. (1979). Values of fasting serum "folic acid" levels. Fed. Proc., (18) 246.
- Hoofnagle, J.H. (1981). Serologic markers of hepatitis B virus infection. In W.P. Greger; Coggins, C.H.; and Hancock, E.W. (eds.) Annual Review of Medicine: Selected topics in the clinical sciences, Vol. 32. Palo Alto, CA: P Annual Reviews,, p. 1-11.
- Hosseini, G.; Ryan, R.J.; Mayberry, M.E.; & Hockert, T. (1971). Radioimmunoassay for triiodothyronine (T₃). I. Affinity and specificity of the antibody for T₃, J. Endocrinol, 33, p. 509.

- Hsu, T.H. & Yang, S.S.L. (1982). thyroid function tests. In B. Rothfeld (ed.) Nuclear Medicine: Endocrinology. Philadelphia: Lippincott, p. 34-54.
- Hughes-Jones, N.C. (1983). Nature of the reaction between antigen and antibody. Br. Med. Bull., 19, p. 171.
- Hunter, W.M. (1967). Radioimmunoassay. In D.M. Weir (ed.), Handbook of experimental immunology. Oxford: Blackwell, p. 608.
- Hunter, W.M. (1981). Preparation and assessment of radioactive tracers. Brit. Medical Bulletin, 30(1), 18-23.
- Hunter, W.M. (1984). Radioimmunoassay and saturation analysis. Preparation and assessment of radioactive tracers. Brit. Med. Bulletin, p. 18-23.
- International Atomic Energy Agency: In vitro procedures with radioisotopes in medicine, Vienna, 1970, I.A.E.A.
- International Atomic Energy Agency: Radioimmunoassay and related procedures in medicine, Vienna, 1984, I.A.E.A.
- International Atomic Energy Agency: Standardization and quality control In Radioimmunoassay and Related Procedures in Medicine (Berlin Symposium, 1977), Vol. 2, pp. 3-167. Vienna, International Atomic Energy Agency, 1978.
- Ishikawa, E. & Kato, K. (1978). Ultrasensitive enzyme immunoassay. Scand. Journal of Immunol., (8) 43-55.
- Jaffe, B.M. & Behrman, H.R. (1984, Eds.). Methods of hormone radioimmunoassay. New York: Academic Press.
- Jaffe, B.M.; Newton, W.T.; & McGuigan, J.E. (1980). The effect of carriers on the production of antibodies to the gastrin tetrapeptide. Immunochemistry, (7) 715.
- Judd, H.L. (1981). Ovarian function throughout life. In G.E. Abraham (ed.) Radioimmunoassay systems in clinical endocrinology New York: Marcel Decker.
- Kabakoff, D. (1980). In E.T. Maggio (ed.) Enzyme-immunoassay. Boca Raton, FL: CRC Press.
- Kanagasabapathy, A.S.; & Welby, M.L. (1974). The use of merthiolate for TBG-blocking in the radioimmunoassay of triiodothyronine. Clin. Chem. Acta. (55) 267.

- Karlsson, F.A.; Wibell, L.; & Wide, L. (1977). Hypothyroidism due to thyroid-hormone binding antibodies. New England Journal of Medicine, (296) 1146-8.
- Karush, F. (1962). Immunologic specificity and molecular structure. Adv. Immunol., 2, p. 1.
- Kato, K.; Hamaguchi, Y.; Fukui, H.; et al. (1985). Enzyme-linked immunoassay. I. Novel method for synthesis of the insulin-beta-D-galactosidase conjugate and its applicability for insulin assay. Journal of Biochem., (78) 235-7.
- Kirby, C.C. (1975). A look at radiologic technology education. Radiologic Technology, 47, 88.
- Kirkham, K.E. & Hunter, W.M. (1971, Eds.). Radioimmunoassay methods. Edinburgh: Churchill Livingstone.
- Kitagawa, T. & Aikawa, T. (1976). Enzyme coupled immunoassay of insulin using a novel coupling reagent. Journal of Biochem., (79) 233-6.
- Kochupilla, N. & Yalow, R.S. (1978). Preparation, purification, and stability of high specific activity 125-I-labelled thyronines. Endocrinology, (102) 128-35.
- Korcek, L. & Tabachnick, M. (1976). Thyroxine-protein interactions. Interaction of thyroxine and triiodothyronine with human thyroxine binding globulin. Journal of Biol. Chem., (251) 3558-62.
- Krugman, L.G.; Hershman, J.M.; Chopra, I.J.; et al. (1985). Patterns of recovery of the hypothalamic-pituitary-thyroid axis in patients taken off chronic thyroid therapy. Journal of Clin. Endocrinol Metab., (41) 70.
- Kundu, N. & Grand, M. (1976). Radioimmunoassay of 15 alpha-hydroxyestriol (estratetrol) in pregnancy serum. Steroids, (27) 785.
- Lam, R.L. & AVECILLA, L.S. (1980). Baccalaureate level education in radiologic technology: Results of a survey. Radiologic Technology, (52) 52.
- Landon, J.; Livanou, T.; & Greenwood, F.C. (1987). The preparation and immunological properties of ¹³¹I-labeled adrenocorticotrophin. Biochem. Journal, (105) 1075.
- Landsteiner, K. (1945). Specificity of serological reactions. Springfield, IL: Thomas.

- Larsen, P.R. (1982). Direct immunoassay of triiodothyronine in human serum. Journal of Clin. Invest. (51) 1939.
- Lau, K.S.; Gottlieb, C.; Wasserman, L.B.; et al. (1985). Measurement of serum vitamin B₁₂ level using radioisotope dilution and coated charcoal. Blood, (26) 202.
- Leclercq, R.; Taljedal, I.B.; & Wold, S. (1982). Evaluation of radioisotope data in steroid assays based on competitive protein binding. Clin. Chem. Acta, (36) 257-59.
- Lemarchand-Beraud, T. (1976). Radioimmunoassay of thyroid-stimulating hormone (TSH) in plasma. In H. Breuer; D. Hamel; and H.L. Kruskemper (eds.) Methods of hormone analysis. New York: Wiley.
- Lock, J.P. & Schalch, D.S. (1986). Anterior pituitary: Gonadotropic hormones. In B. Rothfeld (ed.) Nuclear Medicine: Endocrinology. Philadelphia: Lippincott, p. 34-54.
- Luft, R. & Yalow, R.S. (1974, Eds.). Radioimmunoassay: methodology and application in physiology and in clinical studies. Stuttgart: Georg Thiesme.
- MacRae, J. (1974). Further experiments with artificially provided radioactive substances. Phys. Rev., (45) 497.
- Maiolini, R.; Ferrua, B.; & Masseyeff, R. (1975). Enzymoimmunoassay of human alpha-fetoprotein. Journal of Immunol. Methods, (6) 355-62.
- Maiolini, R. & Masseyeff, R. (1975). A sandwich method of enzymoimmunoassay. I. Application to rat and human alpha-fetoprotein. Journal of Immunol. Methods, (8) 223-34.
- Maitra, S.K.; Yoshikawa, T.T.; Guze, L.B.; & Schotz, M.C. (1986). Determination of aminoglycoside antibiotics in biological fluids: A review. Clin. Chem., 25(8) 1361-71.
- Malan, P.G.; Cox, M.G.; Long, E.M.R.; et al. (1978). A multibinding site model-based curve-fitting program for the computation of RIA data. In Radioimmunoassay and Related Procedures in Medicine (Berlin Symposium, 1977), Vol. 1, p. 425-36. Vienna, International Atomic Energy Agency.

- Malan, P.G.; Cox, M.G.; Long, E.M.R.; et al. (1978). Curve-fitting to radioimmunoassay standard curves: Spline and multiple binding site models. Ann. Clin. Biochem., (15) 132-34.
- Malan, P.G.; Cox, M.G.; Long, E.M.R.; et al. (1978). Development in curve-fitting procedures for radioimmunoassay data using a multiple binding site model. In F. Siemaszko (ed.) Computing in Clinical Laboratories, New York: Wiley & Sons, p. 238-90.
- Marbach, P.; Gotz, U.; Veteau, J.P.; et al. (1978). RIA analysis by means of non-linearized response functions. In Radioimmunoassay and Related Procedure in Medicine (Berlin Symposium, 1977), Vol 1, pp. 383-397. Vienna, International Atomic Energy Agency.
- Marchalonis, J.J. (1979). An enzymic method for the trace iodination of immunoglobulins and other proteins. Biochem. Journal, (113) 299.
- Marshner, I.; Erhardt, F.; & Scriba, P.C. (1984). Calculation of the radioimmunoassay standard curve by spline function. In Radioimmunoassay and Related Procedures in Clinical Medicine and Research (Istanbul Symposium, 1977), Vol. 1, p. 425-36. Vienna,, International Atomic Energy Agency.
- Master plan for radiologic technology programs. Joint Review Committee on Education in Radiologic Technology, 1981.
- Mayberry, W.E.; Bilstad, J.M.; & O'Rourke, M.F. (1981). Radioimmunoassay for human throtropin. Ann. Intern. Med. (74) 471.
- McBride, K.E. (1977). Task analysis of medical technology administration and supervision as a foundation to a curriculum ladder. Dissertation, Univ. of Houston, p. 14.
- McFarlane, A.S. (1958). Efficient trace-labelling of proteins with iodine. Nature, 182, p. 53.
- Mikhail, G.; Wu, C.H.; Ferin, M.; et al. (1979). Radioimmunoassay of plasma estrone and estradiol. Steroids, (13) 731.
- Miles, C.N. & Randle, P.J. (1978). The preparation and properties of purified ¹²⁵I-labelled antibodies to insulin. Biochem. Journal, (108) 611.

- Miles, L.E.M. & Hales, C.N. (1968). Labelled antibodies and immunological assay systems. Nature, 219, p. 186.
- Michell, J.L.; Harden, A.B. & O'Rourke; M.F. (1980). The in-vitro resin sponge uptake of triiodothyronine-¹³¹I from serum in thyroid disease and pregnancy. Journal of Clinical Endocrinol. Metab. (20) 1474.
- Midgley, A.R., Jr. (1986). Radioimmunoassay: A method for human chorionic gonadotropin and human luteizing hormone. Endocrinology. (79) 10.
- Mincey, E.K.; Thorson, S.C.; Brown, J.L.; et al. (1982). A new parameter of thyroid function, the effective thyroxine ratio. Journal of Nuclear Medicine, (13) 165.
- Mitsuma, T.; Colucci, J.; Shenkman, L.; et al. (1982). Rapid simultaneous radioimmunoassay for triiodothyronine and thyroxine in unextracted serum. Biochem. Biophys. Res. Commun., (46) 2107.
- Mitsuma, T.; Gershengorn, M.; Colucci, J.; et al. (1981). Radioimmunoassay of triiodothyronine in unextracted human serum. Journal of Clin. Endocrinol. Metab., (33) 364.
- Moss, A.J.; Dalrymple, & Boyd; G.V. (1976 & 1986). Practical radioimmunoassay. Mosby Co., p. 48.
- Murphy, B.E.P. (1984). Application of the property of protein-binding to the assay of minute quantities of hormone and other substances. Nature (201) 679.
- Murphy, B.E.P. & Pattee, C.J. (1984). Determination of thyroxine utilizing the property of protein-binding, Journal of Clin. Endocrinol. Metab., (24) 187.
- Murphy, B.E.P. (1987). Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. Journal of Clin. Endocrinol. Metab., (27) 973-990.
- Nakamura, U.; Copra, I.J.; & Solomon, D.H. (1984). Cent. Health Sci. Journal of Nuclear Medicine, 18(11) 1112-5.
- Natarajan, T.K. (1969). A new image display and analysis system for radionuclide imaging. Radiology, (93) 823.
- National Committee for Clinical Laboratory Standards, Guidelines for Standards Assess the Quality of Radioimmune Assay Systems, 1976.

- Nauman, J.A.; Nauman, A.; & Werner, S.C. (1987). Total and free triiodothyronine in human serum. Journal of Clin. Invest., (46) 1346.
- Nelson, J.C.; Johnson, D.E.; & Odell, W.D. (1982). Serum TSH levels and thyroidal response to TSH stimulation in patients with thyroid disease. Ann. Intern. Med., (76) 47.
- Nickoloff, E.L. (1979). Radioisotopes as interfering substance4s in radioimmunoassays. Ligand Quarterly, 2(3) 22.
- Nicod, P.; Burger, A.; Staehell, V.; et al. (1976). A radioimmunoassay for 3, 3', 3'-triiodo-L-thyronine in unextracted serum: Method and clinical results. M. Clin. Endocrinol Metab., (42) 823-9.
- Niswender, G.D. & Midgley, A.R., Jr. (1980). Hapten-radioimmunoassay for steroid hormones. In F.G. Peron and B.V. Caldwell (eds.) Immunologic methods in steroid determination. New York: Appleton-Century-Crofts.
- Nuclear medicine technology essentials questionnaire. Joint Review Committee on Education in Nuclear Medicine, 1987.
- Odell, W.D.; Abraham, G.E.; Skowsky, W.R.; et al. (1981). Production of antiserum for radioimmunoassay. In W.D. Odell & W.H. Daughaday (eds.) Principles of competitive protein-binding assays. Philadelphia: Lippincott, p. 57.
- Odell, W.D. & Daughaday, W. H. (1971, Eds.). Principles of competitive protein-baonding assays. Philadelphia: Lippincott.
- Odell, W.D.; Ross, G.T.; & Rayford, P.L. (1986). Radioimmunoassay for human luteinizing hormone. Metabolism, (15) 287.
- Oellerich, M. (1980) Enzyme immunoassays in clinical chemistry: Present status and trends. Journal of Clin. Chem. Clin. Biochem., (18) 197-208.
- Ohnysty, J. (1978). Scanning a new image: Proposal for professional standards. Radiologic Technology, (49) 448.

- Oliver, G.C., Jr.; Parker, B.M.; Brasfield, D.L.; et al (1968). Measurement of digitoxin in human serum by radioimmunoassay. Journal of Clin Invest; (47) 1035.
- Orskov, H. (1967). Wick-chromatography for the immunoassay of insulin. Scand. J. Clin. Lab. Invest., 20, p. 297.
- Pangaro, L.; Burman, K.D.; Wartofshy, L.; et al. (1980). Radioimmunoassay for 3,5,3'-triiodothyronine. Journal of Clin. Endocrinol Metab., (50) 1075-81.
- Paradisi, R.; et al. (1980). Radioimmunoassay of three estrogens and three androgens in the same plasma sample after extraction and chromatographic separation. Acta Endocrinol, (94) 229.
- Parker C.W. (1971). Nature of immunological responses and antigen-antibody interaction. In D.W. Odell and W.H. Daughaday (eds.) Principles of competitive protein-binding assays. Philadelphia: Lippincott, p. 15.
- Pasternak, C.A. (1975, Ed.). Radioimmunoassay in clinical biochemistry. London: Heyden & Son.
- Patel, U.C.; Berger, H.G.; & Hudson, B. (1971). Radioimmunoassay of serum thyrotropin: Sensitivity and specificity. Journal of Clin. Endocrinol Metab., (33) 768.
- Paul, W.E. & Odell, W.D. (1974). Radiation inactivation of the immunological and biological activities of human chorionic gonadotropin. Nature, (203) 979.
- Perkary, A.E.; Harshman, J.M.; & Parlow, A.F. (1975). A sensitive and precise radioimmunoassay for human thyroid-stimulating hormone. Journal of Clin. Endocrinol Metab., (41) 676.
- Perlstein, M.T. (1978). Evaluation of commercial RIA kits. I: Recovery. Ligan Quarterly 1, (4) 4.
- Perlstein, M.T. (1979). Evaluation of commercial RIA kits. II: Parallelism effects of protein matrices and mathematical treatment of data in validating an assay. Ligand Quarterly, 2 (1) 6.
- Playfair, J.H.L.; Hurn, B.A.L.; & Schulster, D. (1983). Production of antibodies and binding reagents. Brit. Medical Bulletin, 30(1) 24-31.

- Pratt, J.J.; Woldring, M.G.; & Villerius, L. (1978). Chemiluminescence-linked immunoassay. Journal of Immunol. Methods, (21) 179-84.
- Purcell, R.H.; Holland, P.V.; Walsh, J.H.; et al (1969). A compliment-fixation test for measuring Australia antigen and antibody. Journal of Infect Dis, (120) 383.
- Ransom, J.P. (1986). Practical competitive binding assay methods. St. Louis: C.V. Mosby.
- Ratcliffe, J.G. (1984). Separation techniques in saturation analysis. Brit Medical Bulletin, 30(1) 32-7.
- Ratcliffe, W.A.; Challand, G.S.; & Ratcliffe, J.G. (1974). A critical evaluation of separation methods in radioimmunoassays for total triiodothyronine and thyroxine in unextracted human serum. Ann. Clin. Biochem., (11) 224-9.
- Rayford, P. L.; Miller, T.A.; & Thompson, J.C. (1980). Secretin, cholecystokinin and newer gastrointestinal hormones. Second of two parts. New England Journal of Medicine, 294(21) 1157-64.
- Read, C.H. & Bryan, G.T. (1980). Immunological studies of human growth hormone. Ciba Found Coloq. Endocrinol, (13) 68.
- Read, C.H. & Stone, D.B. (1958). An immunological assay for minute amounts of human pituitary growth hormone. AMA Journal of Dis. Child., (96) 538.
- Refsum, H.E. & Stromme, S.B. (1979). Serum thyroxine, triiodothyronine and thyroid-stimulating hormone after prolonged heavy exercise. Scand. Journal of Clin. Lab. Invest., (39) 455.
- Rodbard, D. (1971). Statistical aspects of radioimmunoassays. In W. Odell & W.H. Daughaday (Eds.) Principles of Competitive Protein-Binding Assays. Philadelphia: Lippincott, pp. 204-2.
- Rodbard, D. (1978). Data processing for radioimmunoassays: An overview in clinical immunochemistry, p. 477-94.
- Robard, D. (1980) Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Nat. Inst. Child Health Hum. Dev., Natl. Inst. Health, Bethesda, MD, USA. Clin. Chem., 20(10) 1255-70.

- Rodbard, D. (1984). Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clin. Chem., 20, p. 1255.
- Rodbard D. (1987). Statistical quality control of radioimmunoassays. In J. W. McArthur & T. Colton (eds.), Statistics in Endocrinology. Cambridge, MA: MIT Press, pp. 411-429.
- Rodbard, D. & Bertino, R.E. (1983). Theory of radioimmunoassays and hormone-receptor interactions: Simulation of antibody divalency, cooperativity, and allosteric effects. In B.W. O'Malley and A.R. Means (eds.) Receptors for Reproductive Hormones. Advances in Experimental Medicine and Biology, Vol. 36. New York: Plenum, p. 327-41.
- Rodbard D, Bridson; W., & Rayford; P. L. (1979). Rapid calculation of radioimmunoassay results. Journal of Lab. Clin. Med., (74) 770-781.
- Rodbard, D. & Cooper, J.A. (1970). A model for the prediction of confidence limits in radioimmunoassays and competitive protein binding assays. In International Atomic Energy Agency: In vitro procedures with radioisotopes in medicine, Vienna, I.A.E.A., p. 659.
- Rodbard, D. & Feldman, H.A. (1975). Theory of proteinligand interaction. In B.W. O'Malley and J.G. Hardman (eds.) Hormone action. Part A, steroid hormones. Methods in enzymatology, Vol. 36. New York: Academic Press, p. 3-16.
- Rodbard D. & Frazier, G.R. (1975). Statistical analysis of radioligand assay data. In B.W. O'Malley and J.G. Hardman (eds.), Hormone Action: Part B, Peptide Hormones. Methods in Enzymology, Vol 37. New York: Academic Press, pp. 3-22.
- Rodbard, D.; Munson, P.J.; & Delean, A. (1978). Improved curve-fitting, parallelism testing, characterization of sensitivity and specificity, validation, and optimization for radioligand assays. In Radioimmunoassay and Related Procedures in Medicine (Berlin Symposium, 1977), Vol. 1, p. 469-504. Vienna, International Atomic Energy Agency.
- Rodbard, D.; Rayford, P.L.; Cooper, J.A., et al. (1978). Statistical quality control of radioimmunoassays. Journal of Clin. Endocrinol Metab., (28) 1412.

- Roitt, I.M. & Torrigiani, S. (1987). Identification and estimation of undergraded thyroglobulin in human serum. Endocrinology, 81, p. 421.
- Rosa, V.; Pennisi, F.; Bianchi, R.; Federighi, S.; & Donato, L. (1987). Chemical and biological effects of iodination on human albumin. Biophys. Acta, 132, p. 486.
- Rosenfield, S. (Nov., 1987). Ltr. from Program Director of Nuclear Medicine at St. Louis Univ.
- Rosselin, S; Assan, R.; Yalow, R.S.; & Berson, S.A. (1986). Separation of antibody bound and unbound peptide hormones labelled with iodine-131 by talcum powder and precipitated silica. Nature, 212, p. 355.
- Roth, J. Peptide hormone binding to receptors; a review of direct studies in vitro. Metabolism, 22, p. 1059.
- Roth, J.; Klein, L.A.; & Petersen, M.J. (1986). Vasopressin antibodies and a sensitive radioimmunoassay. Journal of Clin. Invest., (45) 1064.
- Roth, J.; Glick, S.M.; Yalow, R.S.; et al. (1963). Secretion of human growth hormone: Physiologic and experimental modification. Metabolism; (12) 477.
- Rothenberg, S.P. (1961). Assay of serum vitamin B₁₂ concentration using Co⁵⁷-B₁₂ and intrinsic factor. Proc. Soc. Exp. Biol. Med, (108) 45.
- Rothenberg, S.P. (1981). Assay of serum vitamin B₁₂ concentration using Co⁵⁷, B₁₂, and intrinsic factor. Proc. Soc. Exp. Biol., (108) 45.
- Rothenberg, S.P. (1985). A radioenzymatic assay for folic acid. Nature, (206) 1154.
- Rothenberg, S.P.; DaCasta, M.; & Lawson, J. (1984). The determination of erythrocyte folate concentration using a two-phase ligand-binding radioassay. Blood, (43) 437.
- Rothenberg, S.P.; DaCosta, M.; & Rosenberg, Z. (1972). A radioassay for serum folate; use of a two-phase sequential-incubation, ligand-binding system. New England Journal of Medicine. (286) 1335.
- Rothfeld, B. (Ed., 1983 & 1985). Nuclear medicine in vitro, 3rd ed. Lippencott.

- Rubenstein, K.E. (1978). Homogeneous enzyme immunoassay today. Scand. Journal of Immunology, (8) 57-62.
- Rubenstein, K.E.; Schneider, R.S.; & Ullman, E.F. (1982). "Homogeneous" enzyme immunoassay: A new immunochemical technique. Biochem. Biophys. Res. Commun., (47) 846-51.
- Ruoslahti, E. & Seppalla, M. (1985). Radioimmunoassay of alpha-fetoprotein. Clin. Biochem. Anal, 5 (Handbook Radioimmunoassay), p. 543-69.
- Saar, N.; Bachmann, A.W.; & Gordon, R.D. (1981). Radioenzymic measurement of norepinephrine, epinephrine, and dopamine: Stability, enzymic activity, and sensitivity. Clinical Chem., (27) 626-8.
- Sakurada, T.; Rudolph, M.; Fang, S.L.; et al. (1978). Evidence that triiodothyronine and reverse triiodothyronine are sequentially deiodinated in man. Journal of Clin. Endocrinol Metab., (46) 916-22.
- Sanchez-Franco, F.; Carcicedo, G.L.; Martin-Zurro, A.; et al. (1984). Transient lact of thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH) in treated hyperthyroid patients with normal or low serum thyroxine (T_4) and triiodothyronine (T_3). Clin. Endocrinol Metab., (38) 1098.
- Scanlon, M.F.; Ress-Smith, B.; & Hall, R. (1978). Thyroid-stimulating hormone: Neuroregulation and clinical application. Parts 1 & 2. Clin. Sci. Mol. Med., (55) 1, 129.
- Schall, R.F., Jr. & Teneso, H.J. (1983). Alternatives to radioimmunoassay: Labels and methods. Organon Diagnostics, El Monte, CA, USA. Clin. Chem., 27(7) 1157-64.
- Scharpe, S.L.; Cooreman, W.M.; Bomme, W.J.; et al. (1976). Quantitative enzyme immunoassay: Current status. Clin. Chem., (22) 733-8.
- Seibert, M. (1981). Trends in radiologic technology education. Radiologic Technology, (46) 346.
- Sekadde, C.B.; Slaunwhite, R.W.; & Aeltour, T. (1983). Rapid radioimmunoassay of triiodothyronine. Clin. Chem., (19) 1016.
- Seth, J.; Rutherford, F.J.; & McKenzie, I. (1975). Solid phase radioimmunoassay of thyroxine in untreated serum. Clin. Chem., 21(10) 1406.

- SMITH R.G. & Sestili, M.A. (1980). Methods for ligandreceptor assays in clinical chemistry. Clinical Chemistry, (26) 543-50.
- Smith, R.G. & Sestili, M.A. (1981). Methods for ligand-receptor assays in clinical chemistry. Obstet. Gynecol, Baylor Coll. MEd. Houston, TX, USA. Clin. Chem., 26(5) 543-50.
- Snow, A. Ltr. from Director, Department of Nuclear Medicine, San Francisco Hospital.
- Snyder, P.J.; Muzyka, R.; Johnson, J.; et al. (1980). Thyrotropin-releasing hormone provokes abnormal follicle-stimulating hormone (FSH and luteinizing hormone responses in men who have pituitary adenomas and FSH hypersecretion. Journal of Clin. Endocrinol Metab., (51) 744.
- Soares, J.R.; Zimmermann, E.; & Gross, S. (1986). Direct Radioimmunoassay of 16-glucosiduronate metabolites of estriol in human plasma and urine. FEBS. Lett., (61) 263.
- Solomon, D.H.; Bennotti, J.; et al. (1972). A nomenclature for tests of thyroid hormones in serum; report of a committee of the American Thyroid Association. Journal of Clin. Endocrinol. Metab., (34) 884.
- Sonksen, P.H. (1974, Ed.). Radioimmunoassay and saturation analysis. British Medical Bulletin, 30(1).
- Spector, S. & Parker, C.M. (1970). Radioimmunoassay. Science, (168) 1347.
- Springwell, P.H. (1981). An unreactive tyrosine residue in insulin and the exclusive iodination of the A chain. Nature, (101) 1372.
- Stanczyk, F.Z.; Miyakawa, I.; & Gobelsmann, U. (1980). Direct radioimmunoassay of urinary estrogen and pregnanediol glucuronides during the menstrual cycle. American Journal of Obstet. Gynecology, (137) 443.
- Sterling, K.; Bellabarba, D.; Newman, E.S.; et al. (1979). Determination of triiodothyronine concentration in human serum. Journal of Clin. Invest., (48) 1150.
- Sterling, K. & Brenner, M.A. (1986). Free thyroxine in human serum protein-bound iodine and resin uptake of ¹³¹I-

- triiodothyronine. Journal of Clin. Endocrinol Metab., (25) 39-45.
- Sterling, K.; Bernner, M.A.; et al. (1971). The significance of triiodothyronine (T_3) in maintenance of euthyroid status after treatment of hyperthyroidism. Journal of Clin. Endocrinol. Metab., (33) 729.
- Sterling, K. & Milch, P.O. (1974). Thermal inactivation of thyroxine-binding globulin for direct radioimmunoassay of thyrodothyronine in serum. Journal of Clin. Endocrinol. Metab., (38) 866.
- Sterling, K.; Refellof, S.; et al. (1980). T_3 thyrotoxicosis; thyrotoxicosis due to elevated serum triiodothyronine levels. Journal of the American Medical Association, (213) 571.
- Straus, E.; Wu, N.; Quarishi, Mah; et al (1981). Clinical applications of the radioimmunoassay of secretory tuberculo protein. Proc Natl Acad Science USA, (78) 3214.
- Sunderman, F.W., Jr. (1985). Current concepts of "normal values," "reference values" and "discrimination values" in clinical chemistry. Clin. Chem., (21) 1873.
- Svendsen, R. & Sorenson, B. (1984). The concentration of unconjugated estrone and 17 beta-oestradiol in plasma during pregnancy. Acta Endocrinol, (47) 237.
- Thorell, J.I. (1978 & 1984). Radioimmunoessay design and related techniques. Mosby Co., p. 4.
- Thorell, J.I. (1983, Ed.). Radioimmunoassay design and quality control. Pergamon Press.
- Thorell, J.I. (1986). Use of immunosorbent in the preparation of "antigen-free" plasma; an adequate diluent for standards in radioimmunoassay. Clin. Chem. Acta., 22, p. 579.
- Ullman, E. & Khanna, P. (1981). Fluorescence excitation transfer immunoassay. In S.P. Colowick and N.O. Kaplan (eds.) Methods in enzymology, Vol. 74. New York: Academic, p. 28-60.
- Ullman, E.F.; Schwarzberg, M.; & Rubenstein, K.E. (1976). Fluorescent excitation transfer immunoassay: A general method for determination of antigens. Journal of Biol. Chem., (251) 4172.

- Utiger, R.D. (1985). Radioimmunoassay of human plasma throtropin. Journal of Clin. Invest., (44) 1277.
- Utiger, R. (1979). In B.M. Jaffe and H.R. Behram (eds.) Methods of hormone radioimmunoassay, 2nd ed., New York: Academic.
- Vagenakis, A.G. (1979). Regulation of TSH secretion. In G. Tolis, et al. (eds.) Clinical endocrinology: A pathophysiological approach. New York: Raven.
- Vaitukaitis, J.L. (1979). Specific human chorionic gonadotropin assay. In B.M. Jaffe and R.H. Behrman, eds., Methods of hormone radioimmunoassay. New York: Academic, p. 817-29.
- Vaitukaitis, J.L.; Ross, G.T.; Reichert, L.E., Jr.; et al. (1972). Immunologic basis for within and between species cross-reactivity of luteinizing hormone. Endocrinology, (91) 1337.
- Van Weeman, B.K. & Schuurs, A.H.W.M. (1981). Immunoassay using antigen enzyme conjugates. Febs. Lett., (15) 232-6.
- Van De Wiel, D.F.; Goedemans, W.T.; De Vries, J.A.; & Woldring, M.G. (1974). Radioimmunoassay of vitamin B₁₂ using a polyurethane sponge and competitive protein binding assay using *Lactobacillus leichmannii*. In International Atomic Energy Agency: Radioimmunoassay and related procedures in medicine, Vol. 2, Vienna, I.E.A.E., p. 185.
- Vinnik, A.I.; Kalk, W.J.; McLean, H.; et al. (1975). Fasting blunts the TSH response to synthetic thyrotropin-releasing hormone (TRH). Journal of Clin. Endocrinol Metab., (40) 509.
- Voller, A. (1980). Heterogeneous enzyme-immunoassays and their applications. In E.T. Maggic (ed.) Enzyme-Immunoassay. Boca Raton, FL: CRC Press, p. 181-96.
- Walters, G.O.; Jacobs, A.; Worwood, M.; Trevett, D.; & Thomson, W. (1985). Iron absorpction in normal subjects and patients with idiopathic hemochromatosis. Relation with serum ferritin concentration. Dep. Haematol. Welsh Natl. Sch. Med., Cardiff, Wales, Gut., 16(3), p. 188-92.
- Weeke, J. & Orskoy, H. (1975). Ultrasensitive radioimmunoassay for direct determination of free

- triiodothyronine concentration in serum. Scand. Journal of Clin. Lab. Invest., (35) 237.
- Wenzel, K.W. & Meinhold, H. (1984). Radioimmunoassay for T_3 and T_4 in unextracted sera. In International Atomic Energy Agency: Radioimmunoassay and related procedures in medicine, Vienna, I.A.E.A., p. 127.
- Werner, S.C.; Acebedo, G.; & Radichewich, I. (1974). Rapid radioimmunoassay for T_4 and T_3 in the same sample of human serum. Journal of Clin. Endocrinol. Metab., 38(3) 493.
- West, A. (Dec., 1987). Ltr. from Program Director of Nuclear Medicine, Univ. of Alabama, Birmingham.
- Wagner (Ed., 1978). Principles of nuclear medicine. Philadelphia: Sanders, p. 121.
- Weaver, C.K. & Cargille, C.M. (1981). Radioimmunoassay: A simplified procedure for dose interpolation. Journal of Lab. Clin. Med., (77) 661-664.
- Wide, L. (1985). Clinical significance of measurement of reaginic (IgE) antibody by RAST (radioallergosorbent technique). Dep. Clin. Chem., Univ. Hosp. Uppsala, Swed. Clin. Allergy, 3 Suppl., p. 583-95.
- Wide, L. & Porath, J. (1986). Radioimmunoassay with the use of Sephadex-coupled antibodies. Biochim. Biophys. Acta, 130, p. 257.
- Wilkinson, R.; Luetscher, J.A.; & Nokes G.W. (1971). Iodination of angiotensin I for use in radioimmunoassay. Journal of Clin. Endocrinol. Metab., (33) 545.
- Wisdom, G.B. (1986). Enzyme-immunoassay. Clin. Chem., (22) 1243-55.
- Witherspoon, L.R. (1986). The evaluation of commercial radioimmunoassay kits. Dep. Nul. Med., Ochsner Med. Inst, New Orleans, LA, USA. Contin. Educ. Lect. (Soc. Nucl. Med, Southeast Chapter) 15, p. 15.
- Witherspoon, L.; Shuler, S.E.; Garcia, M.M.; et al. (1979). Effects of contaminant radioactivity on results of ^{125}I -radioligand assay. Clin. Chem., (25) 1975.
- Witherspoon, L.R.; Schuler, S.E.; Garcia, N.M.; & Zollinger, L.A. (1986). Assessment of serum myoglobin as a marker

for acute myocardial infarction. Journal of Nuclear Medicine, 20(2) 115-9.

- Witherspoon, L.R.; Schuler, S.E.; Genre, C.F.; et al. (1982). Two radioimmunoassays compared with isoenzyme electrophoresis for the detection of serum creatine kinase-MB in acute myocardial infarction. Journal of Nuclear Medicine, (23) 136-42.
- Woodhead, J.S.; Addison, S.M.; & Hales, C.N. (1983). Immunoradiometric assay and related techniques. Brit. Medical Bulletin, 30(1) 44-8.
- Yalow, R.S. (1971). Radioiodination of peptide hormones: Procedures and problems. In W.D. Odell and W.H. Daughaday (eds.) Principles of Competitive Protein-Binding Assays. Philadelphia: Lippincott, p. 298.
- Yalow, R.S. (1973). Radioimmunoassay; practices and pitfalls. Circ. Res., 32, suppl. 1, p. 116.
- Yalow, R.S. & Berson, S.A. (1960). Immunoassay of endogenous plasma insulin in man. Journal of Clin. Invest., (39) 1147.
- Yalow, R.S. & Berson, S.A. (1968). General principles of radioimmunoassay. In Hayes, R.L., Goswitz, F.A., and Pearson-Murphy, B.E., editors: Radioisotopes in medicine; in vitro studies, AEC Symposium Series 13, Oak Ridge, Tenn., U.S. Atomic Energy Commission, p. 7.
- Yalow, R.S. & Berson, S.A. (1968). Special problems in the radioimmunoassay of small peptides. In protein and polypeptide hormones, Vol. I, Excerpta Medica Foundation, p. 71.
- Yalow, R.S. & Berson, S.A. (1970). Review paper; general aspects of radioimmunoassay procedures. In International Atomic Agency: In vitro procedures with radioisotopes in medicine, Vienna, I.A.E.A., p. 455.

APPENDIX A
JOINT REVIEW COMMITTEE'S NATIONAL SURVEY

QUESTIONNAIRE FOR
UPDATE OF THE NMT
ESSENTIALS

The JRCNMT would appreciate a moment of your time to respond to the enclosed questionnaire, relevant to the education and practice of Nuclear Medicine Technology. The information derived from this and future assessments will guide us in our review of education programs and future revisions of the Essentials and Guidelines.

Please indicate in the space provided, your answers to the following questions.

Demographic Data

1. Present Position(s), Check all which apply to you

Staff Technologist	Physician
Senior Technologist	Scientist
Chief Technologist	Radiation Safety Officer
Administrator	Clinical Instructor
Educator	Radiopharmacy Technologist
Student	Other

2. Training and education in nuclear medicine

<u>OJT</u>	<u>CAHEA</u>	<u>ADV. DEGREE</u>
RT	BS/BA	M.S.
MT	AS/AA	M.D.
RN	Certificate	Ph.D.
HS Certificate		

- 2a. Years in Practice:

3. Type(s) of institution where presently employed: Check all which apply
 - Private Hospital
 - University Hospital
 - Community Hospital
 - Government (Federal or State)
 - Private Practice
 - Mobile Service

4. What is the size of your principal institution:
 - Over 100 beds
 - 101-200 beds
 - 201-500 beds
 - over 800 beds
 - Does not apply

5. Location:
 - Large City
 - Suburb
 - Small City

6. Zip Code:
7. Where is nuclear medicine practiced in your institution?
 Nuclear Medicine is separate department
 Nuclear Medicine is part of Radiology
 Nuclear Medicine is part of Pathology
 Nuclear Medicine is part of Internal Medicine
 Other _____ Specify _____
8. Does the Nuclear Medicine Department at your institution perform: Check all that apply
 Imaging procedures
 In Vitro procedures
 In vivo non-imaging procedures
9. How many nuclear imaging procedures does your institution perform each month:
 Under 100
 101-200
 201-400
 401-600
 601-800
 801-1000
 Over 1000
10. How many immunoassays does your institution perform each month:
 Under 100
 100-500
 501-1000
 1001-5000
 5001-10,000
 Greater than 10,000
11. How many nuclear medicine technologists are currently employed at your institution?

Nuclear Medicine Practice

RADIOPHARMACY

12. Where are radiopharmaceuticals prepared?
 in your institution's Nuclear Medicine Department
 in your institution's Pharmacy Department
 outside commercial centralized pharmacy
 other _____ Specify _____
 Do you anticipate any changes? ____yes ____no
 Comment:

13. What is the professional background of the principal individual responsible for radiopharmaceutical preparation?
 nuclear medicine technologist
 radiographer
 medical technologist
 other Specify _____
14. Who is responsible for radiopharmaceutical quality control procedures?
 nuclear medicine technologist
 radiographer
 medical technologist
 radiopharmacist
 radiochemist
 other Specify _____

IMMUNOASSAY

15. Have you performed immunoassay procedures in the last two years?
 _____ yes _____ no
16. Where are radioassays performed at your institution?
 nuclear medicine
 pathology
 outside commercial lab
 do not do
17. Which of the following methods are used for immunoassay? (indicate % of tests done by each method)
 RIA _____ %
 EIA _____ %
 Fluorescent _____ %
18. Do you anticipate any changes in the involvement of Nuclear Medicine Technologists in Immunoassays at your institution?
 _____ yes _____ no
 If yes, please comment:

APPENDIX B
NUCLEAR MEDICINE PROGRAM DIRECTOR NATIONAL SURVEY

School's Name _____

Individual's Name _____

Title _____

Please Circle the answer that best represents your philosophy in terms of the projected In Vitro laboratory manual.

- Yes/No 1. Do current texts, workbooks or laboratory manuals meet the needs of your nuclear medicine students with respect to In Vitro procedures?
- Yes/No 2. Do you believe an In Vitro nuclear medicine undergraduate level laboratory manual is needed to properly train your students?
- Yes/No 3. Does a laboratory In Vitro manual need to be written to meet this need?
- Yes/No 4. Should such a manual provide both a discussion of theoretical constructs and laboratory exercises for students?

Section 1: The following series of statements deal with components of basic principle and theories to be discussed within laboratory manual. (Please write the appropriate numerical value in the space provided for each item mentioned.)

1. Must be part of: should be thoroughly discussed or reviewed
2. Must be part of: does not need thorough discussion
3. Should be part of: some aspects are vital to In Vitro education
4. Could be part of: nice to know
5. Is irrelevant

- _____ Elements of R.I.A., antibody-antigen reaction.
- _____ Growth, development, historical changes in In Vitro laboratory technology.
- _____ Properties and methodologies of typical R.I.A. tests.

- _____ Components of R.I.A. equations of equilibrium, chemical properties, physiology.
- _____ Basic blood components and interferring substances.
- _____ Separation of free from bond techniques.
- _____ Calculations of results, assessment and evaluation of resultant values
- _____ Evaluation of different techniques and kits.
- _____ Quality control on instrumentations, equipment in laboratory.
- _____ Typical R.I.A. testing procedures.
- _____ Labeling of radioactive material.
- _____ Production and purification of labeled isotopes.
- _____ Preparation of patent samples - blood or urine.
- _____ Gamma well counters and liquid scintillation.
- _____ Alternative immune testing procedures, enzyme, liminescency, gas spectroscopy.
- _____ Proper intravenous injection techniques.
- _____ Statistics and computer data presentations in R.I.A.
- _____ Blood volumes.
- _____ W.B.C. labeling.
- _____ R.B.C. survival.
- _____ B-12 absorption testing, schilling testing.
- _____ Iron recovery.

Please add any principle or theory not included in this list:

Section II: The rest of the data collection relates to the testing procedures which need to be part of a successful nuclear medicine In Vitro laboratory manual. (Please write the appropriate numerical value in the space provided for each item mentioned.)

1. Must be part of: significant, thoroughly discussed or reviewed
2. Must be part of: but not have to be thoroughly discussed

- 3. Should be part of: some aspects are vital to In Vitro education.
- 4. Could be part of: but not vital to In Vitro education
- 5. Can be excluded from: In Vitro nuclear medicine manual

_____	Thyroid	_____	Drug testing
_____	Blood volume	_____	CEA
_____	Iron recovery	_____	I.G.E.
_____	Cortisol	_____	Monoclonal antibodies
_____	Testosterone	_____	Norepinephrine
_____	Estrodiol, estrone	_____	Digoxin
_____	CEA	_____	LH
_____	Peptide	_____	TBG's
_____	RBC survival	_____	Folic acid
_____	FSH	_____	Alpha feto protein
_____	LSH	_____	HCG
_____	Insulin	_____	Vit. B-12
_____	TSH	_____	Insulin
_____	PtH	_____	Schilling test
_____	WBC labeling	_____	Thyrotropin
_____	(Fe) recovery	_____	Creatine
_____	Renin, aldosterone	_____	Aminoglycoside
_____	ACTH	_____	Gonadotropin
_____	Angiotensin		

Please add any other testing procedure that could enhance nuclear medicine In Vitro education.

I wish to have results reported to me.



APPENDIX C
IN VITRO NUCLEAR MEDICINE LABORATORY MANUAL

**IN VITRO NUCLEAR
MEDICINE
LABORATORY
MANUAL**

(developed from a national survey)

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Chapter 1

INTRODUCTION TO RADIOIMMUNOASSAY

Objectives

The student should

- be able to understand the basic advantages of radioimmunoassay over other tests;
- comprehend basic antigen-antibody reactions;
- be able to identify the 4 basic steps in a R.I.A. reaction;
- understand the purpose of RIA, including percent bound antigen concentration relationship;
- define and describe standards, labeled antigen, ligands, cross-reactivity, haptens, antigen production;
- describe a typical RIA exam, including controls;
- be familiar with isotopes used, binders (Ab), titer, affinity, iodination;
- give an example of basic RIA procedure;
- understand the concept of non-competitive assay.

Introduction

Prior to 1960, certain substances in the blood and other body fluids were extremely difficult to measure. This was to soon change and a new world of medicine would soon flourish. Two researchers, Yalow and Berson, reported a method for the

quantitative measurement of insulin in the bloodstream. The technique was known as “competitive binding assay”.

The technique they developed made use of an insulin binding protein using I-131 and guinea pig antibodies.

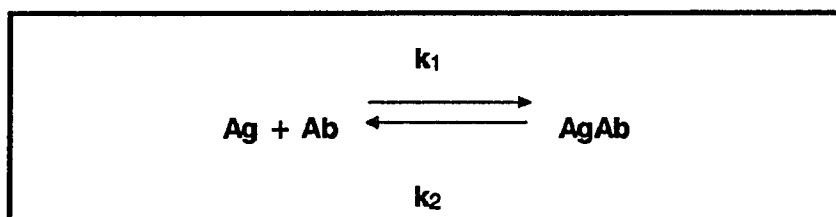
Before “RIA”, substances in biological fluids were measured by chemical analysis or bioassay. These methods, however, lacked sensitivity, specificity, and accuracy. The methods were laborious, expensive and time consuming. Radioimmunoassay techniques became widely used for measuring many substances, particularly hormones. Much of the progress in endocrinology during the past decade has occurred because of the availability of RIA techniques.

In the 1970’s and 1980’s, much of the invitro laboratory procedures’ rapid growth and development were due to:

- production of more and better antibodies;
- different labeling techniques;
- solid phase techniques;
- new methodologies;
- new instrumentation;
- and more accurate data reduction protocols.

The advantages of RIA over other systems include greater sensitivity, accuracy, specificity, and precision. RIA is synonymous with these other terms in principle: “saturation analysis”, “competitive radioassay”, “competitive binding assay”, “displacement analysis”, and “radiosteroassay”.

RIA is based on the principle that antibodies are produced to specifically interact with foreign substances which enter the body. They are the main basis of the body’s immune system. RIA is based on the concept that certain substances to be measured, **antigens**, will react with these specific antibodies. This reaction is outlined as follows:

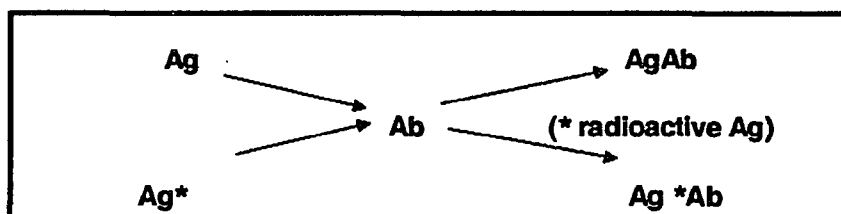


where k_1 represents the **rate constant or association constant**, k_2 represents the **rate constant or dissociation constant**. At the start of the reaction with free Ag and free Ab,

the rate of association is high (k_1). Until the rate is equal in both directions, the reaction will continue until equilibrium is reached and no further changes in concentration will result. K can be demonstrated as follows:

$$\frac{AgAb}{[Ag][Ab]} = k \text{ (equilibrium or affinity concentration)}$$

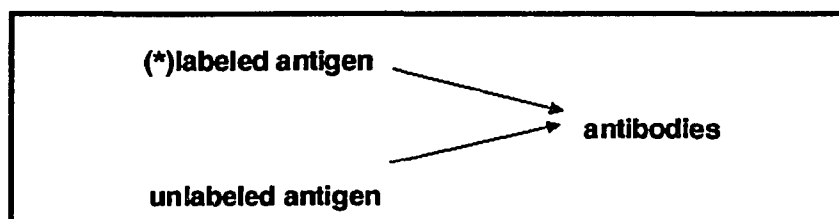
Low concentrations of an antigen can be detected by determining the ability of these antigens to be bound by a specific antibody. Competition of a non-labeled hormone or antigen (Ag) with a radio-labeled antigens for the same binding sites on an antibody (Ab) causes a depression of the amount of bound, labeled material in the final solution.



By studying a series of labeled antigens as standards, known amounts of unlabeled hormone (in unknown patient serum) can be measured because it offers similar competition with labeled hormone molecules for the antibody. In other words, the labeled and unlabeled antigens will compete equally.

The antigen to be measured is known as a "ligand". The antibody is a gamma-globulin protein with receptor sites for the antigens. The radioactive tracer is known as a "radioligand".

THE STEPS IN THE REACTION ARE AS FOLLOWS:



Step 1

where both labeled and unlabeled antigens, again, are allowed to interact with the antibody in solution. The result is two more substances, namely bound labeled antigen and bound unlabeled antigen.

Step 2

Incubation period - this time allows the process to reach equilibrium (the constants are equal).

Step 3

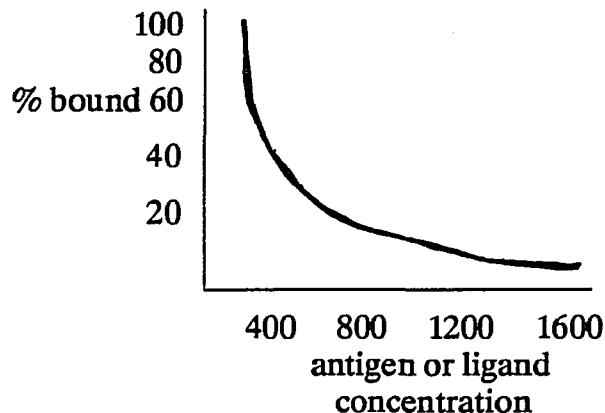
The separation of bound and free or unbound antigens.

Step 4

Determining the amount of radioactivity present using the appropriate instrumentation.

Adding larger amounts of known quantities of the original antigen will result in the formation of less of the labeled bound. The excess unlabeled form competes successfully against the small amount of labeled antigen for the binding sites on the antibody.

After the free and bound substances are separated, the amount bound can be measured.



This chart demonstrates the reaction principle that the *greater* the amount of ligand or patient antigen present, the *lower* the percent bound of the added radioactive antigen or ligand.

Antigens, in more detail, are specific types of ligands. Ligands may be purified from natural or synthetic materials and an ample supply of these purified ligands must be used to gain

the desired reaction. One of the most important qualities of a ligand is that it be identical to the endogenous ligand being measured (the patient sample). If they are dissimilar, the assay's specificity, sensitivity, and accuracy will be affected. The ligand is capable of stimulating an antibody response and must consist of the following properties:

- it must be foreign to the host;
- must have a molecular weight of 5000 or greater;
- must be pure;
- and again, must be identical to the endogenous ligand.

Elements used in RIA reaction are incubated to allow the reaction to occur. The competition between labeled and unlabeled ligands is allowed to occur simultaneously until equilibrium is reached the ligands and the binder. This is known as "equilibrium saturation". The sensitivity obtained by the RIA reactions as a whole depend somewhat on the specificity of the antibody as well as the antigenicity of the antigen, but depends primarily on the equilibrium constant or affinity constant. The higher the affinity, the greater the sensitivity of the antibody and the more rapid the attainment of equilibrium. The greater the affinity, the greater the concentration of antigen-antibody complex formed and the smaller the dissociation constant.

Separation of bound from free reactants once equilibrium is reached is determined by making use of the procedure which will give the greatest amount of separation with the least amount of effort, but in the meanwhile not sacrificing sensitivity. Procedures such as gel filtration, chromatography, precipitation of the bound form (double antibody technique) and absorption of the free phase by charcoal are a few of the methods used. Regardless of the technique used, there should be no effect on the antigen-antibody reaction.

Determining the amount of radioactivity present is done by using gamma counters for traces of I-125 and I-131, for example, and liquid scintillation is used for H-3 and C-14 and other beta emitting radionuclides. The radioactivity measured reflects the relative amount of radioactive bound fraction and radioactive unbound fraction. The results are usually reported as a dose-response curve.

The dilution of antiserum binding in a predetermined percent of tracer which is usually equal to the concentration to be used on the assay is called **titer**. The dilution is that amount which will bind 50% if the antigen.

The term **immunogen** is used synonymously with the ligand. Also, the patient sample (antigen or ligand) can also be termed **analyte**.

Certain characteristics of the labeled antigen must also be present. It must be highly purified, it must be able to be radiolabeled without loss of immunoreactivity. Many antigens are polypeptides which contain tyrosine residues that are fairly easy to label with iodine. Often I-125 is used as the tracer, since a higher specific activity (act/mass) can be gained.

Antigens are produced from an **antiserum**. This antiserum is usually from guinea pigs or rabbits in sufficient amounts for RIA purposes. The actual antibody used in testing usually represents a high dilution of the original sera; the final dilution may be 1:1,000,000 and 1ml of the antiserum will suffice for 2,000,000 assays.

The **labeled antigen** can be labeled with not only I-125, but also I-131, C-14, H-3, P-32, Se-75, Fe-59, Co-57. These isotopes are several of the more historical substances. More widely used today is I-125, though Co-57, Fe-59, Se-75, and H-3 may be used. The ideal radionuclide must conform to the following characteristics:

- high abundance;
- high counting efficiency;
- moderately long half-life;
- be bound easily to the antigen;
- and consist of a low or negative radioactive risk.

The shorter the half-life and the greater the specific activity, the greater the sensitivity.

The **tagging process** as performed by the manufacturers of the "kits" are done by several methods. When labeling with H-3, this process is completed via *in vivo* or *in vitro* H-3-lab precursors, neutron activation, chemical synthesis, and the Wilzbach technique (an exchange reaction where H-3 is substituted for stable H on the molecule).

The iodination of an antigen is accomplished directly or indirectly by conjugation of a pre-labeled tyrosine molecule to an antigen. The I-125 is substituted for H in the aromatic chain of tyrosine residues by conversion of unreactive I⁻ to a highly reactive I⁺ state.

For large protein molecules, direct labeling is usually not a problem since they contain a tyrosine residue. The amount of labeling depends on the specific activity of iodine or the number and location of tyrosine residues.

The most popular method of iodination is called the "**chloramine-T**" method. It is useful in the iodination of peptides and proteins. Chloramine-T is found to oxidize I-125-labeled NaI for reactions with tyrosine residues.

Conjugation labeling makes use of **haptens or bridges**. First coupling of iodine to a carrier molecule is followed by conjugation to an antigen by the hapten. Conjugation labeling is successful for several reasons:

- it does not expose the ligand to chemical damage;
- it can be applied to ligand without tyrosine residues;
- and it is a simple procedure.

Quality assurance regarding these radiolabeled ligands or antigens include:

- **radiochemical purity**—chromatography is used to check the maximum binding or nonspecific binding;
- **immunoreactivity**—checked by the use of excess antibody and a small amount of labeled ligand (if a significant amount of label does not bind it denotes nonimmunoreactivity);
- **specific activity**—measurement of the curves containing radioactive isotope/weight of both labeled and unlabeled ligand—the degree of specific activity can affect assay sensitivity;
- **stability**—this category includes half-life, storage, chemical breakdown, and loss of immunoreactivity.

The stability of the antigen used as the standard is particularly important because they are used in assays over a long period of time. Of importance in the maintenance of stability is an awareness of interfering substances. These substances may prevent nonidentity of the standard and include urea, heparin, bilirubin, buffers, and the effects of temperature and pH.

There are three types of binders common in RIA. They are the **antibody, circulating binding protein, and cell receptors**. The most important characteristics of binders include affinity capabilities, specificity, and availability.

The **antibody**, again, is an immunoglobulin produced in response to an antigen or immunogen. The proteins are categorized as albumin, alpha, beta, or gamma globulin. There are five distinct classes of globulins, all of which have a common structure but a different function. They are known as IgG, IgA, IgD, and IgE. The IgE, for example, is responsible for allergic reactions, while IgM is present at the onset and eventually disappears. Specificity of the antibody for an antigen interaction is influenced by the heterogeneity of antibodies for the same antigen; that is, the cross-reactivity for similar substances. The RIA system depends on the Ag-Ab reaction and not upon the biological activity of the antigen or ligand. A specific concentration of the antibody must be used, and the dilution is often chosen which will be sufficient to bind 50% of the labeled antigen, with the resultant bound to free ratio (B/F) being 1:1.

Certain substances, called **haptens**, are used in the production of antibodies for use in RIA. They are relatively small molecules unable to initiate an antibody response by themselves, but which can combine with larger molecules to produce an antibody response.

Polyclonal antibodies are produced in response to a variety of antigenic determinants on an immunogen which is injected into an animal. Each antigenic determinate produces an antibody with its own affinity and specificity. This is the **antiserum**. Small amounts of immunogen are used at regular intervals and booster shots are given. The immunogen must be as pure as possible and given in a combination of mycobacteria, minerals, and emulsifiers that enhance the immune response and allows for slow absorption into the system, known as **Freud's adjuvant**. The route of administration is usually subcutaneous and the selection of host animal depends on a variety of variables: volume of antisera needed, foreignness of the immunogen and the availability of the animal. After the booster injections are given, usually every 6-8 weeks, the antiserum gained is purified.

The antibody gained is a fairly specific three dimensional configuration. Certain portions of the molecule are exposed or are available to react with other molecules, known as the **lock and key phenomenon**. Cross-reactivity becomes important at this point; the antibody may have a low avidity for the antigen resulting in the inability to discriminate different antigens because of their structural similarities. The greater

the specificity of an antibody, the less likely the chances of cross-reactivity.

Binding proteins, the second type of binder mentioned, are used in assays for thyroid hormones, cortisol, estrogens, and progesterone. The disadvantages of binding proteins are:

- a low affinity constant (less sensitive);
- poor specificity;
- temperature dependency;
- and high concentration of binder.

Cell receptors, the third type of binder, are important when dealing with protein hormones. The effects of many protein hormones are exerted by first coupling specific receptor sites on cell membranes. The receptor activity measured ultimately becomes a measurement of biologic activity. Difficulties arise due to:

- diverse tissue;
- low concentration present;
- unstableness;
- and can be easily damaged.

Because of the reasons stated above, antibodies remain the major choice of binders available.

The **ideal competitive radioassay** should consist of the following criteria:

- nonradioactive and radioactive ligands or antigens should be chemically and immunologically indistinguishable;
- the reaction should continue until equilibrium is reached ($k_1 = k_2$);
- antigen and antibody binding sites should react in a 1:1 ratio;
- the antibody should be specific for a single antigen;
- the amount of antigen should be in excess of the antibody;
- and antibody binding sites should be independent of one another.

In review, the purpose of binding assays is to determine the concentration of the antigen in patient serum or plasma or other body fluids. To perform this function, a dose response

curve must be constructed using **serial dilutions** of a known standard, known as a standard curve.

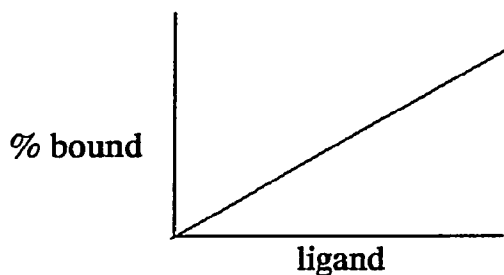
For an example of a **typical RIA procedure** see laboratory exercise section.

In **non-competitive assays**, the antibody is usually labeled instead of the ligand. This method is particular useful when the ligand cannot be easily labeled, as is the case with hepatitis-associated antigen or other viruses.

The receptor preparation is incubated with the sample containing the ligand to be assayed until all ligand present is bound to the receptor. Now, a second set of receptors is added, which are soluble but have a radioactive tracer labeled to their outer end. The radioreceptor adheres to any bound ligand present in the sample.

The ligand must be sufficiently large to have two or more molecular regions that can act as combining sites (divalent or polyvalent). Following the incubation with radioactive receptors, the matrix is washed and all non-bound radioactivity is removed.

The radioactivity remaining bound to the matrix is now measured. The activity bound to the solid phase is proportional to the amount of non-radioactive ligand present:



REVIEW

Section 1

1-10 Matching

- | | |
|---------------------|--|
| ___ 1. pt. sample | a. <i>in RIA it is considered the incubation period</i> |
| ___ 2. antigen | b. <i>minimum quantity detectable</i> |
| ___ 3. affinity | c. <i>capacity to discriminate (Ag)</i> |
| ___ 4. accuracy | d. <i>reference tubes that react with Ab similarly to patient sample</i> |
| ___ 5. ligand | e. <i>the substance that is bound</i> |
| ___ 6. precision | f. <i>the substance capable of inducing formation of (Ab)</i> |
| ___ 7. sensitivity | g. <i>repeatability of measured quantity</i> |
| ___ 8. specificity | h. <i>strength of binding to receptor</i> |
| ___ 9. standard | i. <i>closeness to true or real value</i> |
| ___ 10. equilibrium | j. <i>substance that is interpolated into standard curve</i> |
| | k. <i>substance that is not immunogenic itself</i> |

Section 2

VERY briefly describe or explain the following questions:

1. List and describe the 4 basic procedural steps in any RIA test.

a. _____

b. _____

- _____
- _____
- c. _____
- _____
- _____
- d. _____
- _____
- _____

Section 3

////////////////////

Multiple Choice

1. RIA has become a widely used technique for measuring many substances because _____.
 - a. high specific activity
 - b. wide range of isotopes available
 - c. ability to detect small quantities
 - d. very fast process (less than 15 min.)
 - e. all of the above
2. *Antibodies are* _____.
 - a. receptor sites
 - b. usually gamma-globulin protein
 - c. bivalent or polyvalent molecules
 - d. all of the above
3. Competitive protein binding is inversely related to ligand present, why?
 - a. the titer is at 75% dilution
 - b. Ab receptor sites are saturated
 - c. *Ag, Ag (pt. sample) compete for binding sites on Ab
 - d. only the Ag (pt.) is labeled and can be measured

4. The principle of radioimmunoassay is based upon the fact that _____.
 - a. both labeled and unlabeled antigens will combine to form a measurable entity
 - b. only labeled antibody and labeled antigen will combine to form a measurable entity
 - c. only unlabeled antigen will compete with the labeled antibody to form a measurable entity
 - d. both labeled and unlabeled antigen will compete with a known amount of antibody to form a measurable entity
5. The term radioimmunoassay is synonymous with _____.
 - a. saturation analysis
 - b. competitive-binding analysis
 - c. displacement analysis
 - d. all of the above
 - e. a and c only
6. In general, the basic components of an RIA reaction are _____.
 - a. labeled antibody, unlabeled antigen
 - b. antibody, labeled antigen
 - c. antibody, labeled antigen, unlabeled antigen
 - d. labeled antibody, unlabeled antibody, antigen
7. Which one doesn't belong: haptens are substances
 - a. foreign in origin
 - b. sm. molecules
 - c. can induce Ab formation
 - d. linked to larger molecule
8. The isotope most commonly used in RIA procedures is
 - a. I-131
 - b. C-14
 - c. H-3

- d. I-125
9. Antiserum used to produce (Ab) in RIA _____ .
- come from sm. animals such as guinea pigs and rabbits
 - used in a final dilution of 1:1,000,000
 - used in a final dilution of 1:500
 - a and b
 - a and c
10. The titer of the antibody is a term which refers most specifically to _____ .
- avidity
 - affinity
 - concentration
 - stability
11. An example of non-competitive assays is _____ .
- digoxin
 - T-7
 - HCG-B
 - hepatitis (virus)
 - a and b only
12. Non-competitive binding is different than competitive because _____ .
- radioactivity is labeled to Ab
 - radioactivity to bound ratio is 1/2
 - radioactivity labeling is to Ag
 - a and b
13. In non-competitive binding the ligand is _____ .
- directly related to % bound
 - indirectly related to bound
 - inversely related to amt. of counts recorded
 - dependent if its solid phase

14. In order for (Ag) to induce (Ab) it must be of foreign origin and relatively high molecular weight.

- a. true
- b. false

15. $\text{Ligand} + \text{Ab} \xrightleftharpoons[k_2]{k_1} \text{L1} + \text{Ab} - \text{K1}$ is

- a. overall rate of reaction
- b. association constant
- c. dissociation constant
- d. dependent on amount of tyrosol residues

16. In a competitive binding radioassay, competition exists between _____.

- a. a ligand and an antigen
- b. an antigen and an antibody
- c. a ligand and a radiolabeled ligand
- d. a ligand and an analyte

17. After the ligand, radioligand, and binder are given time to equilibrate _____.

- a. the radiolabeled ligand must be separated from the binder
- b. the radiolabeled ligand must be separated from the nonbinder
- c. the bound ligands must be separated from the free ligands
- d. all of the above

Section 4

T or F

- ___ 1. A hapten is defined as the substance to be bound.
- ___ 2. In a competitive assay between Ag and Ab the greater the amount of ligand or patient antigen present, the lower the percent bound.

- 3. *Patient (Ag) or ligand must be identical to other (Ag) and have a molecular weight of 500.*
- 4. *The measure of the strength of the bound antigen is called affinity.*
- 5. *In non-competitive assays such as hepatitis-associated antigen or other virus, the labeled molecule could be either the Ag or Ab.*
- 6. *In producing antiserum, freud's adjuvant is used to enhance the immune response and allow for slow absorption into the animal's system.*
- 7. *In non-competitive assays, the antibody or the antigen are labeled as count rates increase and concentration decreases.*

Chapter 2

ASSAY CHARACTERISTICS AND PROCEDURAL EVALUATION

Objectives

- Understand the uses and meanings of: total counts tubes, blank tubes, standards and controls.
- Be able to describe the differences between pooled and commercial serum sources of controls.
- Develop a working knowledge and purpose of the College of American Pathologists, American College of Nuclear Physicians, and the 'Center for Disease Control.
- Be able to define accuracy, sensitivity and specificity and how they are measured in RIA testing procedures.
- Describe other considerations to be considered when evaluating testing procedures.

Assay Characteristics

The following is a discussion of the components of a typical RIA test and their function in determining final resultant values.

Total Activity or Total Counts Tubes

Usually the first tubes utilized in any test. These tubes contain only the radioactive tracer and are used as a comparison to all other tubes.

Blank Tubes - 2 Types:

- Standard "O" Tubes
- NSB-Non-Specific Binding Tubes

Blanks: Blanks, or solutions containing only portions of the assay system, are often necessary in the assay. An antibody blank contains labeled ligand (Ag^*), antibody, and antigen-free serum. Its use allows assessment of the antibody - labeled antigen interaction as well as the efficiency of the separation step. A second blank consisting of labeled ligand and antigen-free serum (without antibody or unlabeled ligand) gives information about the non-specific precipitation of labeled hormone and the trapping of "free" ligand in the bound fraction.

Standards or Calibrators

Known amounts of different concentrations used to analyze and compare with pt. unknowns and controls.

The purposes of standards are 1) to establish a curve of dilutions on known amounts of substances to which the unknowns may be compared, 2) to ensure a stable interassay comparison, and 3) to compare the results obtained with other laboratories or established quantities in other assay systems.

Controls

A control may be a standard or any known concentration of test material that serves as a comparison between successive runs (inter-assay), whereas the usual standard involves varying concentration used to evaluate individual samples within a single run (intra-assay). Controls may vary in concentration ranges so that high, medium, and low ranges are covered. Controls and standards are solutions that contain known amounts of material, but their end uses may differ.

The manufacturers recommend levels of control Sera for each different type of RIA assay. This is commonly referred to as target values.

These controls are used as a means of setting individual precision limits of reproducibility from day to day on specific tests being performed.

Sources of Control

- **Pooled Serum**—Manufacturers may send a laboratory a serum containing many different Ag (Antigen) which are to be used for numerous RIA assay procedures.
- **Commercial**—A serum containing only one specific type of control assay.

External Surveys used as control measures for laboratory assay quality control.

College of American Pathologists and the American College of Nuclear Physicians help laboratories establish and maintain quality control. JCAH of hospital may waive a formal evaluation of an RIA if they are accredited by either organization.

The laboratory receives monthly unknown control serum from these bodies and reported their results quarterly. A composite answer from all reference labs that comply will provide verifiable information pertaining to controls used.

If the laboratory is significantly off composite control answer, they will have to document corrective actions to alleviate the problem.

Center for Disease Control

can be utilized as stop-gap for new procedures or new kits. Example: Can provide positive (negative) serum samples for new AIDS tests on the market.

Pt. Unknown Sample

These are the serum from the patient. One wants to find their actual value of antigen in body. These values are determined by interpolating counts from patient test tubes and comparing them with counts of known standard values of concentration.

Procedure Evaluation and Validation

Accuracy

Closeness to true *or* real value

In our RIA tests the *true values* are the standards or calibrators provided to labs by the manufacturer of these kits.

These reference standards are weighted and measured by the National Board of Standards which defines what calibrators are acceptable and will be allowed to be used.

The National Institutes of Health and the World Health Organization manufactures some standards that are used as reference standards. Kits produced by RIA manufacturers need to meet the criteria and specifications set up by one of these bodies.

Recovery Studies

When a manufacturer sends a laboratory a known amount of an antigen or reagent. The RIA lab does not know the true value and must test for the amount of antigen and recover the quantity. They then send in their analysis to the manufacturer to see how close they were to the "true value."

Precision

Degree of agreement of repeated measurement of a quantity *or* "reproducibility." Two types:

- **Within Assay Run**—In any particular RIA test, all reagents are run in duplicate tubes. A coefficient of variance value of less than 10% is acceptable.
- **Between Assay Runs**— A comparison of standard curve from one assay run to another. Secondly, a cumulative data collection of all high, medium and low controls run on that type of assay.

Sensitivity

Minimum quantity detectable

The manufacturer in his packet insert will describe and analyze the smallest value that can be accurately interpreted and reported out for a particular assay. This is usually described in terms of some value (B) compared to the standard "O" value (B_0) - B/B_0 .

Specificity

Capacity to discriminate antigens of similar structure Can be described and analyzed in terms of:

- **Dilutional Parallelism** – when a laboratory dilutes the pt. samples and attempts to see if and/or where the ratio of resultant values changes significantly.

	Cts
Example: Dilution 1:1 - 10,000	
1:2 - will cts be cut in half?	
1:3 - will cts be cut by one-third?	
1:4 - will cts be reduced by one-fourth?	

- **Cross Reactivity**—asks the question: What other similarly structured antigens will interfere with analysis of a given assay?
- **Non-Specific Binding**—NSB tubes when labeled ligand (Ag^*) and antigen-free serum (without antibody or unlabeled ligand) are put together in the same test tubes. The lab is looking for information about the non-specific precipitation of labeled hormone and the trapping of "free" ligand in the bound fraction.

Other Considerations when evaluating procedures include:

- **Cost**—Is it too expensive to run this kit in your laboratory?
- **Personnel Time**—Do you have the workers that have sufficient time to run this testing procedure?
- **Equipment Required**—Do you have all the necessary materials available?

- **Manufacturers**—Are they reliable, established, reputable?
- **Service**—Can they supply the kits and materials needed?

REVIEW

Section 1

1. Discuss the purpose of:
 - a. Recovery study -
 - b. Dilutional parallelism -
 - c. Cross-reactivity -
2. Explain why these components are utilized in an RIA test.
 - a. Total counts tubes _____
 - b. Blanks _____
 - c. Standards _____
 - d. Controls _____
 - e. Pt. unknown _____

Section 2

1. The property of antisera whereby antibodies react with two or more antigens of similar structure and which reduces the specificity of the assay is known as
 - a. sensitivity
 - b. cross-reactivity
 - c. avidity
 - d. antigenicity
2. Controls need to
 - a. be part of standard curves
 - b. set laboratory acceptable levels of reproducibility
 - c. be able to induce formation of (Ab) or (Ag)
 - d. all of the above

3. Cross-reactivity results from some (Ab) molecules low avidity for (Ag) and being unable to discriminate between different (Ag) because of their structural similarities.
 - a. True
 - b. False
4. Cross-reactivity is
 - a. not desirable in RIA
 - b. similar to haptens
 - c. used as reference standards
 - d. all of the above
5. When running a standard RIA test, what goes into the total count tubes?
 - a. standard O only
 - b. tracer and Ag-free serum
 - c. tracer and standard O
 - d. tracer only

Section 3

=====

T or F

- ___ 1. *The total cts tubes are the second blank tubes consisting of labeled ligand and antigen-free serum.*
- ___ 2. *Calibrators can be used to ensure a stable inter-assay comparison and compare results obtained with other laboratories.*
- ___ 3. *Two sources for control are the pooled serum and the Center for Disease Control.*
- ___ 4. *The College of American Pathologists work as a stop-gap for new procedures or new kits. (Example: provide positives for new AIDS tests)*
- ___ 5. *A precision test within assay run is a comparison of standard curves from one assay run to another.*
- ___ 6. *Sensitivity can be analyzed by comparing some valve (b) to the standard "O" valve.*
- ___ 7. *Non-specific binding is analyzed by using dilutional parallelism.*

- 8. *Cross-reactivity is when similar structures interfere with analysis of a given assay.*
- 9. *Non-specific binding are test tubes that have only labeled Ag and antibody.*
- 10. *Most hospitals using RLA procedures send their results to the National Institute of Health for evaluation.*
- 11. *Precision is the capacity of Ag to discriminate.*
- 12. *Affinity is closeness to true value.*
- 13. *In most testing procedures, the order of analysis of test tubes is unimportant as long as the technologist knows which is which.*
- 14. *Controls are used to establish a curve of dilutions on known amounts of substances to which the unknowns may be compared.*

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Chapter 3

SEPARATION TECHNIQUES IN RIA

Objectives

- Understand the overall purpose and usefulness of any separation technique.
- Demonstrate a knowledge and understanding of the purpose and methodology of:
 - the absorption technique
 - non-specific precipitation
 - immunoabsorbent technique
 - sandwich technique
 - double antibody technique
 - magnetic separation method
- Be able to recall the uses of electrophoretic method and gel filtration separation techniques.

Separation Methods in Radioimmunoassay

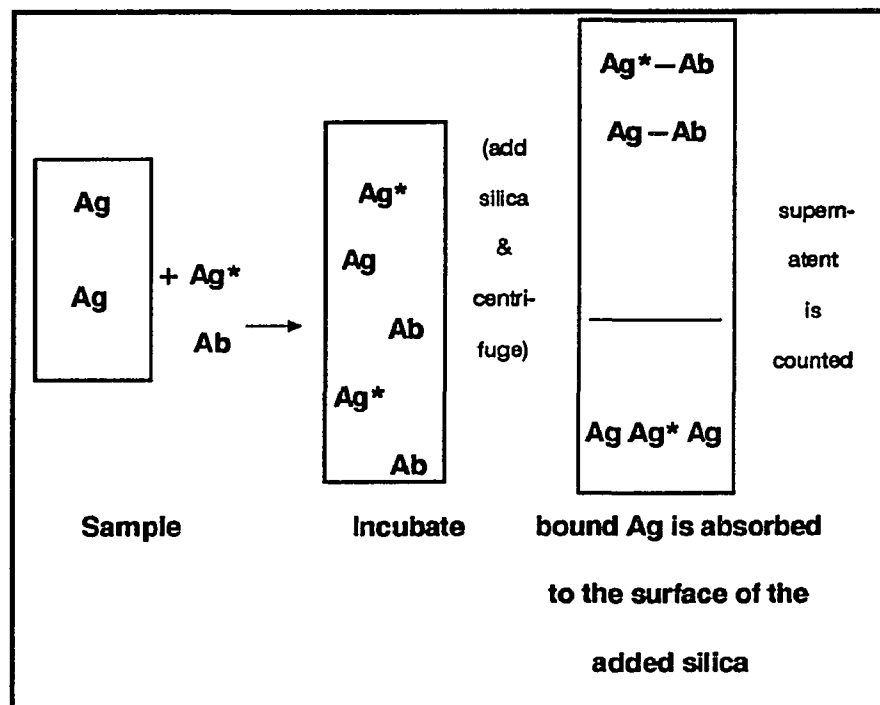
There are two basic methods of isotopic dilution performed in the presence of specific binding agents. **Radioimmunoassay** utilizes techniques that employ the principle of isotopic dilution of labeled antigens and unlabeled antigens in the presence of specific antibodies, with the desired end being the separation of bound, labeled ligand (Ag) from the unbound, free, labeled ligand (Ag). **Radioassay** utilizes specific binding proteins instead of specific antibodies.

The selection of a suitable separation procedure is based upon the properties of the antigen and nature of the biological material to be assayed. Successful separation techniques should be rapid, inexpensive and simple. Also, common available reagents should be used and there should be a minimal margin for error in the procedure.

There are a number of separation techniques available, each of which is discussed in the following paragraphs:

Absorption Techniques are based on the removal of the unbound, labeled ligand (Ag) from the system after the conclusion of the reaction. Many physical and chemical factors determining the absorption, including size, charge of antigen, composition and concentration of other proteins present pH and ionic strength of the solution. The advantage of this technique is the reduction of the concentration of free antigens to zero very quickly, thus eliminating kinetic influences that would cause dissociation of the complex.

Some common absorbents are charcoal, talc, and silicates. A common characteristic shared by all of the absorbents is rapid binding of unbound Ag / Ag^* . Silicates specifically are of value because of their ability to absorb peptide hormones. Silicates with high silica content are added to the incubation period or phase. The absorption is rapid and centrifugation is simple, the end result of centrifugation being the packing of silica on the bottom of the test tube.



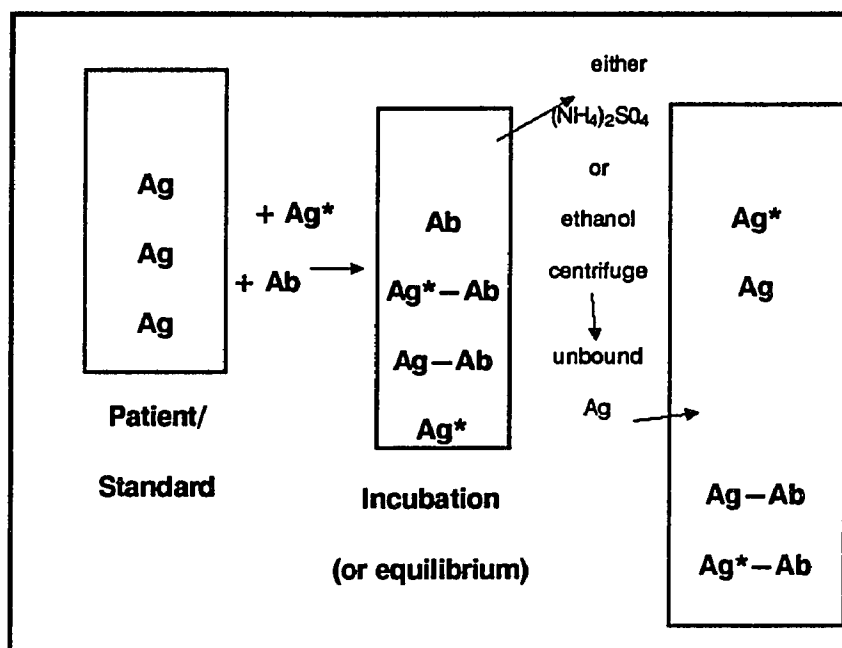
Separation of bound from free labeled Ag is the process known as the **Electrophoretic Method**. This method uses differential migration in an electric field to accomplish the separation of labeled Ag from the Ag-Ab complex.

Chromatoelectrophoresis depends initially on placing a sample containing 1) undamaged, free hormone, 2) bound hormone, and 3) damaged hormone on a paper strip that demonstrates selective preference for the absorbent of the labeled, undamaged hormone.

The labeled, undamaged hormone is returned at the origin, while other components migrate under the influence of buffer flow of evaporation and the electric field on the strip. The entire process, though, has some major disadvantages: it is too complex and time consuming, needs a high voltage, and must be performed under cold temperatures.

Non-Specific Precipitation is based on the principle that at critical concentration of inorganic salt or organic liquid, the Ab (globulins) become insoluble, while free Ag remains in solution. The procedure precipitates all Ab present including labeled Ag and Ab attached.

Separation of both free fraction of labeled Ag and bound can be achieved by filtration or centrifugation.

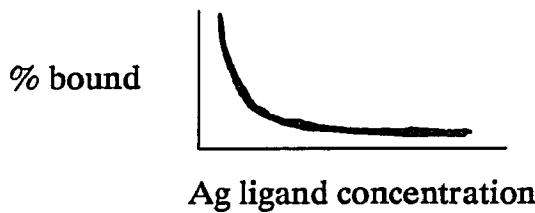
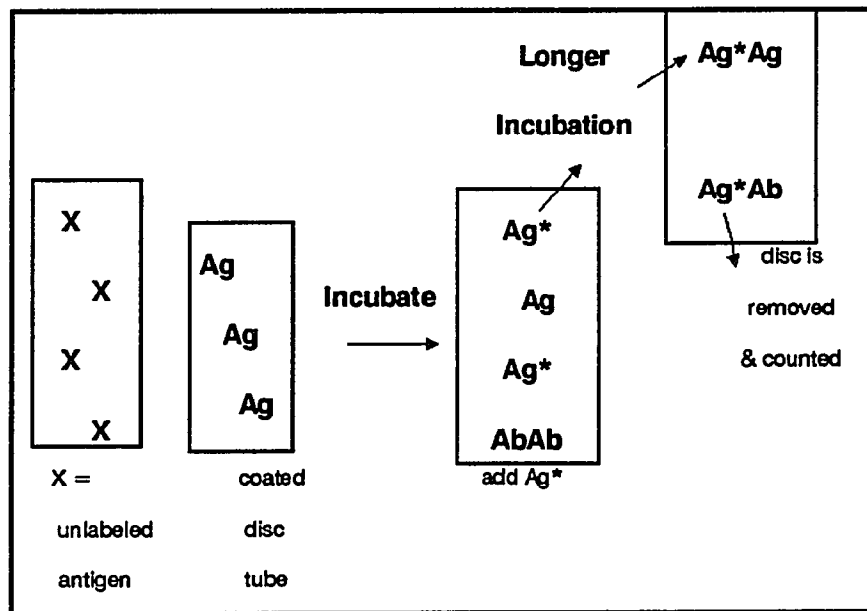


What does **precipitated** mean? It means to cause a substance to settle (heavier particles) in solid particles.

Gel Filtration, another separation technique, separates materials according to size by the elution of these materials through beds of porous beads. The adjustment of various sizes and types of molecules can be done by correcting the pore size distribution of the beads.

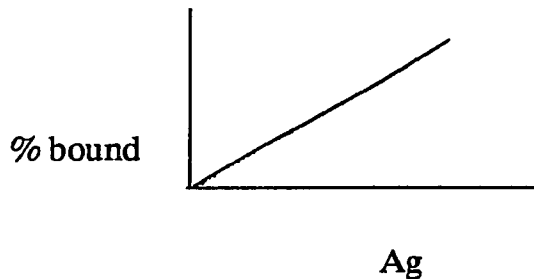
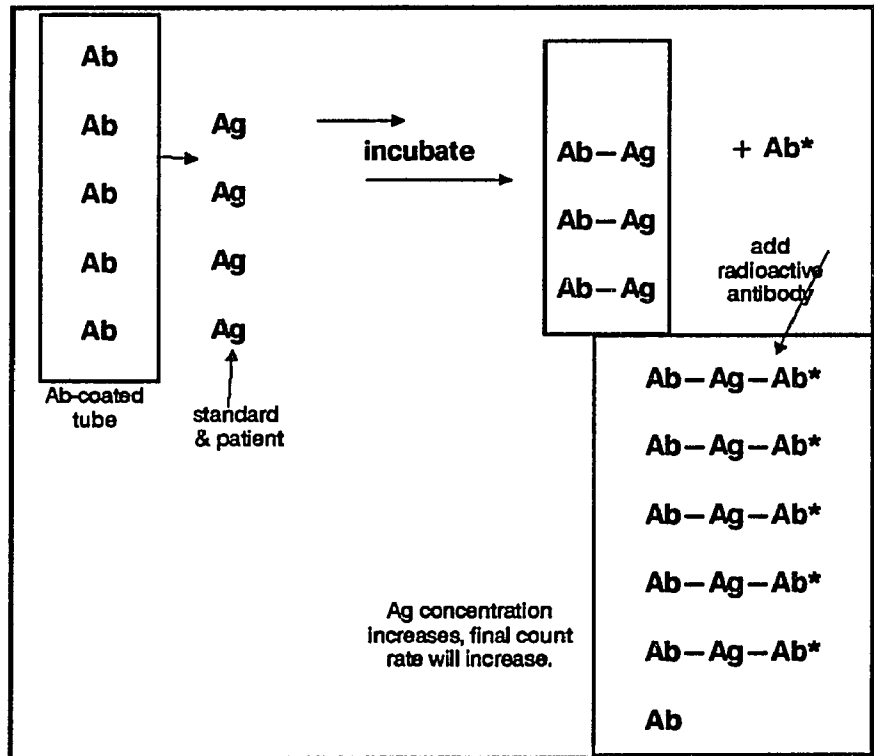
The incubated mixture can pass through the column in such a way that the bound portion of the Ag-Ab complex is eluted, leaving the unbound Ag in the resin.

The **immunoabsorbent** technique is similar to the solid phase technique; the test tubes are manufactured labeled with Ab. In the immunoabsorbent technique, an insoluble **polymer** is coupled to an Ag binding agent. A polymer is a compound of high molecular weight derived either by a combination of smaller molecules or by condensation of small molecules (eliminating the OH- molecule).



(High count rates reflect low *unlabeled Ag* concentration.)
 The Ab is absorbed on polyethylene or polypropylene tubes.

Another useful method is known as the **Sandwich Technique** and is used for the detection of hepatitis B and viruses. A plastic tube is pre-coated with a specific antibody. A standard or unknown sample is added to the Ab coated tubes and allowed to incubate. If the antigen is present, it will react with the specific antibody sites. After incubation, the labeled Ag is added and is followed by a second incubation. The tubes are then washed and counted and any unbound radioactivity is washed away. This is an example of a **noncompetitive binding assay**.



(Direct relationship in the noncompetitive binding assay)

The **Double Antibody Technique** utilizes a second Ab to precipitate the initially formed Ag-Ab complex.

Ag	Ab-Ag	Ag-Ab₁-Ab₂
Ab₁ + Ag*	Ab-Ag	Ab₂ Ag*-Ab₁-Ab₂
Ag	Ag-Ag	
specific Ab	competitive binding assay	double antibody separation

The second antibody is capable of recognizing the antigenic sites on the first antibody molecule (Ab₁).

Ab₁-from a small animal such as a guinea pig or rabbit

Ab₂-from a large animal; uses preparation of immuno-globulin against those of the small animals

The advantage of the double antibody technique is in its effective separation of free from bound hormone. This technique differs from non-specific separation in that there is little precipitation of the free, unbound, labeled Ag in the Ag-Ab₁-Ab₂ complex.

The **Magnetic Separation Method** also utilizes two antibodies, as well as **polymer beads** with the capability of being magnetized. When a magnetic field is introduced, separation can begin. Antibody A is bound to the Antibody B (which is attached to the beads), and it is this process which allows the separation of free and bound Ag to take place. The beads move to the bottom when the magnetic field is applied.

The advantages of this process are reduced incubation periods, centrifugation is not longer needed, and it is simple.

REVIEW

Section 1

1. Briefly describe these RIA separation techniques (choose only 3 out of 5):

a. Absorption technique:

b. Non-specific precipitation:

c. Immunoabsorbent:

d. Double antibody technique:

e. Magnetic separation technique:

2. From one (1) of the *above*, draw out the process and discuss each aspect. Is this technique competitive or non-competitive and why?

Section 2**T or F**

- 1. *During non-specific precipitation, the bound portion is on the bottom of the tubes.*
- 2. *In non-specific precipitation, the principle that at critical concentrations of inorganic salt or organic liquid the (Ab) concentration becomes insoluble while (Ag) remains in solution.*
- 3. *The absorption technique utilizes a paper strip that demonstrates selective preference for the absorbent of the labeled.*
- 4. *In non-specific precipitation the unbound Ag is on the bottom of the test tube after separation.*
- 5. *The magnetic separation method utilizes a double antibody technique.*

Section 3**Multiple Choice**

1. When the final product after separation is Ab-Ag-Ab* and there is a direct relationship between counts and antigen, the separation technique used was
 - a. double antibody
 - b. immunoabsorbent
 - c. sandwich
 - d. magnetic
2. A technique that utilizes a polymer, removes a disc to count, and is an indirect measurement of antigen is
 - a. sandwich
 - b. absorbent
 - c. double antibody
 - d. immunoabsorbent
3. In the absorption technique, the most common material used is
 - a. polypropylene

- b. $(\text{NH}_4)_2\text{SO}_4$
 - c. silicates
 - d. peptide proteins
4. Which does not belong? Selection of suitable separation procedure is based upon
- a. being rapid
 - b. inexpensive
 - c. fairly simple
 - d. commonly available reagents
 - e. none of the above
5. ___ is absorbed to the surface of the added silica material.
- a. Unbound Ab
 - b. Radioactive Ag
 - c. Radioactive Ab
 - d. Unbound Ag
6. The sandwich technique is used for
- a. detection of hepatitis B antigens
 - b. non-competitive binding
 - c. Ab coated tubes
 - d. all of the above
7. Chromatoelectrophoresis, solid-phase, double-antibody immunoprecipitation, filtration, and centrifugation are all methods used to
- a. iodinate the labeled antigen
 - b. separate bound from free antigen
 - c. purify the labeled antigen
 - d. produce antibodies for use in the assay

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Chapter 4

STATISTICS IN IN VITRO LABORATORY TESTING

Objectives

Know the meaning and uses of:

- the mean
- frequency distribution
- normal distribution
- median
- mode
- range
- standard deviation
- percent error
- confidence intervals
- normal ranges
- fitting the standard curve

Statistics Needed

In vitro RIA tests depend upon statistics principles very heavily. Some important questions that need to be considered are:

- How long should a sample be counted?
- What do you do with a sample with low count rates and high background counts?

- When is the quality of results compromised?
- How do we know the instruments are accurate and reliable?
- How long can we use a test kit without compromising our test results?

Central Tendencies

Mean: $\frac{\text{the sum of all numbers}}{\text{number of items}}$

Frequency distribution: examination of all the different individual counts

Normal distribution: resembles a bell-shaped curve, where the majority of counts are clustered in the middle

Median: middle point

Mode: most frequent occurring value

Measure of Dispersion

Range: difference between largest minus the smallest

Standard deviation:

$$\frac{\sum(Y-\bar{Y})^2}{n-1}$$

Fitting the Standard Curve

The standard curve is estimating point value samples of known concentration of the material under study. Then under conditions identical to those used by patient samples from known results, curves can be constructed that allow estimation of patient values. The patient values are called interpolated into the actual known standards.

The Use of the Normal Distribution

Example of its use in statistics:

Number of Cts	(1SD) 68%	Range	Percent Error
100	+ 10	90-110	10%
1,000	+ 32	968-1,032	+ 3.2%
10,000	+ 100	9,900-10,100	1.0%
100,000	+ 317	99,683-100,317	+ 0.3%

In routine N.M., the decision must be made how many cts. be accumulated to have reasonable confidence that cts. within acceptable degree of error.

95% confident - within + 2% error

10,000 cts. - represent 68% chance of 1% error

2SD = 95%

3SD = 99%

$\frac{2SD}{+200}$

$\frac{\text{range}}{9,800-10,200}$

$\frac{\text{percent error}}{+ 2\%}$

Regardless of time required, 10,000 cts. should always be accumulated.

Relationship between number of cts, recent errors, and desired confidence level:

$$\frac{V}{\sqrt{N}} = \% \text{ error} \quad 68\% \text{ confidence } N = 10,000 \quad N = \# \text{ of cts.}$$

$$95\% \text{ confidence } N = 40,000 \quad V^2$$

$$99\% \text{ confidence } N = 90,000 \quad V^2$$

68% - preliminary studies

95% - routine studies

99% - research studies

Suppose: We wanted to know + 1% error or 99% confidence.

How many cts. would be needed?

$$(n) = 90,000 \times (1)^2 = 90,000 \text{ cts.}$$

95%, 2% error

$$(n) = 40,000 \times (2)^2 = 10,000 \text{ cts.}$$

As the number of cts. (n) collected increases, the relative percent error decreases.

Error: *The deviation of an observed value from the absolute or true value:*

- Determinate errors - equipment malfunction
- Indeterminate or random errors - operator has no control

(See lab exercises on normal distributant percent error)

Quality Control and Normal Ranges

The results obtained in any type of lab must be valid. Proved Q.C. measures should be routinely used in order to ensure valid results.

Q.C.: Any and all measures taken to ensure a valid result.
Precision, accuracy, reproducibility, standard controls, sensitivity, specificity

REVIEW

Section 1

Based on the following set of count rates, find the mean, median, mode, 1SD, 2SD, 3SD

1. 10,352
2. 9,681
3. 10,945
4. 11,842
5. 10,369
6. 9,677
7. 10,001
8. 10,356
9. 10,945
10. 10,258

Section 2

Multiple Choice

1. The minimum number of counts for any sample testing in nuclear medicine is
 - a. 5,000 cts./min
 - b. 10,000 cts./min
 - c. 10,000 cts. regardless of the time required
 - d. depends upon the amount of efficiency one desires

- d. depends upon the amount of efficiency one desires
2. The number of counts needed in routine studies can be derived from
 - a. $N = 10,000/V$ squared
 - b. $N = 40,000/V$ squared
 - c. $N = 90,000/V$ squared
 - d. depends upon type of scan one is performing
3. If a technologist is receiving 900/ct./sec on a patient for a spleen scan, how long must he count to be + or - 2% error: _____.
4. As the number of counts in any test increases,
 - a. the relative percent error increases
 - b. the relative percent error decreases
 - c. percent error will not change
5. If a test is performed in nuclear medicine department with 5,000 ct./sec., how long must the test take in order to be 95% confident and + or - 2% error:
 - a. 20 seconds
 - b. 2 minutes
 - c. 2 seconds
 - d. 5,000 of x 200 seconds

Chapter 5

BASIC IN VITRO EQUIPMENT AND DETECTION INSTRUMENTATION

Objectives

- Have a working knowledge of pipettes, vortexers, centrifuges, refrigerator, timers, test tubes, and incubators in a RIA laboratory setting.
- Know the basic principles and concepts of a gamma well counter and a liquid scintillator, including their applications and purposes.
- Understand the counting of iodine I-125 and its many inherent problems.
- Demonstrate knowledge and understanding of the various components of gamma scintillation detection and liquid scintillation.

Basic In Vitro Equipment



Pipettes

A device which no RIA laboratory can do without is the pipette. Pipettes are used to withdraw specific quantities of solution, as a sample or ingredient of an RIA kit. Pipettes are available in various sizes and volumes or they may be equipped with a control which can be set to control the amount of solution to be collected. The tips of the pipettes also vary in size and are usually made of glass or plastic, and

most are disposable. Most of these devices are semi-automatic; that is, they are operated by pushing a (button) at the top of the pipette while in solution and releasing to remove the pipette. The tip is then placed into the desired tube and the (button) pushed again to release the solution. Pipettes are also available which have rubber suction devices or designed so that the user must use mouth suction, but this kind is not appropriate for collecting samples or radioactive materials due to the inherent danger of swallowing or transferring contaminated solutions. Automatic pipettes are also used in those laboratories which do large quantities of tests.

Vortexers

Since most tests require the mixing of samples and reagents, electronic instruments called vortexers have become indispensable. The test tubes can be placed in the vortexer for a predetermined time (some require that the tube be held at the top until the mixing is done) to assure adequate agitation and to increase the chances of component interaction.

Centrifuges

The centrifuge is used for the separation of elements, namely the supernate and precipitate. The tubes are placed in the centrifuge, being careful to balance the weight from side to side so as not to cause uncontrolled vibration of the instrument. Care must also be taken to control the time, temperature (if possible; some centrifuges do not have this capability), and speed inside. Its principle is based on that of centrifugal force, with the heavier elements ultimately ending up at the bottom and the lighter elements on top. Most will have controls which the operator can set to a desired number of revolutions per second or minute.

Refrigerators

Refrigeration is essential for the storage of certain kits which require temperatures below normal room environment in order to maintain their stability. Certain samples, such as blood, also require refrigeration to keep them fresh and usable for a longer period of time. These refrigerators must be lead-lined (due to the radioactive materials being stored inside) and should have the capability of being locked for

safety and protection. Thermometers should also be easily accessible and monitored to avoid the costly destruction of samples and kits.

Timers

The use of timers may be necessary in some laboratories should some instruments not contain one. Devices such as vortexers and centrifuges may already have timers; but should they not or this component is malfunctioning, a timer would be essential.

Test Tubes

Test tubes come in various sizes and materials. Plastic tubes are lighter in weight, basically non-destructible, and are less expensive than glass since disposability can be important. Test tube holders and racks are also available in various sizes, materials, and capacities. Tubes and racks should be able to be identified easily and/or labeled appropriate (by using an easily removable marker if nondisposable, for example).

Incubators

An incubator is used should the test tube require a constant temperature.

In Vitro Detection Instrumentation

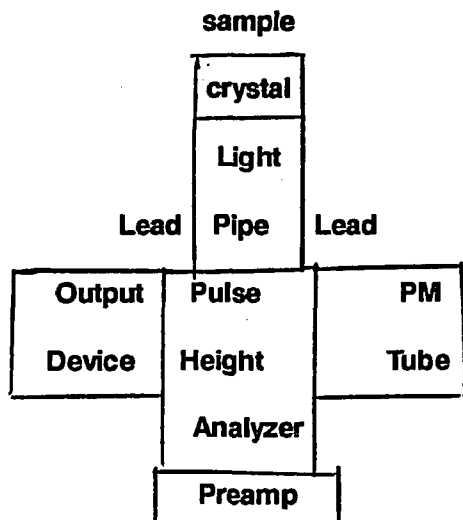
- Gamma well counters
- Liquid scintillation

Primary strength of RIA is its ability to measure extremely small quantities of material because of the presence of radioactivity. Radiation means spontaneous decomposition of a nucleus that is accompanied by emission of radiation types common in RIA, gamma rays, x-ray, beta emissions.

Gamma (γ) is most desirable, as contrasting with beta radiation I-125, I-131, Co-57 are the most commonly used gamma radionuclides. Solid scintillation detector (well counter) can be used.

For some applications the counting of beta particles must be performed. The beta emitting radionuclides commonly used tritium ^3H and ^{14}C - liquid scintillation counting is most frequently used.

Detection of Radiation by NAI System



This type of detection is considered two (2) π . It relates the positioning of the sample within the NAI counter.

NAI Crystal (TL) Thallium Impurities

Gamma Detection System

Iodine is an element of high (Z) number the properties of an absorption event by photoelectric absorption is greatly enhanced.

Thallium impurities— When a (γ) or (x-ray) photon is absorbed, visible light is emitted by the crystal. The amount of light emitted is proportional to the energy given up to the photoelectron or the recoil by the incident photon.

The technologist must select the proper window in the pulse height analyzer for the specific energy range of the radioisotope one wishes to count. If PHA accepts a pulse, this pulse is then passed to the output device — commonly scaler. Simply sums the cts. which have been passed by the PHAs. The output of the scaler with modern instruments usually prints the results on some form of hard copy output-coupled

by computer that will give both total cts. and ct. rate accumulated.

If more than one isotope is counted, two PHA are needs.

Example: B-12, folates (CO57, I125)

Instrument should be calibrated separately for each radionuclide measured. 125I, 131I, 57CO

Counting of I-125

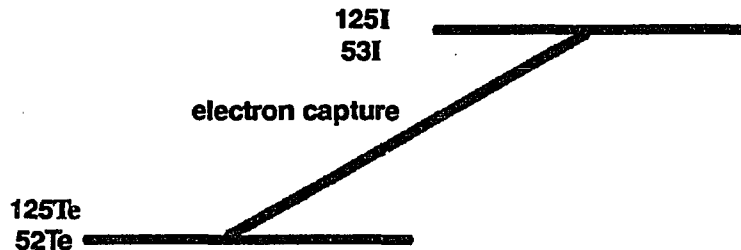
Counting iodine 125 has many inherent problems. First, because of its very low energy, it has many self-absorption problems, which stem from the fact that the energy may be absorbed in the sample if the sample is too large, if the container holding the sample is too thick, if its position within the well is improper. All of these situation may drastically reduce the count rate. Second, setting up the spectrum has a unique problem.

When the spectrum of 125I from a sodium iodide detector is plotted, the spectrum shows two basic energy peaks. One peak is at approximately 28 KeV, and the second peak is at approximately 60 KeV. The first primary energy peak is composed of basic characteristic X-rays and the primary 35 KeV energy peak. Because of the inherent resolving problem of a sodium iodide detector, the individual peaks from the X-ray and the primary gamma ray cannot be resolved. Thus a resulting peak of 28 KeV is observed. The second peak, which is sometimes called the sum-peak, is the sum of the characteristic x-ray from 125I and radiation from the daughter product of 125I (tellurium 125). The resulting sum is approximately 60 KeV. When 125I is counted, the spectral settings either can be set to count just the primary peak, which is a larger peak, or it can be set up to count both peaks.

The Decay Scheme of I-125 Looks Like:

The Decay Scheme of I-125 Looks Like:

Electron Capture: $e + p \rightarrow n + \gamma + \text{energy}$



ENERGY PEAKS:

Primary peak (larger)

28KeV

60KeV

Liquid Scintillation Counters

Used for low energy beta emitters, sample is actually dissolved in the scintillation itself.

Advantage—Excellent geometry. Literally surrounded by scintillator (4π) geometry.

Disadvantage—Problem of solubility of material to be counted. Sample cannot be brought into solution. Counting by liquid scintillation becomes more difficult.

Four Components:

- **Solvent:** toluene and diozane. This is an aromatic solvent.
- **Solubilizer:** protosol, hydroxide. This requires treatment with relatively strong agents. It functions to dissolve the sample for biological liquid counting.
- **Fluor:** a primary and secondary. **Primary:** (PPO) 2,5 diphenyloxazole. Light emitted by primary fluor is a wavelength not detected by some types of P.M. tubes. **Secondary:** (POPOP) 2,5 phenoyloxade benzene. The second fluor is used to shift the wavelength to emit light.

- **Radioactive sample:** usually H-3 or C-14 **Quenching Problems**—Anything that interferes with the visualization by P.M. tubes of the emitted light.
Chemical Quenching—Scintillation solution absorbs the energy of radiation without the emission of detectable light (inorganic acids, organic compounds).

Color Quenching—Samples that are red or yellow, such as blood or urine, may cause problems. Red color absorbs the bluish light emitted by fluor, causing reduction in amount of light reaching P.M. tubes and reducing count rates.

Section 2

1. The principal gamma energy and half-life of I-125 is
 - a. 159 KeV, 13 hours
 - b. 364 KeV, 8 days
 - c. 173 KeV, 2.8 days
 - d. 35 KeV, 60 days
2. I-125 shows two basic energy peaks, 28 KeV and 60 KeV. 28 KeV is based on
 - a. characteristic X-ray and primary energy peak
 - b. inherent resolving problem
 - c. I-131 interference
 - d. all of the above
 - e. a and b only
3. The most common causes for counting failure include
 - a. high voltage drift
 - b. cracked NaI crystal
 - c. preamp to scaler malfunction
 - d. loss of control of window setting
 - e. none of the above
4. Geometric factors do *not* include
 - a. volume of sample solution
 - b. type of NaI arrangement (side window)
 - c. position of sample in counter
 - d. none of the above

Section 3

T/F Section

- 1. *A gamma counter works in this sequence:*

Nal → pre-amp → P.M. tubes → PHA → scaler

- 2. *The primary peak (larger) for I-125 is approximately 60 KeV and decays by electron capture.*
- 3. *I-125 window settings are 25-60 KeV.*
- 4. *Liquid scintillation counters are used to count beta emitting radionuclides such as I-125, I-131, and C-14.*
- 5. *I-125 has a sum-peak of approximately 35 KeV and decays by positron emission.*

It can be predicted that RIA and related techniques will be applied increasingly to the detection of active infectious disease. Straus, et al (1981), have reported an RIA for a tuberculo-protein that can make the diagnosis of active tuberculosis more rapidly and in a much less expensive, simpler, and far safer fashion than with the use of classic microbiologic methodology (p. 3214). Even were this technology applicable only to the diagnosis of tuberculosis, it would remain a major breakthrough. However, it should serve as a model for the development of assays for proteins associated with other slow-growing organisms, the diagnosis of which may be difficult with standard microbiologic methodology.

Future of RIA

This review of the development of a small sampling of applications of RIA emphasizes the fact that in science new truths become evident when new tools become available. For more than a quarter century, RIA has been an important tool, fruitfully employed in diverse areas of biomedical investigation and clinical medicine. Can we predict what the relative usage of radioactive and non-radioactive labels in immunologic methods for diagnosis (immunodiagnos-tics) will be in the future? A Wall Street investment concern has attempted such a prediction (Dunne, 1981). They have estimated that in 1980 the market segment for RIA in

immunodiagnosics was \$332 million, for non-radioisotopic therapeutic drug monitoring \$43 million, and for all other non-radioisotopic immunoassays \$28 million; in 1990, they predict markets of \$785 million, \$625 million, and \$838 million respectively for these three methodologies. Thus, from a commercial point of view RIA usage is expected to more than double during the decade of the 1980s, but its fraction of the total immunodiagnosics market is expected to drop from over 80 percent to about 35 percent. Why? First of all, many of the new applications simply do not require the exquisite sensitivity of RIA. For instance, in therapeutic drug monitoring the concentrations being determined are generally a million-fold higher than the concentrations of peptide hormones. Furthermore, assays for drugs generally need not be quantitative over a multiple hundredfold range. It is often necessary only to determine whether the concentration is high enough to be efficacious and not so high as to be toxic. In the field of infectious diseases, where immunodiagnosis will play a major role, the choice between radioisotopic and non-radioisotopic methodology again would depend on the sensitivity required; in the presence of massive infection, the massive infection the sensitivity of RIA is not likely to be necessary. However, in a number of instances, those promoting non-radioisotopic labels are not necessarily suggesting that these have any technical advantage, but rather that

regulatory procedures and fear of radiation at any level would make radioisotopic label less desirable. Nonetheless, RIA is likely to remain the method of choice for what has been its first application, the measurement of peptide hormones in plasma and other body fluids, since their concentrations are well below $10^{-10}M$, and the sensitivity of RIA is essential (Dunne, 1981).

RIA and related methodologies have opened new vistas in science and medicine. RIA is now used in thousands of laboratories around the world to measure hundreds of substances of biologic interest. Even now, a quarter century after the introduction of RIA, there remain many additional fields that can be explored with its help.

Review of In Vitro Literature

A selective review of the nuclear medicine In Vitro literature was performed on over 300 articles, text books, and manuals. This researcher examined the literature and categorized the materials into a content analysis chart. The five areas were: Level of Material, Type of Material, Possibility of Providing Laboratory Exercises, General Principles, Concepts Discussed, and Specific Testing Procedures Mentioned.

The following is presented as a content analysis chart and a summary of resultant values obtained.

Content Analysis of In Vitro Nuclear Medicine

Literature. Column indexes and abbreviation conventions:

Column 1, Bibliography

Column 2, Level of Material

under = undergraduate

grad = graduate

tech = technical

Column 3, Form of Material

Column 4, Laboratory Exercises Provided

Column 5, Concepts Discussed

Column 6, Specific Testing Procedures

01	02	03	04	05	06
Berson, S.A., and Yalow, R.S., editors: Method in investigative and diagnostic endocrinology, vol. 2A and 2B, Amsterdam, 1973, North-Holland Publishing Co.	tech;text				endocrine tests
Cameron, E.H. D., Hiller, S.G., and Griffiths, K.: Steroid immunoassay, Cardiff, U.K. 1985, Alpha Omega Publishing Co.	tech:arti- :cle			basic principles	steroid testing
Diczfalusy, E., editor: Immunoassay of Gonadotrophins. Karolinska Symposia No.1, Acta Endocrinol. (eds) 142(suppl.): 1979	tech:arti- :cle			basic reactions	gonadotropins
Hayes, R.L., Goswitz, F.A., and Pearson Murphy, B.E., editors: Radioisotopes in medicine; in vitro studies. A.E.C. Symposium Series 13, Oak Ridge, Tenn., 1978; U.S. Atomic Energy Commission	grad:arti- :cle			radiolabeling; basic principles/chemical reactions	
International Atomic Energy Agency: In vitro procedures with radioisotopes in medicine, Vienna, 1970, I.A.E.A.; International Atomic Energy Agency: radioimmunoassay and related procedures in medicine, Vienna, 1984, I.A.E.A	un- :der	text	exercises	basic procedures	
Jaffe, B.M., and Behrman, H.R., editors: Methods of hormone radioimmunoassay, New York, 1984, Academic Press Inc.	tech:text			basic principles; concepts	
Kirkham, K.E., and Hunter, W.M., editors: Radioimmunoassay methods, Edinburgh, 1971, Churchill Livingstone.	tech:text			methodology, equipment kit; evaluations	
Luft, R., and Yalow, R.S., editors: Radioimmunoassay; methodology and application in physiology and in clinical studies, Stuttgart, 1974, Georg Thieme Verlag	grad:text			many principles; concepts	types of tests
Odell, W.D., and Daughaday, W.H., editors: Principles of competitive protein-binding assays, Philadelphia, 1971, J.B. Lippincott Co.	tech:text			basic principles	
Pasternak, C.A., editor: Radioimmunoassay in clinical biochemistry, London, 1973; Heyden & Son Ltd.	grad:text			basic concepts	bio-chemistry
Ranson, J.P.: Practical competitive binding assay methods, St. Louis, 1986, The C.V. Mosby Co.	tech:text			many principles; concepts	numerous tests
Rothfeld, B., editor: Nuclear medicine in vitro, Philadelphia, 1984, J.B. Lippincott Co.	grad:text			many principles; concepts	numerous tests
Sonksen P.H., editor: Radioimmunoassay and saturation analysis, Br. Med. Bull. 30(1): 1974.	tech:arti- :cle			separation technique	
Bassiri, R.M., and Utiger, R.D.: The preparation and specificity of antibody to thyrotropin releasing hormone, Endocrinology 90:722, 1972.	tech:arti- :cle			preparation, calibration	thyropin releasing hormone
Berson, S.A., and Yalow, R.S.: Kinetics of reaction between insulin and binding antibody, J. Clin. Invest. 36:873, 1957.	grad:arti- :cle			reactions	insulin

Berson, S.A., and Yalow, R.S.: Immunoassay of protein hormones; dependence of sensitivity of assay on energy of antigen-antibody reaction. In Pineus, G., Thimann, K.V., and Astwood, E.B., editors: The hormones, vol.IV, II D, New York, London, 1964, Academic Press, Inc., pp. 567-572	tech:arti- icle	basic Ig - Ab reactions
Berson, S.A., Yalow, R.S., Bauman, A., Rothchild, M.A., and Newerly, K.: Insulin- ¹²⁵ I metabolise in human subjects; demonstration of insulin binding globulin in the circulation of insulin treated subjects, J. Clin. Invest. 33:170, 1966	grad:arti- icle	basic reactions insulin
Bolton, A.E., and Hunter, W.M.: The labeling of proteins to high specific radio-activities by conjugation to a ¹²⁵ I-containing acylating agent, Biochem. J. 133:529, 1973.	tech:arti- icle	radiolabeling
Catt, K., Niall, H.D., and Tregear, G.W.: Solid phase radioimmunoassay of human growth hormone, Biochem. J. 100:31, 1966	tech:arti- icle	separation technique
Catt, K., and Tregear, G.W.: Solid phase radioimmunoassay in antibody-coated tubes, Science 158:1570, 1967	un- der icle	separation technique
Chard, T., Kitau, M.S., and Landon, J.: The development of a radioimmunoassay for oxytocin; radioiodination, antibody production and separation techniques, J. Endocrinol. 46:296, 1980	grad:arti- icle	radiolabeling separation technique oxytocin
den Hollander, F.C., and Schuurs, A.H.: Discussion. In Kirkham, K.E., and Hunter, W.M., editors: Radioimmunoassay methods, Edinburgh, 1971, Churchill Livingstone, p. 419	tech:text	methodology, basic principles
Desbuquois, B., and Aurbach, G.D.: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays, J. Clin. Endocrinol. Metab. 33:732, 1971.	tech:arti- icle	separation technique reaction concept peptide hormones
Ekins, R.P.: The estimation of thyroxine in human plasma by an electrophoretic technique, Clin. Chim. Acta 5:463, 1980.	tech:arti- icle	separation technique; calculation results T-4 test
Ekins, R.P.: Theoretical aspects of saturation analysis. In International Atomic Energy Agency: In vitro procedures with radioisotopes in medicine, Vienna, 1980, I.A.E.A., p.323.	tech:man- ual	exercise varied basic for use of: principles/ separation separation technique unique
Ekins, R.P.: Radioimmunoassay design. In Pasternak, C.A., editor: radioimmuno- assay in clinical biochemistry, London, 1975, Hayden & Son Ltd.	grad:text	interactions for reactions in chemical levels
Ekins, R.P., Newman, G.B., and O'Riordan, J.L.H.: Theoretical aspects of saturation and radioimmunoassays. In Hayes, R.L., Goswitz, F.A., and Pearson Murphy, B.E., editors: Radioisotopes in medicine; In vitro studies, A.E.C. Symposium Series 13, Oak Ridge, Tenn., 1978, U.S. Atomic Energy Commission, p.59.	tech:arti- icle	separation techniques

Chapter 6

EQUIPMENT QUALITY CONTROL

Objectives

- Know the three basic parameters of equipment performance evaluation:
 - Counting efficiency
 - Statistical bias
 - Background cts.
- Be able to demonstrate an understanding of the purpose of these 3 testing procedures.
- Be able to quantitatively analyze and set up these 3 testing procedures.
- Demonstrate a knowledge of normal ranges and their functions.
 - Be able to set up a data reduction system for:
 - point-to-point analysis
 - log-logit assays
 - single-point assays
- Know the different kinds of RIA testing procedures that match up to the appropriate data reduction methods.
- Understand how to assess antibodies, labeled ligands, standards and different RIA testing kits.

Equipment Performance Evaluation.

Three parameters can affect overall accuracy and/or reproducibility of test results:

- Counting efficiency (accuracy)
- Statistical bias (reproducibility)
- Background cts.

Ct. Efficiency: Number of cts. registered compared to the number of potentially effective emissions from the source.

$$\text{CPM} = \frac{\text{Ct. Per Min.}}{\text{Disintegrations Per Min.}}$$

The ratio should be nearly constant for a given detector, nuclide, sample size, base and window setting, gain and high voltage.

Ct.: A standard having a similar decay scheme. A long half-life and record the results (see lab exercise).

Statistical Bias: The most critical performance test of any counting equipment is when large number of samples are to be counted and compared to some standard value.

Test is performed to ensure that variation in a series of individual cts. are likely to be due to random nature of radioactive decay and not by other influences.

Using a single source (long - many weeks)

T 1/2 - ¹²⁵I ct. a series of 1 min. cts.

This set of cts. is compared to theoretical variation that one would expect from random sampling:

Chi-Square Test

$$\chi^2 = \frac{\sum (X - \bar{X})^2}{\bar{X}} \quad \text{-- Compare result to tabulated P values}$$

P values should fall between .10-.90 if outside these limits, the most common causes for non-reproducible counting failure:

- High volt. drift
- Cracked NaI crystal

- Loss of optical coupling between P.M. tubes and crystal
- Loss of control of window setting

Background Checks

Observed background to quickly alert for contamination or other radioactive sources in area.

Sample Collection

Most assays are performed on the blood components (plasma or serum).

Collection must be reviewed by specific assay protocol. Proper attention to timing of collection of samples TSH and cortisol is necessary to account for nature of the secretion of these hormones.

Posture may affect serum secretion, such as plasma renin.

Food intake may affect certain test results. Human growth hormone is markedly affected by stress.

Collection involves immediate clotting and centrifugation and possible freezing -20C for some specimens.

Normal Ranges

For any assay, should be established in each laboratory.

N.R. usually refers to healthy persons. It has become rather difficult to relate to natural phenomenon, such as aging, actives.

Statistical principles use 95% confidence limits to define normal population or test results.

Expected measurement for 1 individual in 20 to fall outside of the normal range.

Fairly large population must be sampled. Population should be illness-free (by as many criteria as possible) when defining the normal population.

Other factors: race, age, sex, etc.

Setting up Your Equipment Parameters

Background

In all cases background is subtracted automatically prior to print-out of counts or any calculations.

Window Settings

There are two preprogrammed isotope values which can be used to set the window, I-125 and Co-57. Ranges for these isotope values can be entered in one or two ways. The appropriate keys representing the isotope can be entered. Or the range can be entered by pressing the appropriate numeric keys. For example, the range for I-125 equals 15 to 70 KeV. By pressing the numeric keys then pressing the decimal point, the range is entered.

These isotopes have these equivalent ranges:

$$\text{I-125} = 15 \text{ to } 70 \text{ KeV}$$

$$\text{Co-57} = 45 \text{ to } 235 \text{ KeV}$$

When unknowns are run, the standard deviation and coefficient of variation are calculated as a function of dosage.

$$\text{Standard Deviation (SD)} = \sqrt{\frac{\sum (X - \bar{X})^2}{n-1}}$$

$$\text{Coefficient of Variation (\%CV)} = \frac{(\text{SD})}{\bar{X}} \times 100$$

$$\text{Mean} = \frac{\text{Sum of Individual Counts}}{\text{\# Replicates}}$$

$$\text{Chi-square} = \frac{\sum (X - \bar{X})^2}{\bar{X}}$$

Methods of Data Reduction

Protocol: Point To Point

Point to Point assays are entered similarly to log-logit assays:

- Total count tubes (if any).
- Non specific binding (NSB) tubes (if any).
- Zero standard (BO) tubes (if any).
- Lowest standard
- .
- .
- .
- .
- Highest standard. Point to Point assays can be run without:
 - Total count tubes
 - NSBs
 - Bos

Standard values for concentrations other than 0 (zero) must be entered in ascending order (from lowest to highest).

Respective counts for those standards can be entered in either a consistently ascending order or descending order. If the counts are not entered in consistently ascending or descending order, an error message will be printed: **RECHECK STNDS**. The assay calculations will revert to the counter mode and only counts will be printed.

The method of data reduction for Point to Point assays uses the respective averages from each set of adjacent standards to plot a series of straight lines.

Average counts from a given set of unknowns will then be read from a series of straight lines and the corresponding concentration is determined.

If an individual unknown tube has a count rate corresponding to a concentration that is less than the lowest concentration or higher than the highest concentration, it will be deleted. If replicates are being run and one or more tubes of a given sample are deleted, the calculation will continue based only

on the undeleted tubes. When this occurs the operator should validate the data.

Protocol: Log-logit (curve Fit) Assays

Log-Logit assays must include:

- Either a total count tube(s) and/or B₀ tube(s) (zero standard tubes).
- At least three standards other than zero.

Standard values (concentrations other than zero) must be entered in ascending order (lowest to highest concentrations). Assays must be loaded and run in the following sequence:

- Total count tubes (if any).
- Non specific binding (NSB) tubes (if any).
- B₀ (zero standard) tubes (if any).
- Lowest standard
-
-
-
- Highest standard

DATA REDUCTION

The following formulas are used in the data reduction process:

$$\text{Total counts} = \text{Free counts} + \text{Bound counts} + \text{NSB}$$

$$B_0 = \text{Bound counts at zero} - \text{NSB}$$

$$\text{Logit} = \ln \frac{y}{1-y} \quad \text{where } y = B/B_0 \text{ or } B/T$$

$$\text{Pipetting Error} = \sqrt{\frac{(SD)_T^2 - T}{T}}$$

where $(SD)_T^2 = \text{Variance of total count tubes}$

The curve fitting procedure is a log-logit transformation using all data points other than the zero standard.

Each concentration is converted to its \log_{10} . Each standard count ratio (B/B_0 or B/T) is converted to a logit.* A least squares linear regression is then performed. The unknowns are determined by converting each ratio to a logit. If more than one ratio exists per patient, a mean of the patient's logits is calculated. The unknown can be read off the regression line. The following will be printed:

Concentration of the unknown
SD
% CV If more than one reading/patient

Special checks of the data are performed during data reduction.

- All standards and unknowns must be:
 - $> NSB$
 - $< Total$
 - $< B_0$

If standards do not meet these criteria, the assay must be re-run. When unknowns do not meet these criteria, they are deleted.

- When counting bound counts, or when free counts are converted to bound counts, the counts must decrease as the standards increase.

Each standard count is compared to the mean of the previous standard value. If it is not less than the previous mean, an error message **RECHECK STNDS** will be printed, identifying the standards as questionable and the assay will continue.

- Unknowns and controls which are not within the range of standards entered are either extrapolated to zero or reported higher than the highest standard. Such controls appear as zeros in Q.C. data.

Protocol: Single Point Assays

A Single Point assay must be loaded and run in the following sequence:

- Reference control tubes (standards)
- Controls (knowns, unknowns, Q.C. tubes)
- Unknowns

Single Point assays, such as Triobead, have only one standard value. If more than one replicate is used of the standard value, the average count of the replicates is used and a straight line is plotted through that data point (counts vs. dosage or % bound, etc.) and the zero concentrations point.

If more than one tube is included for each patient, a mean is calculated.

Controls and unknowns are read from that line and reported in the same units as the standard. For example, if the standard is entered as 100%, each unknown is reported as a percentage of the standard.

The patient's T3 value is then calculated using this equation:

$$\text{Unknown \%} = \frac{\text{Unknown Count Mean}}{\text{Control Count Mean}} \times \text{Reference Value in \%}$$

If multiple replicates of either or both standards and unknowns are run, the standard deviation and coefficient of variation are calculated and printed.

Q.C. data stored for each assay includes:

- Date
- Lot Tech. No.
- Reference Control Counts
- Reference Value in %
- Control Counts

If a T3 test, such as Triobead, is being run and T7 values are desired, the respective T4 value for each patient must be manually entered. The patient's T3 value, when derived, is multiplied by the T4 value. The units of T7 value are the same as T4 value.

If T7 values are desired for some but not all patients, a zero should be entered in response to the operator prompt asking for T4 values for those patients whose T7 value is not necessary.

=====

Assessment

Binding Agents — Antibodies requirement are specificity of reaction and sensitivity.

Actual specificity is determined by comparing known substances that might cross-react with the binder substance that show identical dose-response curves can be assumed to be immunologically similar, but actually may not be identical compounds.

Specificity of a system is usually estimated by adding known interfering substances and comparing the dose-response curves.

Labeled Ligand – Assessment is concerned with:

- specific act of the substance
- purity of the substance
- identity of the labeled ligand with its corresponding unlabeled substance (use of chromatography)

Standards – Purposes of standards are:

- establish a curve of dilution on known amounts of substances to which the unknown may be compared
- ensure a stable interassay comparison
- compare the results obtained with other lab or established quantities

Kit Evaluation

Some tests are requested by MDs so infrequently that it may be advantageous to send samples to large commercial labs.

Isotope needed – important consideration:

liquid scint. ^3H , ^{14}C and gamma counters - ^{125}I , ^{131}I ,
 ^{57}Co

Stability of the reagents (components) will determine the shelf life of an individual kit. Lyophilized (freeze dried) agents generally provide the longest shelf life. Frequency and the number of tests performed influence kit selection.

Reliability of delivery, cost, policy of replacement, ease of instructions, provisions for free kits.

Type of equipment needed, separation techniques, pipetting, cting, temperature requirements, time requirement.

Kit should be chosen based on:

- clinical needs

- equipment, personnel, and time available
- component characteristic of kit
- cost
- manufacturer's consideration, reliability, availability

Kit evaluation should include:

- specificity of assay
- sensitivity of assay dose-response curve (shape of curve)
- inclusion of controls to judge interassay variation and clarity
- protocol itself, separation technique, equipment, time required
- components of kit: shelf life, storage, replacement

REVIEW

Section 1

1. Name at least three (3) quality control measures used in gamma well counter equipment and their specific functions. **Please show all work:**

2. Calculate efficiency of a given gamma counter based on the following data:

CPM of counter = 31,501

CS-137 = T_{1/2} 30 years - calibrated 9/71 - 1 uci

2.22×10^4 DPM = 1 uci

3. Very briefly, describe these data reduction methods used in RIA testing.

- a. Point to point:
- b. Log-Logit:
- c. Single Point:

Section 2

Which does *not* belong?

1. Important components of kit evaluation include:
 - a. cost
 - b. clinical needs
 - c. equipment, personnel, and time available
 - d. manufacturer's availability
 - e. a and b only
 - f. none of the above
2. The kit itself should include:
 - a. protocol
 - b. specificity of assay
 - c. components of kit
 - d. inclusion of control to judge interassay variation
 - e. none of the above
3. The three parameters that can affect overall accuracy and/or reproducibility of test results include:
 - a. counting efficiency
 - b. gamma absorption test
 - c. statistical biases
 - d. background counts

True/False

- 4. *The results obtained in any type of lab must be valid. Proven quality control measures should be routinely used in order to ensure valid results.*
- 5. *One of the aspects of assessment of labeled ligand is concerned with the purity of the substance.*
- 6. *Counting efficiency is the number of counts registered compared to potential number of effective emissions.*

- 7. A test to measure the variation of a series of counts from one source is the background check.
- 8. Point to point data reduction method allows for the development of a nicely uniformed curve.
- 9. Chi-square test is used to check accuracy of a gamma well counter.
- 10. A coefficient of variance value within assay runs must be greater than 10% to be acceptable.
- 11. Point to point assays can be run without total count tubes, NSBs and BO.
- 12. Log-Logit data reduction method is used for one standard only. Example: HCG-B testing.
- 13. In single point assays:

$$\text{Unknown \%} = \frac{\text{Unknown Ct. Mean}}{\text{Control Ct. Mean}} \times \text{Reference value in \%}$$

- 14. Coefficient of variance = $\frac{\text{Mean}}{\text{Chi-Square}}$
- 15. Point to point data reduction utilizes a least square linear regression.

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CHAPTER 7

HEMATOLOGY

Objectives

- Be able to provide a definition, including function and purpose, of the following terms:
 - erythrocytes
 - leukocytes
 - thrombocytes
 - specific gravity
 - hematocrit
 - plasma
- Understand the basic principles and methodology needed for blood sample collection.
- Be able to demonstrate a basic understanding of blood fluid precautions.
- Be able to demonstrate how to perform a blood volume determination.
- Know the principle of dilution and its purpose.
- Understand the uses and basic procedure for plasma volume and RBC volume determination.
- Know the basic procedure and purpose of red cell survival testing and iron metabolism studies.

Blood is a part of the main transport system that delivers O₂, nutrients, hormones, and antibodies to the tissues.

Blood makes up approximately 7-8% total body weight.

Fluid fraction—

- plasma

Formed elements —

- red cells (erythrocytes) (RBC)
- white cells (leukocytes) (WBC)
- platelets (thrombocytes)

Specific gravity — Formed element which values are greater than 1, the plasma cells tend to settle if allowed to stand.

- Because of abundance of red cells $5,000,000/\text{mm}^3$
- in relation to leukocytes $8,000/\text{mm}^3$
- Relative small size of the platelets

Volume of packed erythrocytes expressed as percentage of blood sample — **hematocrit**

- Normally averages 45%
- Supernatant plasma (plasmacrit) 55%

Plasma — Complex, watery fluid contain various ions as well as inorganic and organic molecules.

Solid constituent by **plasma proteins** 7.5gm/100 ml

Plasma protein helps maintain the constancy of the blood reactors.

Albumin factor together with the proteins concerned in blood clotting are manufactured in the liver.

Globulins, the fibrogens, are formed by reticuloendothelial systems, plasma cells, lymphoid nodules.

RBC — Circular, non-nucleated, biconcave manufactured — RB marrow — vertebrae, sternum, ribs. Hemoglobin content — red cells carries O_2 . Red cells usually survive for about 120 days.

WBC — Leukocytes, are nucleated (50-70%).

- Granulocytes — formed in RB marrow - survive for less than 2 weeks.
- Lymphoid tissue (lymphocytes) — 20-40%, survive 2-200 days, essential for the development of immunity

- Monocytes—2-8% phagocytic, play major role in production of Abs.

Platelets—Small, non-nucleated, granulated bodies formed in RB marrow, survive 10 days, provide support for endothelium of injured vessels and formation of hemostatis plugs, particularly in blood coagulation and clot retraction.

Blood Sample Collection

Quality control involves not only actual assay consideration, but also the sample itself should be subjected to a similar scrutiny. Because most assays are performed on blood components (plasma or serum), these samples are the most important to consider. It should be emphasized, however, that many other body fluids and organ structures may be analyzed by the principles of radioimmunoassay and related techniques. For example, urine may be analyzed for its content of insulin, thyroxine, etc. We will confine this discussion to blood samples, but other samples should have similar attention when they are analyzed.

Patient Factors

Collection of samples must be preceded by a review of the specific assay protocol. Several assays require special procedures for collection. For example, proper attention to timing of collection of samples for HTSH and cortisol is necessary to account for the circadian nature of the secretion of these hormones. Posture may affect serum secretions, such as plasma renin, and food intake will obviously affect insulin levels. Human growth hormone is markedly affected by the presence of stress. These factors must be either known or controlled at the time of specimen collection.

Sample Factors

Prompt handling of the specimen is desirable, and thorough mixing of any anticoagulant material should prevent undesirable clotting. Since many assays require serum, clotting of the collected blood followed by centrifugation and freezing (-20°C optimal) of the serum specimen should be expedited. Some substances may be rapidly destroyed in whole blood (such as ACTH), while others (such as angiotensin) may be

inactivated by enzymes and require the addition of enzyme inhibitors. Hemolysis causes increased enzyme release and may accentuate enzymatic destruction of specific substances being measured. Anticoagulant effects on the assay should be evaluated prior to sample collection. The total amount of sample available should be adequate for the specific assay.

Normal Range

The statement has already been made that the normal range for any assay should be established in each laboratory. This section will review the concept of a normal range and how the laboratory can approach its determination.

The normal range in medical terms usually refers to healthy persons. It becomes rather difficult to relate a state of health (or ill health) to natural phenomena such as aging, activity, etc. Often the characteristics of healthy and ill persons overlap, further complicating matters. Thus the simple establishment of a "normal range" may entail serious arbitrary decisions on the part of the laboratory, which may directly affect the usefulness of the determination.

Many factors affect levels of assayable substances besides ill health. Age has been mentioned. Other factors include alterations by conditions unrelated to the substance being measured, as occurs in unrelated diseases in the same individual; lack of longitudinal study of the individuals; circadian variations (as with ACTH); and effects of posture. To establish a true "normal" population, these factors need to be determined and standardized.

A typical application of statistical principles is to use the 95% confidence limits to define the "normal" population or test results in that population. Using these limits, the operator would expect the measurement for one individual in 20 to fall outside of the normal range (one in 40 would exceed the upper limit, and one in 40 would be below the lower limit). This technique of looking at a population of values in a "normal" population and forming a mean value and its standard deviation is the most frequently used method for deriving a normal range for the determination.

Blood and Body Fluid Precautions

Health care workers need to consider ALL patients as potentially infective and to adhere rigorously to infection control precautions for minimizing the risk of exposure to blood or body fluids of ALL patients. Body fluids include: urine, feces, oral secretions, sputum, vomitus, saliva, semen, wound drainage and all other moist body substances.

The Centers for Disease Control recommend using the following precautions when caring for ALL patients:

1. Gloves should be worn when:
 - a. touching blood, body fluids, mucous membranes or non-intact skin of all patients.
 - b. handling items or surfaces soiled with blood or other body fluids.
 - c. performing venipuncture or other vascular access procedures.

Gloves should be changed after contact with each patient and hands should be washed, with particular attention to the area around the nails and in between the fingers, immediately after gloves are removed.

If a glove is torn or a needlestick injury occurs, the glove should be removed, hands washed, and a new glove used as promptly as patient safety allows.

2. Masks/protective eyewear or face shields should be worn to prevent exposure to mucous membranes of the mouth, nose or eyes during all procedures that are likely to generate droplets of blood or other body fluids.

3. Gown or apron made of materials that provide an effective barrier should be worn during procedures that are likely to generate blood or body fluid splashes.

4. Needles should not be recapped, bent or broken or otherwise manipulated. Puncture-resistant containers should be located as close as practical to the use area.

Special care should be taken to avoid needlestick injuries when discontinuing IVs, IV meds or giving injections. Get help when giving injections to uncooperative patients.

5. Ventilation devices should be available for use in areas in which the need for resuscitation is predicted.
6. Health care workers with exudative lesions or weeping dermatitis should refrain from all direct patient care and from handling patient care equipment until the condition resolves.
7. For all invasive procedures (surgical entry into tissues, cavities, organs or repair of major traumatic injuries), cardiac catheters and angiograms, vaginal and C-section delivery gloves, surgical masks, protective eyewear or face shields and aprons or gowns should be worn.
8. All specimens of blood and body fluids should be put in well-constructed containers with a secure lid to prevent leaking during transportation to the lab. Care should be taken when collecting specimens to avoid contamination of the outside of the container.
9. Soiled linen should be handled as little as possible with minimum agitation to prevent gross microbial contamination of the air and of the person handling the linen. All soiled linen should be bagged at the location where it was used.
10. Chemical germicides that are approved for use as a hospital disinfectant can be used to clean up spills of blood or body fluids.
11. Hands and other skin surfaces should be washed immediately and thoroughly if contamination with blood or body fluids occurs.

Besides following the usual routine of completing an occurrence report and seeking medical attention, the employee that has had a blood or body fluid exposure should contact the employee health nurse as soon as possible after the occurrence.

Blood Volume Determination

Radioactive tracers used for volume measurements must be **non-toxic** and completely safe for parenteral administration, should mix rapidly and uniformly with the diluting fluid, and remain for a **reasonable time interval**.

Tracer—easily detected and quantitated in high dilutions. Ideally, accurate measurement of circulating blood volume should be performed by simultaneous determination of the volume of both **plasma** and **blood cells**. Two radioactive

tracers of different energy are used to enable their differences by means of PHA.

^{125}I - Human serum albumin - used measure plasma

^{51}Cr - Tagged erythrocytes - used measure red cell volume

Problems with measuring both together:

- time consuming
- tedious calculations to correct for ^{125}I measuring from ^{51}Cr
- use of dual isotopes
- specific equipment

Single tracer can be used for determination of volume of either plasma or red cells using **principle of dilution!**

$$V_1C_1 = V_2C_2 \quad Q = V \times C \quad \begin{array}{l} V = \text{diluting volume} \\ C = \text{tracer concentration} \\ \text{in diluting fluid} \\ Q = \text{total quantity of tracer} \end{array}$$

$$V = \frac{Q}{C}$$

Serum differs from plasma in that it contains no fibrinogen plasma. Since of the volume of one component is determined, the total blood volume can be calculated with the aid of hematocrit.

$$\frac{\text{Total blood}}{\text{Volume}} = \frac{\text{Plasma volume}}{\text{Plasma crit}} = \frac{\text{R.C. volume}}{\text{Hematocrit}} \sim 45\%$$

Average whole-body hematocrit is roughly 92% of venous blood hematocrit.

Problems: different size vessels, different organs

Discrepancy exists: plasma trapped within cells

Hematocrit is multiplied by correction factor 0.90 (.92 x .98)

Plasma Volume Determination

^{131}I human serum albumin (RISA or IHSA)

- Venous blood sample is obtained to estimate background radioactivity.
- Test dose of 5-30 uci RISA transferred into volumetric container and diluted up to 1,000 ml to act as stand.

- Exactly equal amount of tracer is injected in patient (antecubital vein).
- After 15 minutes, a blood sample is withdrawn from patient's other arm in a heparinized syringe.
- Hematocrit is determined and plasma separated by centrifugation.
- Radioactivity in equal volumes of stand. and plasma are measured in canter.
- Background is subtracted.

$$\text{Plasma volume (liters)} = \frac{\text{count rate of standard}}{\text{net count rate of plasma}}$$

Normally 43 ml/kilogram of body weight

¹²⁵I – human serum albumin: main disadvantage is lower counting efficiency

⁵¹Cr – tagged chromic chloride trivalent: 98% labels the plasma protein

Remainder attached RBC

Red Cell Volume Determination

Depends on labeled erythrocytes as the tracer. Sodium chromate (⁵¹Cr) radiochromate is most commonly utilized - hexavalent compound - easily penetrated RC membrane and establish firm tag with hemoglobin - tagged is done (invitro).

- Anticoagulated blood sample (10 ml) is obtained from patient and mixed with 50 uci of radiochromate.
- Mixture left at room temperature 15 minutes (ACD solution added) (stops tagging process). Radiochromate should not be added to ACD solution before use. ACD is going to hinder the chromate and hinder tagging process.
- Add 100 mg (ascorbin acid) (vitamin C) to mixture to reduce radiochromate and thus prevent it from tagging any other erythrocytes.
- Accurate measured volume (0) of tagged mixture is kept as a standard.
- Exact equal amount is injected back into patient.

- After 15-30 minutes, blood sample is withdrawn from patient's other arm.
- Hematocrit of blood sample and standard one determined
- 1 ml of standard, 1 ml of blood sample - counted in well counter

$$\text{RBC Volume} = \frac{\text{Cts of Standard}}{\text{Net Cts of RBC}} \quad V = \frac{Q}{C}$$

Problems with this method

- Errors encountered by the occurrence of extravasation during intravenous injection of tagged cells
- Lack of tagging due to reducing agents
- Formation of clots in standard
- Hemolysis of tagged cells

Clinical Indications for Blood Volume Determination

- After acute blood loss
- After extensive burns
- Preoperatively in patients of extreme ages – children and elderly – before major surgery
- Measure for degree of anemia of a patient
- Postoperatively - need for transfusion therapy

Red Cell Survival

Is measured by following a given group of erythrocytes to determine the time required for their elimination from circulation. Specific erythrocytes easily identified from remainder of RBC by a label or tag.

Two methods

Selective labeling – uses glycine C-14, glycine N-15, glycine H-3, Fe-59, Se-75. The RBC are tagged and followed for the full life span which can extend over 4 months.

- Normal life span = 120 days
- Normal disappearance $T_{1/2}$ = 25-35 days

Difficulties of this method include: radionuclide expense, special preparation, specific equipment is required for counting (liquid scintillation).

Random labeling—uses Cr-51 sodium chromate tagged to TBCs. This method is easier. The RBC survival time is calculated from the percent of surviving tagged erythrocytes as determined at period time intervals.

Procedure: Inject Cr-51, wait 24 hours, take sample, then take sample every other day for 3 weeks.

It is preferable to rely on the results of the first 3 weeks and express the survival in terms of time required for elimination of 50% of the tagged erythrocytes. Since survival is computed from % of surviving tagged cells as determine/volume, a wide fluctuation in blood volume can invalidate results.

Disadvantage: inability to differentiate a short life span from random destruction.

Clinical application: study cases of unexplained anemia and helps determine the nature of defect causing shortened R.C. survival.

Determine site of R.C. sequestration - choice of proper treatment

Iron metabolism studies - uses 10 uci of Fe-59 ferrous citrate. After injection of Fe-59, external counting is performed periodically and simultaneously over the precordium, sacrum, spleen and liver over a period of 2 weeks. Normally within the first few hours, after injection. The tracer dose—ct rates over the precordium—representing blood diminishes. Ct. rates over spleen and liver decrease. Ct. rates over sacrum increase - represent bone marrow - and peak for about two days before rapidly decreasing.

Significant increases in splenic cts.—denotes splenic sequestration of red cells.

If radioactivity over liver/spleen behave similar to that over sacrum—extramedullary (formation of RBC)—erythropoiesis is suggested.

The main indication of ferrokinetic studies is the investigation of obscure hematologic disorders.

REVIEW

Section 1

1. What is
 - a. Plasma
 - b. Specific gravity
 - c. Hematocrit
 - d. Plasmacrit

2. Explain the different functions of RBC, WBC, platelets.

$$V_1C_1 = V_2C_2$$

Initial volume = 15 ml

Initial concentration = $\frac{.6 \text{ uCi}}{\text{ml}}$

New concentration = $\frac{.007 \text{ uCi}}{\text{ml}}$

What is the new volume?

Section 2**Multiple choice**

1. The fluid portion of the blood is called _____.
 - a. serum
 - b. plasma
 - c. fibrogen
 - d. hematocrit
2. The three types of cellular elements present in the blood are _____.
 - a. fibrogen, serum and plasma
 - b. granulocytes, albumin, and monocytes
 - c. eosinophils, globulin, and albumin
 - d. erythrocytes, leukocytes, and thrombocytes
3. The term used to describe the production of blood cells is _____.
 - a. nuclear maturation
 - b. hematopoiesis
 - c. polyphyletic
 - d. unitarian
4. The volume of packed erythrocytes expressed as a percentage of the blood sample is defined as _____.
 - a. plasmacrit
 - b. hematocrit
 - c. albumin fractia
 - d. all of the above
5. The normal hematocrit is approximately what percentage of a given blood volume?
 - a. 10%
 - b. 25%
 - c. 45%

- d. 85%
6. Hematocrit obtained from blood sample is multiplied by .91. This is done:
- because 91% of the blood cells are erythrocytes
 - because 91% of blood is usually plasma
 - to correct for large vein sampling
 - all of the above

7. The following equation:

$$Q = V \times C \text{ where } V = \text{volume of diluent,}$$

$$Q = \text{amount of tracer, } C = \text{concentration of tracer}$$

— explains the relationship between volume and concentration. This equation is based upon:

- Starting's Law
 - the dilution principle
 - law of concentration
 - none of the above
8. In blood volume measurements, the plasma portion of the blood is most commonly labeled with _____ .
- ^{51}Na chromate
 - ^{125}I HSA
 - ^{131}I HSA
 - ^{32}p
9. The RBC survival test can be performed _____ .
- by randomly labeling RBCs in the circulation with sodium chromate
 - to estimate the RBC life span
 - to diagnose hemolytic anemia
 - all of the above

10. Selective labeling of erythrocytes for RBC survival can be achieved with _____ .
- ^{75}Se
 - ^{59}Fe
 - ^3H glycine
 - all of the above
11. In a red cell survival determination, samples are taken from the patient:
- every day for 1 week
 - every other day for 3 weeks
 - every other week for 1 month
 - every week for 1 month
12. If the specific gravity is less than 1, cells will
- tend to settle if allowed to stand
 - recombine after a while
 - tend to remain in solution
 - form particles that will fragment into alpha, beta, and gamma emitters.
13. Leukocytes are in abundance approximately _____ mm^3 in the body while erythrocytes are _____ mm^3 .
- 8,000; 5,000,000
 - 8,000,000; 5,000,000
 - 5,000; 8,000,000
 - 7,000; 16,000,000
14. The supernatant is
- the volume of packed erythrocytes expressed as percentage of whole blood
 - approximately 55% of whole blood normally
 - approximately 45% of whole blood normally
 - a and b

15. RBCs are
- small, non-nucleated granulated bodies formed in red bone marrow
 - granulocytes, nucleated formed in red or yellow marrow
 - circular, non-nucleated, biconcave, manufactured in red bone marrow
 - could be any of the above
16. The hematocrit value of a given blood sample represents
- the ratio between the plasma compartment and the cellular compartment
 - the ratio between the serum compartment and the cellular compartment
 - the percentage of the cellular components to the whole blood
 - the ratio of the RBCs to WBCs
17. The dilution principle can be expressed as
- $Q = VXC$
 - $V = QXC$
 - $V = QXC^2$
 - $V = QXC^2$
18. Radionuclides used for blood volume determination may either be tagged to plasma proteins or to the
- serum
 - WBCs
 - platelets
 - erythrocytes
19. The form of Cr-51 which can permeate the red cell membrane is
- trivalent form
 - bivalent form
 - hexavalent form

- d. all of the above
20. All of the following are types of WBCs *except*
- a. thrombocytes
 - b. granulocytes
 - c. lymphocytes
 - d. monocytes

Chapter 8

SIGNIFICANT IN VITRO TESTING PROCEDURES IN NUCLEAR MEDICINE

Objectives

- Understand the functioning and metabolic effects of the hypothalamus-pituitary-thyroid axiom.
- Be able to describe the function, clinical applications, normal ranges and testing procedures for: TSH, T-3, T-4, T-3 binding capacity.
- Understand the importance of Vitamin B-12 to the human body.
- Demonstrate a knowledge of Vitamin B-12 absorption testing methodology, including blood testing, hepatic uptake testing, and Schilling test.
- Understand the function and methodology of folic acid.
- Demonstrate an overall understanding of tumor testing, including HCG, CEA, and AFP.
- Understand the basic feedback system for pituitary-gonadal axis and the hormones that are involved.
- Demonstrate a knowledge of Indium-III WBC labeling, including the basic tagging procedure.
- Develop an appreciation for the functional uses of various other RIA tests.

Thyroid Functioning and Testing

These tests are often used as a screening device for patients suspected on clinical grounds of having thyroid dysfunction.

Do before radioactive iodine-uptake and invivo thyroid tests.

TSH - thyroid stimulating hormone

T₄ - thyroxine

T₃ - triothyronine

Uptake T₃ - binding capacity

Hypothalamus-Pituitary-Thyroid Homeostasis

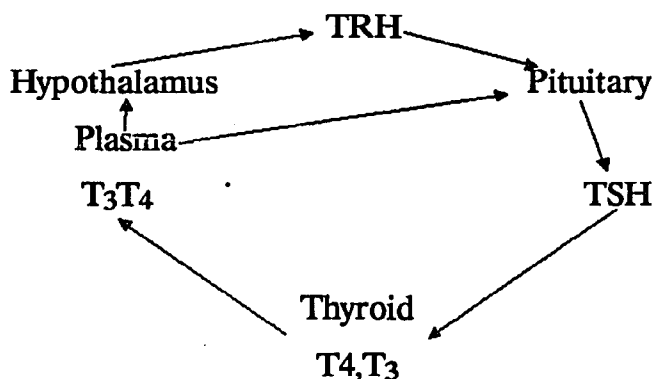
Normal thyroid function depends on effective function at 5 levels:

- Hypothalamus and its release of thyrotropin-releasing hormone (TRH) or (TRF) - thyrotropin-releasing factor
- Pituitary release of (TSH)
- Thyroid's secretion of active hormone T₄, T₃
- Plasma space with its specific thyroid-binding plasma protein
- Target organs and specific cell receptors

TRH is transported from hypothalamus via the portal system of the pituitary region of adenohypophysis where it stimulates basophilic cells to produce and release TSH.

TSH stimulates thyroid gland to increase production and release of active thyroid hormones T₃ and T₄; therefore, plasma T₄ and T₃ are increased. If plasma levels of T₄ and T₃ fall, TSH is secreted.

Thus, TSH is the prime regulator of the rate of function of the thyroid gland and hence of thyroid hormone production and secretion.



TSH – Thyroid Stimulating Hormone

TSH is a glycoprotein – molecule weight approximately 28,000. Protein contains one alpha and one beta polypeptide subunit. Possible cross-reactivity (same alpha species as HCG, LH, FSH).

It is the beta subunit of TSH that determines the specific physiology of the hormone.

TSH is essential to normal thyroid function. Absence of TSH: thyroid gland undergoes involution. It is involved in all levels of thyroid function.

- Production of hormone is stimulated by increasing the amount of thyroid iodide transported into gland and by increasing organic binding of iodide – I₂ (I⁻ = Iodine)
- Increase T₄, T₃ storage – iodotyrosines – into thyroglobulin
- Release of T₄, T₃

TSH is part of a sensitive control system that keeps the serum concentration of thyroid hormones within fairly narrow limits.

TSH under direct control by TRH – via portal system.

TRH stimulates basophilic cells in the adenohypophysis to produce TSH, TSH stimulating thyroid gland to release T₄T₃.

Increased blood levels inhibit release of TSH.

1/2 time of TSH turnover in plasma is approximately 1 hour. Daily secretion rate is about 170 mU.

Test is performed in 24 hours or less—long incubation. Double-Ab precipitation and solid phase tech.—separation tech. HCG is usually added to absorb out alpha chain.

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Clinical Applications

Hypersecretion of TSH occurs when hypofunction of thyroid due to decreased plasma levels of thyroid hormones.

Suspicion of hypothyroid disorders is a main clinical reason for TSH assaying.

Primary TSH overproduction—pituitary tumors and increased TRH secretion.

Secondary TSH (pituitary level)

Hyposecretion of TSH

Secondary (pituitary level)—destruction in pituitary is the cause—postpartum patient necrosis, pituitary tumors

Usually TSH deficiencies are associated with other trophic hormones: gonadotropins, ADH.

Frozen samples—stable for years.

Samples of TSH—does not undergo marked diurnal variation

Pregnancy—TSH is unchanged

TRH stimulation test for hyposecretion of TSH

I.V. dose 200 ug TRH—normal patient prompt increase peak in 30 minutes

Average increase: 10 uU/ml

Normal levels: 0-10uU/ml

15 uU/ml

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Interpretation of Results

Elevated TSH level—greatly elevated in primary hypothyroid myxedema. Levels up to 100 uU/ml are frequently seen in untreated hypothyroid.

Decreased TSH Levels

TSH levels are not measurable in hyperthyroid or hypopituitarism and secondary myxedema. Most assays do not permit difference between normal and decreased TSH levels.

In hypothyroid, response of serum TSH to TRH infusion (I.V.) is greatly increased.

Circulation of increased concentrations of T₄, T₃

Response of TSH to TRH (I.V.) is markedly reduced. Patient with thyrotoxicosis: no effect is observed.

Thus, TRH may be used different diagnosis of thyrotoxicosis (excessive activity of thyroid gland).

Normal response to TRH – good evidence of absence of hyperthyroidism.

Thyroxine (T₄)

Tetraiodothyronine

T₄ is produced by the actinar epithelium of the thyroid gland. A large reserve of T₄ is stored in the thyroid gland within thyroid follicle – sufficient supply for body's needs for 2 weeks.

In bloodstream T₄ is bound to plasma protein.

99.9% of plasma T₄ is bound to TBG.

Protein-bound thyroid hormone is metabolically inactive – free (T₄) that exerts metabolic effects.

T₄ - 1/2 life = 8 days

60 ug T₄ - extracted daily from blood by target tissues

Performance of Test

Serum absorbed with charcoal to remove thyroid (T₄-free serum) is frequently added to standard curve samples.

Separation Technique (Absorption Technique)

Absorption of unbound thyroxine to activated charcoal predominates – also may use double-Ab method.

Time needed for test usually 2 hours.

Clinical Application

Hyperfunction of thyroid gland – thyroxicosis: excessive amount of thyroid hormone is present in peripheral tissues. Denominates in every organ system – accelerated metabolism.

Disease states cause hyperthyroid: diffuse toxic goiter.

Most common: toxic multinodules (Graves disease), goiter (Plummer's disease) and toxic adenoma (epithelial tumor). In these disorders, thyroid loses its normal responsiveness to inhibition of falling TSH level.

Its autonomous or “nonresponsive” (independent of TSH).

Thyrotoxicosis Factitin

Excessive ingestion of T₄ or T₃.

Hypofunction – thyroid tissue fails to provide enough thyroid hormone to peripheral tissues – reduction on metabolism to activity.

Absence of thyroid hormone – ultimately leads to coma, progressive falling body temperature of organ systems and death.

Not commonly – idiopathic (self-originated, spontaneous) – last manifestation of autoimmune thyroiditis.

Hypothy – end stage of treating hyperthyroid may occur at birth leading to cretinism from low levels of circulating TSH.

T₄ sample – stable in serum for several days at room temperature little diurnal variation unaffected by fasting acute febrile illness (pertain to fever).

- Reference Values

normal - 45 to 120 ng/ml

hypo - 5 to 50 ng/ml

hyper - 120-460 ng/ml

Interpretation of Results

Elevated levels — patient has hyperthyroidism.

Decreased levels — hypothyroidism of both primary and secondary types different diagnosis of these disorders is best made using TSH.

Elevated TSH - Decreased T4 - primary hypothyroidism

Decreased TSH - Decreased T4 - secondary hypothyroidism

Increase T4 concentration in blood may be wrongly thought to be due to thyroid overactivity when real cause is increase in binding protein concentration (TBG).

T4 - along with T3 binding capacity may be affected by the following conditions:

Increased	Decreased
TBG — estrogen, pregnancy	hyperthyroidism
hypothyroidism	acromegaly
hepatitis	Down's syndrome
cirrhosis	major illness
	androgens
	corticosteroid

Competes with T4 for binding

- Phenytoin (Dilantin)
- T3
- Dicumanol

T3 Tridothyronine

T3 Tridothyronine — like T4, T3 is produced with thyroid gland by the **Acinar epithelium**. Thyroid secretes T3 in mass amounts that are less than 10% of T4 secreted.

Thyroid secretion of T3 is only a minor source of T3 produced. The majority is produced by **monodeiodination** of T4 in the peripheral tissues.

T3 concentration is influenced by TBG concentration - same as T4. T3 - 1/2 life in plasma 2.0 days.

Daily turnover: 30 ug/day.

Performance of Test

Charcoal absorption is most frequently used separation method. Also solid phase method.

Highly specific antiserum is required for T3. T4 concentration in serum 80 - 100x. T3 serum close structure - makes for some degree of cross-reactivity.

Clinical Applications

Hypersecretion of T3 is *always* found in hypothyroidism as far as is now known.

Frequently T3 levels are much more elevated than T4, indicating T3 is being produced directly from the thyroid in greater abundance than T4. Some patients' hyperthyroidism may be manifested only by T3 elevation. **T3 Thyrotoxicosis**—before T4 begins to elevate T3 thyrotoxicosis is very common hyperthyroidism patients are in iodine deficient areas.

Hyposecretion of T3—accompanies hypothyroidism, may occur in euthyroid patient with acute febrile illness.

Reference Values—normal 1.5 +/- 1 ng/ml

hyper 3.0 ng/ml

Interpretation of Results

Elevated T3 - single best indicator of hyperthyroidism. If T3 is normal, what seems to be hyperthyroid state should be carefully scrutinized.

Decreased Values T3—do not necessarily imply hypothyroidism. May be low euthyroid or acute illness. Tend to decrease in hypothyroidism. Normal T3 - is not infrequent in hypothyroid state, therefore, T3-RIA is not particularly useful for diagnosis of hypothyroidism.

T3 Binding Capacity or T3 Uptake

Since most thyroid hormone circulates bound to protein in plasma, changes in concentration markedly influence thyroid hormone concentration.

An indirect estimate of capacity of plasma protein to bind thyroid hormone is the T3 uptake test. It measures the ability of patient's plasma proteins to compete with a secondary binder such as a resin bed, red cells or rubber sponge matrix.

Technique gives an estimate of unoccupied binding sites of transporting proteins, chiefly TBG.

Adding I-125-T3 to free binding sites of TBG and by the resin.

If free binding sites TBG decrease because of TBG deficiency or because sites are occupied by increase T4 production, I-125-T3 will be greater degree take-up by resin. If free binding capacity TBG increases, example is extremely low T4 levels, or increase (example pregnancy).

Resin will bind less to I-125-T3. Resin uptake should be interpreted in light of total T4 serum concentration.

Free T4 Index—estimate of the amount of free thyroxine hormone present in plasma.

Value from T3 uptake x total T4 have been combined into a free T4 index.

Free T4 index has been shown to be closely related to the actual concentration of free T4 in serum.

Since resin T3 uptake reflects the number of TBG binding sites present and since total T4 concentration indicates total hormone, the product should be proportional to free T4.

Vitamin B-12

Vitamin B-12 is a very potent, cobalt-containing, dietary factor available in many foods such as liver, kidney, muscle, milk, and eggs. It plays a major role in the **synthesis of nucleic acids** and therefore, is particularly important in the process of **cell maturation**. The first cells to suffer from its deficiency are the rapidly dividing cells such as bone marrow and gastrointestinal tract cells.

Proper absorption of vitamin B-12 depends upon adequate secretion of intrinsic factor by glands in the stomach. Vitamin

B-12 intrinsic factor complex mainly absorbs in the terminal ileum. After absorption vitamin B-12 is carried in blood by plasma protein. Then goes to liver as a storage depot. It stays in the liver for months to even years and is slowly released, carried out by normal cellular metabolism. Vitamin B-12 is excreted through urine.

The presence of cobalt atom in each molecule of vitamin B-12 makes it possible to synthesize a radioactive cobalt labeled vitamin B-12 using ^{57}Co , ^{58}Co , ^{60}Co . ^{57}Co is preferred because of its efficiency and absence of beta radiation.

This is called **cyanocobalamin**. It is essential to a variety of enzymatic reactions, normal growth, hematopoiesis, functional states of nervous system.

Intrinsic factor is produced by gastric parietal cells found in the stomach. .1 ug of vitamin B-12 daily, recommended intake 2 ug/day.

Radioisotope of cobalt (^{57}Co , ^{58}Co) are easily substituted for natural cobalt, used for highly specific receptors in RIA.

The binding of cyanocobalamin to exogenous binders must not disrupt vitamin B-12 reactivity.

In the lower normal and lower levels substitution may cross-react giving falsely high levels of measure for vitamin B-12. Most assay give sensitivity in the order of 10-30 pg/ml.

Clinical Applications

Vitamin B-12 deficiency – A characteristic defect develops in cells of many different tissues. The nucleus is unable to mature normally and does not divide appropriately. Affects organ systems with rapidly dividing cells such as hemopoietic cells. Prolong deficiency causes central nervous system problems. Most deficiencies are due to malabsorption of vitamin B-12.

Possible Causes

- Inability of gastric parietal cells to secrete intrinsic factor.
- Gastric resection.
- Inflammatory disease of ileum.
- Bacterial growth in stomach.

- Diet deficiency such as seen in strict vegetarian dieters and their breast fed infants.

The normal ranges in blood serum is 200-900 pg/ml.

Interpretation of Results

Values less than 100 pg/ml are almost always associated with vitamin B-12 deficiency.

Falsely low levels have been reported in patients taking very large doses of ascorbic acid and high concentrations of fluoride in serum.

Elevated values are values greater than 1,000 pg/ml which can be found in hepatic disorders such as hepatitis and leukemia patients in which there is a release of cyanocobalamin.

Folates – Generic term used to describe folic acid. In adults the dietary requirement is about 50 ug/day. The requirement in children is considerably greater at about 100 ug/day. During pregnancy the requirement is 400 ug/day and during lactation (period of secretion of milk) 300 ug/day.

Folates are found in all foodstuffs. Examples are liver, yeast, vegetables and fruits. Folates in food are in the form of polyglutamate. They are broken down within the stomach into monoglutamate form and is rapidly absorbed primarily through the proximal small intestine. Folic acid is rapidly absorbed into the blood and converted into a variety of active folate forms and stored in the liver.

Two isotopes have been utilized to evaluate levels;

- ^3H – triton was first used
- ^{125}I - is now more common.

Clinical Applications – Malabsorption of folate may occur in intestinal disorders.

Most common is folate deficiency in diet. In certain disease states, hyperutilization of folate may occur and result in deficiencies found in pregnant patients, hemolytic anemia, malignancies, and lactation. Some drugs such as oral contraceptives, phenytoin, and ethanol impair absorption of folate.

Like vitamin B-12, folate is essential in rapidly dividing cellular systems.

Sampling Blood — fasting serum should be used, folate levels vary significantly after eating.

The normal ranges 3-20 ng/ml.

Interpretation of Results

Elevated levels are not uncommon in patients with vitamin B-12 deficiency. Ranges less than 3 ng/ml is common in malabsorption states, pregnancy, alcoholism and a malignancy

Other Methods to Measure Vitamin B-12 Absorption

Fecal Excretion Method — Stools collected at least 72 hours after the administered dose of radiation, the net amount of radiation in stools is expressed as % of administered dose. This represents the amount of vitamin B-12 not absorbed. Remainder is absorbed normally in stools at 30%-70% of administered dose with defective absorption, stool % is higher.

Hepatic Uptake Test — days after oral ingestion subject is given a laxative to clear alimentary tract. Two days later, radiation accumulates in four different areas over the liver, left iliac fossa, and thighs (considered background). The ratio between hepatic count rate and that over iliac fossa is then calculated. Normal patient's count over liver and over left iliac fossa is at least 2.5 to 1.

Urine Radioactivity Collection — Schilling Test — most commonly used. The patient is given an oral dose of .5 uci of vitamin B-12. Patient is then given nonradioactive vitamin B-12 which will reach and block specific binding sites before absorbed radioactive vitamin B-12. This non-radioactive B-12 is given intramuscularly. Consequently the absorbed unbound radioactive vitamin B-12 is excreted in the urine. The 1,000 ug of stable vitamin B-12 is injected within 2 hours after oral administration of tracer dose. Urine is collected over the next 24 hours. Radioactivity is estimated and expressed as a percentage of the administered oral dose. Percentage of dose that is absorbed and consequently recovered in urine by flushing with stable vitamin B-12 is normally 7% or more. The main source of error is in the collection of urine, measure-

ment of radiation, and results may also be affected by renal disease. (For preparation procedure, calculations and diagnostic values see Laboratory Exercises.)

In Vitro Testing for Tumors in Body

Measurement of tumor-specific products in body fluids may in the future offer a selective way to detect human neoplasms at a curable stage. Some tumors produce hormones that call attention to their presence, such as gynecomastia (excessive development of mammary glands in males), Cushings Syndrome (overproduction of adrenocortisol from the pituitary effecting the adrenal cortex), and certain trophoblastic (uterus) tumors for examples.

The ideal method for detecting tumor products would be extremely sensitive and specific for neoplasia. The level of the tumor product in the blood should correlate with the total mass of the tumor present.

Such methods would be useful for:

- Screening for cancer in high risk patient populations.
- Assisting in establishing a specific diagnosis of cancer.
- Helping to localize tumors.
- Monitoring tumor response to therapy.

The three most common blood testing methods for tumor detection are:

- CEA - carcinoembryonic antigen
- AFP - alpha-fetoprotein
- HCG - human chorionic gonadotropin

CEA - carcinoembryonic antigen

CEA is a normal product of human development and occurs in the fetal colon. CEA may also be found as a normal constituent of adult tissues, such as the glycocalyx of the adult large intestine.

Gold and Freedman found a tumor-specific antigen (CEA) that was present in perchloric acid extracts of cancer of the colon and fetal intestine, but not the adult intestine. This development showed great promise for diagnosis of

gastrointestinal malignancies. CEA has become a widespread evaluator of patients with a variety of neoplasm.

A double-antibody measurement used to extract perchloric acid, CEA is located in the luminal surface of the mucosal cell.

Two factors appear to be important as a cause for increased serum levels of CEA in malignancies:

- Tumor cells produce a greater quantity of this antigen.
- Rapidly growing tissue results in disruption of blood-tissue barriers, what is produced in tissues is more accessible to the blood.

A variety of malignant tumors produce CEA in abundance some examples include: pancreas, colon, rectum, lung, breast, cirrhosis and gastrointestinal.

Reference Values:

- normal - 2.5 ng/ml
- malignant - 40 ng/ml

Modest elevation occurs in pancreatitis, cirrhosis, chronic bronchitis and inflammatory bowel diseases.

Increased levels - disseminated malignancies, main utilization of measuring CEA is evaluating patients who have already been documented to have tumors checking on effects of therapy. CEA is often utilized as an indicator of recurrence of tumor after surgical treatment of colon cancer.

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AFP - alpha-fetoprotein

Associated with liver malignancies, hepatoma, teratocarcinoma (malignant neoplasm in testes), and metastatic tumors to liver AFP is a normal constituent of the fetus and plays a similar role in fetal life that serum albumin plays in adults.

The normal transition from AFP to serum albumin as the dominant serum protein begins at about 13 weeks of gestation, peak concentrations in AFP in fetus is 3 to 4 ng/ml. In pregnancy and adults it is 100 ng/ml.

AFP is found as a normal constituent of human serum but in diseases of the liver massive elevations may be noted.

Elevated values of AFP are seen in both benign and malignant liver diseases.

Reference values:

3-25 ng/ml - normal adult
up to 120 ng/ml - pregnant women

Values above 1,000 ng/ml in an adult are always associated with primary liver malignancies.

HCG—human chorionic gonadotropin

Certain trophoblastic tumors (uterus) which produce HCG can be detected since this hormone is not present in nonpregnant individuals. Its presence in serum indicates a neoplastic state. Significant HCG levels are associated with tumors of 1mm³ in size.

HCG levels are virtually always present with patients with choriocarcinoma and chorioadenomas.

HCG-B is a common testing method to rule out pregnancy. It can be either qualitative or quantitative in value.

Pituitary—Gonadal Axis

The reproductive system's regulated by three distinct classes of hormones.

- The hypothalamic releasing hormones.
- Gonadotropins which exert no target organ effect except stimulate release of other hormones (anterior pituitary hormones).
 - follicle stimulating hormone (FSH)
 - luteinizing hormone (LH)
 - placental hormone
 - human chorionic gonadotropin (HCG)
- Gonadal steroids—Hypothal. releasing hormone known to be of importance in reproductive physiology is gonadotropin releasing hormone (Gon RH) migrates within the portal venous system to the pituitary where it stimulates production of LH and FSH.

FSH and LH stimulate the gonads to produce a number of sex steroids principally estradiol and progesterone from the ovaries and testosterone from the testes.

Complex interactions between the gonadotropins and the sex steroids that produce the cyclic changes of ovary such as maturation of the follicle ovulation and formation of the corpus luteum are only partially understood.

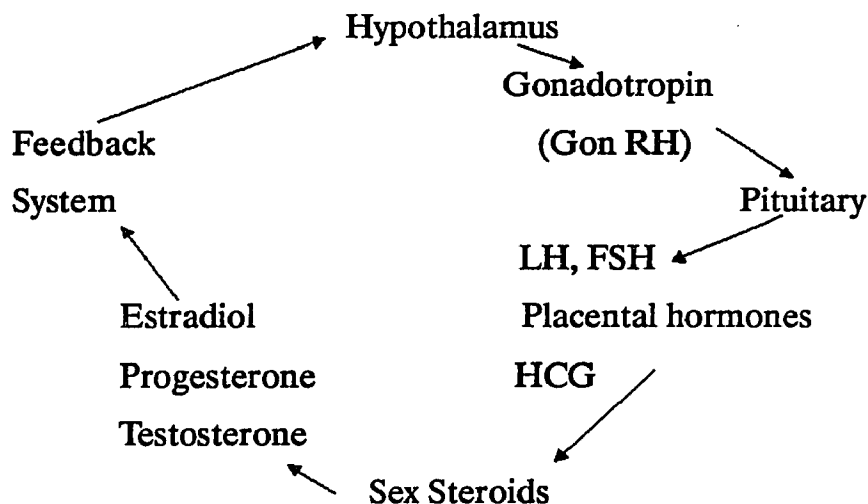
Estrogen and testosterone exert an inhibiting feedback on the secretion of FSH and LH. Increased plasma levels of sex steroids will inhibit release of gonadotropins and decrease levels stimulate release of gonadotropins and decrease levels stimulate release of gonadotropins.

Castration, oophorectomy (removal of ovaries) and menopause are followed by an increase of the release of gonadotropins.

During pregnancy HCG intervenes in this regulatory system and secretion of FSH and LH is suppressed.

Feedback inhibition is directed against anterior pituitary.

Pituitary – Gonadal Negative Feedback System



INDIUM-111 LEUKOCYTES TESTING PROCEDURE AND TECHNIQUE

Introduction:

The clinical use of Indium-111 oxine labeled white blood cells for detection and localization of abscesses and other inflammatory disease is a valuable diagnostic tool.



Physiological Principle:

Leukocytes function primarily in the localization, destruction, and removal of micro-organisms and damaged cells. The leukocytes chemotactic, phagocytic, and microbial destruction functions all play a role in the body defense mechanisms. With the introduction of Indium-111 oxine as a radiolabel for cellular blood elements, it became possible to image the distribution of leukocytes. The lipid soluble oxine carries the Indium-111 across the cell membrane where the Indium separates from the oxine and preferentially binds to cytosolular proteins. The labeled cells retain viability and function. When labeled cells are intravenously administered to the patient they will normally distribute to the liver, spleen and functioning bone marrow, but some will continue to circulate in the blood. Any collection of labeled white cells outside of these areas indicates an area of abnormality to which the patient's blood cells are being attracted.



Description of Technique:

Forty cc's of patient blood are collected with five cc's of acid-citrate-dextrose (ACD) as an anticoagulant. Donor blood may also be used. The blood components are separated by centrifugation. The white blood cell layer is removed, suspended in saline and incubated at room temperature with Indium-111 oxine for 15 minutes. All white blood cells will be labeled unless more sophisticated procedures (generally not available) are used to separate the granulocyte from mononuclear and lymphoid cells. The cells are washed to remove non-incorporated Indium and oxine from the labeled white blood cells which are resuspended in the patient's plasma and returned to the patient via intravenous injection.

Imaging may be started as early as four hours after injection; however, the quality and diagnostic value of the images will be superior in the day after (18-24 hours) the labeling and injection of the white blood cells. Anterior and posterior whole body images along with spot views of the anterior upper

and lower abdomen are routinely obtained along with additional images as indicated. Occasionally, delayed imaging at 48 hours is useful.

Indium-111 oxine - dose 500 uci

- Energy levels 171 kev (primary)
- 252 kev
- T 1/2 67.2 hours

Basic Invitro Labeling Technique

- 40 cc of patient blood is collected with 5 cc's of acid-citrate-dextrose (ACD) anticoagulant
- blood components are separated by centrifugation
- WBC layer is removed - suspended in saline (1% albumin)
- incubate at room temperature for 15 minutes
- WBC's are labeled
- cells are washed
- labeled In-111 WBC are reinjected into patients (for step by step procedure see lab exercise)

Indications for In-111

Useful for spectrum of inflammatory diseases. Abscess localization, acute and chronic osteomyelitis. Activity in inflammatory bowel disease, Crohn's disease, ulcerative colitis, fever of unknown origin, study of choice.

CT and ultrasound has better resolution in abdominal abscesses. When one does not know specific area of abscess, use In-111.

Limitations

Technically demanding, false negatives if chemostatic function has been altered, long-term antibiotic therapy may fail to attract WBC's, gallium scan is preferred, false positives, gastrointestinal bleeding, upper respiratory infection.

Hepatitis

RIA TEST:Antibody to Hepatitis B Surface Antigen I-125
Ausria I-125

Introduction

Three forms of viral hepatitis are currently recognized. Hepatitis A, Hepatitis B which can be differentiated by Serologic Tests. A third form called non A, non B is diagnosed by exclusion.

Hepatitis B causes greatest concern in terms of it spreading from patient specimens to laboratory personnel. As little as 0.0004 ml of blood contaminated with hepatitis B can transmit the disease. Transmission can also occur by oral route, mucous membrane and through minor cuts or scratches in the skin.

Clinical Differences

	Type A	Type B
Incubation	15-45 days Avg. 28 days	28-120 days Avg. 45 days
Symptoms	20-40% (in patients)	40-60%
Onset of Symptoms	usually abrupt	usually insidious
Duration	1-3 weeks	1-4 weeks sporadic
Jaundice	30-35% (of patients)	30-40%

Major Difference — Type B has a tendency to evolve to chronic carrier state in 5 to 10% of cases.

Structural Components & Physiology

Electron Microscopy has shown that in the serum Hepatitis B Surface Antigen (HB_sAg) is constantly associated with spherical particles, tubular forms, and the Dane particle (larger). Hepatitis B virus (HBV) production in the body is characterized by the disproportionate manufacture of HB_sAg as compared with the production of whole virus particles. The complete HBV (Dane particle) consists of a core component enveloped by a lipoprotein Surface matrix (HB_sAg). HB_sAg is produced in vast unassembled amounts in tubular and spherical forms. The number of such incomplete virus par-

ticles in the circulation of a typical HB_sAg carriers is immense and may range up to 100 billion to a trillion particles per milliliter of blood.

Principle

The Ausria II-125 system uses the "Sandwich principle". It is a solid phase radioimmunoassay technique to measure HB_sAg levels in serum. Plastic beads coated with guinea pig antibody are supplied. Patient serum or plasma is added and during incubation Hepatitis B Antigen, if present is fixed to the antibody. When antibody tagged with Iodine-125 is added it binds to any Hepatitis B Antigen on the bead creating an antibody-antigen-antibody sandwich.

Indications

- Testing should be done on a routine basis particularly on patients admitted to the hospital.
- Medical and health care staff that have a high risk of exposure.
- Determine the type of viral hepatitis and type of prevention and prognosis.
- For studies of epidemics.
- Diagnosing of liver disease.
- Screening of blood donors.

Implications: HB_sAg presence is the earliest indicator of the presence of acute infection. Also indicative of chronic infection.

Interpretation of Results

The presence or absence of HB_sAg is determined by relating net cpm of the unknown to the "cut off value". The cut off value is the net cpm of negative control mean (NCX) times a correlation factor.

Specimens with net cpm equal to or greater than the cut off value established with NCX are to be considered reactive for HB_sAg which should be evaluated further.

For the run to be valid, the mean value for the positive controls (PCX) specimens should be at 4 times the NCX.

Disease States and Patient Problems

- The chances of detecting HB_sAg in patients with hepatitis B relate directly to the time of testing, frequency of testing, sensitivity of the method.
- The detection of HB_sAg in a patient with acute viral hepatitis is strong presumptive evidence that the patient has hepatitis B.
- The persistence of HB_sAg for 3 or more months following the onset of acute viral hepatitis suggests that the patient is becoming a chronic carrier or is developing chronic active hepatitis.
- Positive results may be first evidence that the patient has underlying liver disease.

Infrequently Utilized Nuclear Medicine – In Vitro RIA Tests

Cortisol Levels—useful in the diagnosis of Cushing's syndrome (hyperadrenocorticism) and Addison's disease primary adrenal insufficiency.

Secreted by adrenal cortex called zona fasciculata.

Pituitary secretion of ACTH stimulates adrenal cortex to secrete cortisol.

Diurnal variation—diff. am and pm values.

Dexamethasone used to suppress overactive ACTH stimulation.

Hepatitis Testing—used to evaluate viral hepatitis, type A and B at different stages.

- Hepatitis A—transmitted by oral-fecal routes.
- Hepatitis B—spread by contaminated blood or blood products.
- Hepatitis A—positive for acute and recovery period. A-AB, A-B-M.
- Hepatitis B—known as Australia Antigen (positive for forms of Hep-B complex). BS-Ag, Bc-Ab, BS-Ab, Be-Ag.

RIA tests for hepatitis will give qualitative results - not quantitative.

Calcitonin—useful for differential diagnosis of medullary thyroid carcinoma and to monitor therapy.

Produced in parafollicular or C-cells of thyroid gland.

Important in calcium metabolism.

HGH Levels (Human Growth Hormone)—Important in diagnosis of growth retardation.

Pituitary insufficiency—lack of hypothalamic stimulation.

Hypersection in adults (acromegaly).

Hypersection in children (giantism).

PTH (Parathyroid Hormone)—Useful in differential diagnosis and management of hypercalcemia and hypocalcemia, tumors of parathyroid gland.

Regulation of calcium—by regulating the calcium—ion concentration in body fluids.

Gastrin—useful in diagnosis of peptic ulcers, pernicious anemia, Zollinger-Ellison syndrome (extreme gastric hyperactivity).

Functions in body and stimulate gastric acid secretion.

Collected sample should be fasting.

Digoxin—useful in treatment of congestive heart failure, atrial fibrillation, atrial flutter and supra ventricular tachycardias.

Blood drawn 8 hours after last dose.

Must be monitored closely—proper therapeutic range .5-2 ng/ml.

Ck-B (Creatinine-Kinase-B Isoenzyme)—Is most specific and sensitive for acute myocardial infarctions.

Test should be done with N.M. scan evaluating heart infarct.

Myoglobin—elevated levels following myocardial infarction and usually peaks 5-18 hours after onset of chest pain.

Found in skeletal and cardiac striated muscles.

HCG-B Levels (Human Chorionic Gonadotropin - Beta)—Useful in detection of choriocarcinoma, ectopic pregnancy and threatened abortion. Can be detected within first 10 days of pregnancy.

Useful in diagnosis of tumors of ovaries, testes, breast, prostate.

Maximum levels of HCG-B during second-third month.

Can be used for quantitative or qualitative results.

Renin Levels (Plasma PRA) – Useful in diagnosis of primary and secondary aldosteronism in hypertensive and renin-angiotensin system disease states.

Usually measuring angiotensin 1 – rate of angiotensin formation directly related to renin level.

Collected in EDTA – coated tubes (usually purple tops) and early morning draw, and after letting patient walk around.

Gentamicin - used as an aminoglycoside antibiotic with narrow therapeutic range.

IgE – immunoglobulin class responsible for allergic reactions and is elevated in most patients with asthma, hay fever, eczema, and other allergies.

Phenytoin or Dilantin – Is an anticonvulsant drug used to treat epilepsy.

Theophylline – Bronchodilator useful in the treatment of asthma. Must be drawn two hours after dose.

REVIEW

Section 1

Draw and explain the feedback system of the hypothalamus-pituitary-thyroid axiom including the process, function, and type of hormones released at each level.

Thyroid Functioning

Match the *organ* with the substance it *releases*.

- | | |
|-------------------|-------------------------|
| — 1. pituitary | <i>a. T3, T4</i> |
| — 2. thyroid | <i>b. TSH</i> |
| — 3. hypothalamus | <i>c. TRH</i> |
| | <i>d. thyroglobulin</i> |
| | <i>e. barium</i> |

Section 2

1. Secondary hyperthyroidism refers to disorders of the ____ .
 - a. thyroid
 - b. pituitary
 - c. hypothalamus
 - d. adrenal gland
2. T4 (thyroxine) is stored in the ____ .
 - a. acinar epithelium
 - b. thyroid follicles
 - c. adenophysis
 - d. colon
3. Hyperfunction of T4 or thyroid gland is called thyrotoxicosis this is when ____ .
 - a. there is accelerated metabolism throughout the body
 - b. decreased metabolism
 - c. unknown etiology
 - d. too much TBG in system
4. When there is an increase in TSH levels and a decrease in T4 levels it is probably ____ .
 - a. primary hyperthyroidism
 - b. primary hypothyroidism
 - c. secondary hyperthyroidism
 - d. secondary hypothyroidism
5. Elevated T3 testing is ____ .
 - a. the single best indication of hyperthyroidism
 - b. doesn't necessarily imply hyperthyroidism
 - c. must be elevated with TRF stimulation test
 - d. none of the above

6. In T3 uptake testing, the greater the number of binding sites, the (lower) or (higher) the uptake on the resin of I-125-T3.
7. The pituitary release of TSH is influenced by ____ .
 - a. amount of TRH
 - b. amount of thyroid hormone in plasma
 - c. amount of cortisol simultaneously stimulated
 - d. all of the above
 - e. a. and b. only
8. TSH help stimulate thyroid to ____ .
 - a. increase the amount of thyroid iodide transported into the gland
 - b. increase T4, T3 storage
 - c. release T4, T3
 - d. a. and b. only
 - e. all of the above
9. The hormones which the thyroid gland produces include all of the following *except* ____ .
 - a. calcitonin
 - b. thyroxine
 - c. triiodothyronine
 - d. TSH
10. After synthesis, the thyroid hormones T3 and T4 are stored in the ____ until needed for use in the body.
 - a. acinar epithelium of the thyroid
 - b. parathyroid glands
 - c. adenohipophysis
 - d. colloidal thyroid follicles
11. The thyroid hormone which is in the greatest abundance in the bloodstream is ____ .
 - a. triiodothyronine
 - b. thyroxine
 - c. calcitonin

d. TSH

12. The more metabolically active of the thyroid hormones T3 and T4 is ___ .

- a. triiodothyronine
- b. thyroxine

13. The bulk of circulating thyroid hormone is ___ .

- a. free, unbound
- b. TBG bound
- c. TBPA bound
- d. albumin bound

14. TSH is secreted by the ___ .

- a. hypothalamus
- b. pituitary gland
- c. parathyroid glands
- d. thyroid glands

15. The element which is essential for normal thyroid function is ___ .

- a. iron
- b. iodine
- c. chlorine
- d. fluorine

16. Isotopes of iodine which have been used to evaluate thyroid function include all the following *except* ___ .

- a. I-127
- b. I-123
- c. I-131
- d. I-132

17. Euthyroidism refers to the condition of the thyroid as being ___ .

- a. overactive
- b. normal

- c. underactive
 - d. oversecreting
18. Which of the following conditions occurs as a result of thyroid deficiency in infancy?
- a. Down's syndrome
 - b. mongolism
 - c. cretinism
 - d. myxedema
19. Primary hypothyroidism is due to ___ .
- a. failure of the pituitary to secrete TSH
 - b. failure of the hypothalamus to secrete TRH
 - c. failure of the gland itself
 - d. all of the above
20. Secondary hypothyroidism is due to ___ .
- a. failure of the pituitary to secrete TSH
 - b. failure of the hypothalamus to secrete TRH
 - c. failure of the gland itself
 - d. none of the above
21. T₃ by RIA is most helpful in the diagnosis of ___ .
- a. hypothyroidism
 - b. hyperthyroidism
 - c. thyroiditis
 - d. thyroid carcinoma
22. The greatest clinical utility of measuring TSH by RIA is in differentiating ___ .
- a. primary from secondary hypothyroidism
 - b. Graves from Plummer's disease
 - c. Hashimoto's thyroiditis from Reidel's struma
 - d. thyroid adenoma from cyst

23. Serum TSH levels are Generally ___ in the hypothyroidism and ___ in hyperthyroidism.
- decreased, elevated
 - decreased, normal
 - normal, elevated
 - elevated, decreased
24. Calcitonin, a hormone involved in calcium metabolism, is produced in the ___.
- parathyroid glands
 - thyroid gland
 - pancreas
 - anterior pituitary
25. A person with clinical features of acromegaly is likely to be tested for which hormonal aberration?
- elevated TSH levels
 - depressed TSH levels
 - elevated HGH levels
 - depressed HGH levels
26. PTH, which is produced by the parathyroid glands, is important in the regulation of ___.
- iodine
 - calcium
 - tyrosine
 - thyrotropin
27. Plummer's disease is another name for ___.
- toxic diffuse goiter
 - toxic nodular goiter
 - hypothyroid goiter
 - Grave's disease
28. Free thyroxine index (FTI) was developed to adequately reflect thyroid status; the formula used is ___.
- I-131 uptake

T4

b. T4

T3 uptake

c. T3 uptake x T4

d. T3 uptake x TBG

29. If a patient is tested for vitamin B-12 deficiency and received a value less than 7% in phase I and phase II, but urine values above 7% after antibiotics are given, then the most probable disease state is ___ .

- a. pernicious anemia
- b. gastrectomy
- c. blind loop
- d. disease of the ileum

30. Cyanocobalamin is ___ .

- a. important in cell maturation
- b. absorbed in stomach with intrinsic factor
- c. same as folates
- d. both a. and b.
- e. all of the above

31. Which is *not* needed for a Schillings test?

- a. blood sample
- b. urine sample
- c. standard
- d. cyanocobalamin injection

32. If a phase I Schillings test provided a 17% uptake on a patient, one should ___ .

- a. begin phase II using intrinsic factor
- b. consider patient normal and end testing
- c. administer antibiotics to make sure patient does not have sprue or gastro-colic fistula

33. The first procedure performed during Schillings test is ____.
- inject 1 cc (1000 micrograms) of non-radioactive vitamin B-12 (flushing dose)
 - administer cobalt-labeled cyanocobalamin capsule to patient
 - instruct patient to remain fasting for four hours
 - collect blood sample of patient
34. Which does not belong when evaluating vitamin B-12 deficiency?
- inability of gastric parietal cells to secrete intrinsic factor
 - gastrointestinal reflux and esophagal pain
 - gastric resection
 - inflammatory disease of ileum
 - all need to be evaluated
35. When evaluating B-12 by RIA, the isotope used is ____, whereas folic acid utilizes ____.
- cobalt, Tc-99m
 - I-125, cobalt
 - cobalt, I-125
 - I-125, cobalt
36. Normal ranges for vitamin B-12 are ____ while values less than ____ are almost always associated with vitamin B-12 deficiency.
- 200-900 pg/ml, 100 pg/ml
 - 200-800 pg/ml, 200 pg/ml
 - 3-20 ng/ml, 3 ng/ml
 - 100-500 ng/ml, 100 pg/ml
37. The most common test utilized for vitamin B-12 absorption after blood testing is ____.
- hepatic uptake test
 - fecal excretion method

- c. Schillings test
 - d. depends upon the type of absorption one desires
38. CEA testing is most *commonly* associated with what type of carcinoma?
- a. liver
 - b. uterus
 - c. fetal intestine
 - d. colon
39. Elevated levels of AFP between 25-100 pg/ml are associated with ____.
- a. primary liver malignancies
 - b. an indeterminate range that *no* diagnostic value is assessable
 - c. either benign or malignant liver diseases
40. Vitamin B12, which is not stored in the body, is excreted *primarily* via the ____.
- a. feces
 - b. urine
 - c. bile
 - d. all of the above
41. Intrinsic factor is a glycoprotein secreted by the ____.
- a. jejunum
 - b. stomach
 - c. duodenum
 - d. ileum
42. Absorption of vitamin B12 can be studied by one more of the following tests ____.
- a. fecal excretion
 - b. hepatic uptake
 - c. urinary excretion
 - d. all of the above
 - e. b. and d. only

43. The dose of Co-57 vitamin B12 given for a Schilling test is generally ____ .
- 0.5 uci
 - 5.0 uci
 - 50 uci
 - 500 uci
44. The most common cause of vitamin B12 malabsorption is ____ .
- drug therapy
 - small bowel blind loop
 - pancreatic insufficiency
 - deficiency in intrinsic factor
45. Failure to absorb dietary vitamin B12 will eventually lead to ____ .
- pernicious anemia
 - microcytic anemia
 - leukemia
 - hyperchromic anemia
46. Choose one that *does not* belong—Possible causes of malabsorption of Vitamin B12 include ____ .
- inability of stomach to secrete intrinsic factor
 - gastric resection
 - inflammatory disease of ileum
 - Down's syndrome
 - bacterial growth in stomach
47. High levels of HCG-B hormone in the blood corresponds highly with ____ .
- patient with abdomen cancer
 - patient is pregnant
 - patient has fever of unknown origin
 - impossible to diagnose without correlation to bone or liver scan

48. Pituitary-Gonadal negative feedback system means that ____.
- low levels of estradiol or testosterone stimulate hypothalamus to release gonadotropin
 - high levels of estradiol or testosterone stimulate hypothalamus to release gonadotropin
 - LH and FSH (low levels) stimulate sex hormone production
 - pituitary stimulates hypothalamic release of hormones at certain time intervals
49. LH and FSH are released by the ____.
- gonads
 - anterior pituitary
 - hypothalamus
 - a. and b. only
50. The Australia antigen, discovered in 1963 by Blumberg is also known as the antigen of ____.
- hepatitis A
 - hepatitis B
 - hepatitis C
 - hepatitis D
51. The effect of the hormone gastrin, secreted by the gastric antrum, is to ____.
- stimulate gastric acid secretion
 - suppress gastric acid secretion
 - increase gastric motility
 - decrease gastric motility
52. The standard time when blood for digoxin concentration measurements should be drawn is approximately ____ hours after the last digoxin dose was taken.
- 2 hours
 - 4 hours
 - 6 hours

- d. 8 hours
53. The serum Ck-B is elevated in cases of ____ .
- acute myocardial infarctions
 - chronic liver disease
 - ischemic heart disease
 - digoxin toxicity
54. The radiolabel used in Vitamin B12 RIA testing is ____ .
- I-125
 - Se-75
 - Co-57
 - I-131
55. The subunit which is immunologically identical for the glycoprotein hormones FSH, LH, TSH, and HCG is the ____ .
- Alpha subunit
 - Beta subunit
 - Gamma subunit
 - Delta subunit
56. Theophylline, which may be measured RIA, belongs to the class of drugs known as ____ .
- anticonvulsants
 - bronchodilators
 - antibiotics
 - vasodilators

Section 3

True or False

- 1. Acid-citrate-dextrose (ACD) is used to clot blood when making up Indium-111 labeled WBC's.
- 2. Lipid soluble oxine carries the In-111 across the cell membrane where In-111 separates from oxine.

- 3. *Labeled WBC will not distribute to liver, spleen, and functioning bone marrow in normal patients.*
- 4. *In-111 is the isotope and technique of choice (WBC labeling) when investigating abdominal abscesses.*
- 5. *As little as .0004 ml of blood contaminated with hepatitis B can transmit the disease.*
- 6. *Incubation of hepatitis B is approximately 3-4 days.*
- 7. *Digoxin is used to treat congestive heart failure.*
- 8. *IgE is an immunoglobulin class responsible for allergic reactions.*
- 9. *The normal range is 3-250 ng/ml in adults for CEA testing.*
- 10. *Alpha-fetoprotein plays a similar role in fetal life that serum albumin plays in adults.*
- 11. *One reason for high levels of CEA is the rapid growth of tissue resulting in disruption of blood tissue barriers.*
- 12. *Pancreas, colon, rectum, and lung carcinomas are associated with AFP.*
- 13. *Blood testing of carcinoma are useful in evaluating response to tumor therapy.*
- 14. *Liver disease and Cushing syndrome are associated with high levels of HCG.*
- 15. *The recommended intake of vitamin B12 is 2 ug/day.*
- 16. *Folates is a generic term for vitamin B12.*
- 17. *Folic acid is found in liver, yeast, and vegetables.*
- 18. *Vitamin B12 and folates are stored in small intestines until needed by the body.*
- 19. *Decreased levels of TSH with decreased levels of T4 is indicative of secondary hypothyroidism.*
- 20. *T3 uptake is an indirect estimate of capacity of plasma protein to bind thyroid hormone.*
- 21. *CEA levels become increased in pancreas, rectum and lung malignant cancers.*
- 22. *Alpha-fetoprotein is most commonly associated with liver malignancies such as hepatomas.*

Chapter 9

ALTERNATIVE METHODS TO RIA TESTING

Objectives

- Understand the basic differences between heterogenous and homogeneous immunoassay testing.
- Be able to distinguish the advantages and disadvantages of heterogeneous and homogeneous testing.
- Demonstrate an overall understanding of EIA, FIA, FPIA and monoclonal antibody testing including their function and methodology.

Alternative Methods to RIA Testing

All of the discussion within this manual thus far has centered on radioimmunoassay testing. This type of testing is called **heterogeneous** immunoassay. That is when there is no difference between the signals produced by bound or free labeled substances, therefore it is necessary to separate the two before measurement.

Homogeneous Immunoassay

In these types of testing procedures there is a difference between the signals generated by the free and the bound labeled substances. Separation is therefore unnecessary.

Example: EIA, FIA (optical) testing, FPIA testing. Difference between signals arise because the signal is either produced or altered upon binding.

	Heterogeneous	Homogeneous
	more sensitive	no separation technique
Advantages	more specific	easier, faster to perform
	less matrix interference	

Three common homogeneous immunoassay testing procedures will be briefly discussed:

- Enzyme immunoassay (EIA)
- (FLA or LIA) fluorescence and luminescence immunoassays
- Fluorescence polarization immunoassay (FPIA)
- Monoclonal antibodies

Enzyme Immunoassay (EIA)

Substance is labelled with an enzyme rather than I^{125} . This enzyme catalyzes another reaction within reaction matrix. This other reaction produces an optical signal.

EMIT (Enzyme Multiplied Immunoassay Test)

Enzyme GGPDH (Glucose-G-Phosphate Dehydrogenase)

Catalyzes NAD → NADH Nicotinamide Adenine Dinucleotide

But GGPDH is deactivated when bound to antibody measures change of absorbance at 340 nm produced by conversion of NAD to NADH.

When large amount of natural Ag, little Ab remaining to deactivate enzyme. Rate of conversion of NAD to NADH high as measured by increase of absorbance at 340 nm. Conversely low natural Ag high Ab to deactivate enzyme, rate NAD → NADH low.

Direct Relationship

Advantages

- Less expensive to run, can be automated.

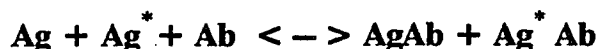
- No separation.
- Not labor intensive like heterogeneous.

Disadvantages

- Not as specific as RIA.
- Reaction medium critical.
- pH, ionic, temperature, other enzyme like lysozyme interfere 95% confidence level false negatives and false positives.

FIA or LIA Luminescence Immunoassays

Ag labeled with fluorophore but not active and an enzyme that release fluorophore - active but enzyme is ineffective when Ag is bound to Ab.



What happens to fluorescence when large Ag - goes up directly proportional.

Fluorescein - fluoresces when light added to system luminol produces light on own - no light to system.

Fluorescence Polarization Immunoassay (FPIA)



Ag* fluorescent labeled with a fluorophore when light at 485 nm (blue) hits fluorophore - raises it to an excited state (e- outer shell) when returning to ground level (e- come back), a different is emitted 525 - 550 nm (green).

How does it tell difference? B/W Ag* and Ag*Ab

Polarize the 485 nm blue light, Therefore absorption of the blue light takes place on a single plane. But during emission of green light, a rotation occurs or a depolarization and the 525 - 550 nm light is emitted on different plane.

But Ag* Ab hinders rotation B/W absorption and emission preventing depolarization of green light. Green light emits on the polarized plane so higher natural Ag, Lower the Ag* Ab and lower the degree of polarized (green) light (inversely proportional) degree of polarized light akin to radioactivity.

Advantages

- Specificity, stability like RIA
- Can be automated, not labor intensive

Disadvantages

- Cost

Monoclonal Antibodies

Generally when inject antigen, animal immune system activates broad range of antibody secreting cells each cell releases one molecular configuration antibody against antigen. Wind up with a mixed population of antibodies that bind with differing affinities—call polyclonal antibodies. Binding is also at different locations. This increases possible cross reactions to substances with similar configurations to a particular locations. To produce monoclonal antibodies, antibody secreting cells are fused with myeloma cells purpose: faster division genetic material of myeloma cells—provides the rapid division. Resulting “hybridomas” are separated with special techniques into separate cell colonies which produce only one antibody population.

REVIEW

Section 1

1. List and describe the four types of homogeneous testing methodologies described in the chapter.

2. List and describe the advantages and disadvantages of heterogeneous and homogeneous tests.

Section 2

1. Which blood testing technique is considered heterogeneous?

- a. fluorescence
- b. enzyme
- c. sandwich
- d. RIA

2. Enzyme immuno testing is the most common form of blood sample testing because ____ .
 - a. it's cheap and not labor intense
 - b. of it's sensitivity
 - c. of it's reliability
 - d. it produces the fewest false negative results
 - e. all of the above
3. Enzyme testing ____ and ____ . RIA testing ____ and ____ .
 - a. results are a direct relationship between counts and Ag
 - b. results are presented an indirect between counts and Ag
 - c. high sensitivity, high specificity
 - d. not labor intensive
4. Two substances that may interfere with analysis of drug testing are ____ .
 - a. opiates, free T₄
 - b. chlorine, salts
 - c. iodine and morphine
 - d. any of the two above
5. Which is not true of florescence blood sample testing?
 - a. absorbed florescence is polarized in blue light, emitted in green light
 - b. emission of light is due to rotation of plane or depolarization
 - c. high specificity, sensitivity and high cost
 - d. all of the above are true

Laboratory #1

Lab Safety & Pipetting Laboratory

SELECTING RADIOISOTOPE COUNTERS

In equipping a radioimmunoassay laboratory probably the most important piece of equipment is the auto-gamma scintillation spectrometer. All of the assays performed will depend upon the reliability and efficiency of this instrument. Considerations involving the selection of a gamma counter include: (1) the number of assays to be performed, (2) availability of service for the instrument, (3) capability of the instrument (e.g., does it have a single pulse height analyzer or more than one; does it have a rapid, clear printing system, etc.), and (4) the financial resources of the laboratory. If beta counting is to be performed, a high quality liquid scintillation system is mandatory.

THE LABORATORY FACILITY

As in any good laboratory, the RIA facility includes many components: adequate lighting and counter space, abundant cabinet storage for chemicals and glassware, convenient electrical outlets, a hood with an outside air escape (especially working with volatile chemicals or pathogenic antigens), vacuum, air, and gas sources, and a place for disposing of all radioactive waste.

General Laboratory Equipment

An assortment of glassware consisting of beakers, graduated cylinders, and Erlenmeyer flasks, should be on hand. Additional equipment includes graduated serologic pipettes (1, 5, and 10 ml); Pasteur pipettes; test tubes, both glass and plastic (polypropylene or polystyrene); and test tube racks.

A triple beam balance is necessary for weighing chemicals and balancing tubes for centrifugation. An analytical balance is occasionally required for certain assays for more accurate weight determination. Three or four timers capable of timing up to two hours are also useful. Magnetic stirrers and magnetic stirring bars are necessary for complete mixing of the many different solutions in the assays. A vortex mixer for test tube or vial mixing should also be available.

Many assays require careful regulation of the temperature; consequently, a water bath capable of maintaining temperatures from 37° to 100°C should be available. In addition to heat, ice is needed for a number of applications. Distilled water in plentiful supply represents an integral part of preparing any buffer or solutions needed in the assays.

A pH meter should be used to check the pH of many assay solutions. We recommend a pH meter with an expanded scale and single electrode.

A refrigerated centrifuge equipped with a horizontal head is the centrifuge of choice.

A refrigerator and freezer must also be available for storage of reagents, sera, etc.

Specific RIA Equipment

Automatic pipettes with disposable tips capable of delivering accurate volumes are required for all procedures (including commercial kits). The pipettes should be available in the following sizes: 5, 10, 20, 50, 100, 200, 500, 1,00 ul. Also needed is an automatic jar type pipette that can deliver volumes of 1 to 10 ml. This device allows the rapid delivery of known volumes of solutions used in the assays. A relatively new type pipette is the automatic pipetting station. This instrument was designed for high volume laboratories that perform very large numbers of studies. While expensive, this type of instrument promotes a greater degree of efficiency and reliability for the larger laboratory than is possible with conventional pipettes.

A calculator is needed to compute the results of the different assays. Also, a good bookkeeping system is critical. The receipt of the sample, the date of the assay, and the result must be carefully recorded and available for immediate retrieval.

Laboratory Safety and Pipetting problems with radiation safety include:

Personnel, monitoring, loss of data validity, and environmental pollution. Of these factors, the most prominent is lab contamination with radioactivity.

Lab Facility & Selection of Equipment in R.I.A.

1. Gamma scintillation spectrometer (counter). All of the assays performed will depend upon the reliability and efficiency of this instrument.
2. Adequate lighting and counter space.
3. Adequate cabinet storage for chemicals and glassware.
4. Beakers, graduated cylinders and flasks.
5. Graduated serologic pipettes (1, 5, 10 ml), tubes, test racks.
6. Triple beam balance for weighing and balancing tubes in centrifuge.
7. Magnetic stirrers, vortex mixers.
8. Temperature controls – water baths, distilled H₂O.
9. pH meter – check pH of many assay solutions.
10. Refrigerated centrifuge, refrigerator, and freezer to store reagents.

Specific R.I.A. Equipment

- Pipettes – 5 – 1,000 ul size.
- Automatic pipettes with huge volumes.
- Calculator to compute the results of different assays.

Contamination Control

1. Limit amount of radioactive material to just amount needed.
2. Surfacing materials should be chosen with contamination control in mind.
3. Areas designated for opening, transferring and handling radioactive materials.
4. Removable absorbent material or non-absorbent easy to clean material.
- 5.* Wear lab coats.
6. No eating or drinking.

Personnel Monitoring – film badge or TLD may be required depending upon type and amount of radioactive used.

Radioactive Material – Inventory, Storage, Disposal – all radioactive materials require legal documentation of each isotope - received and disposed - monitoring of expiration date.

Storage of radioactive expired kits may become prohibitive due to long half lifes.

The amount of activity that may be discharged into the sewage is restricted by the amount per unit volume and by the total amount of activity per year. Each isotope has its own specific limit.

Pipetting Laboratory

The purpose of this laboratory exercise is to allow students the opportunity to practice with a variety of different pipettes. Students will be able to demonstrate competence in their pipetting technique by the end of this lab.

1. Have students set-up racks of test tubes and number them 1-10.
2. Using various pipette sizes 10 ul - 200 ul students will practice pipetting H₂O into various test tubes 1-10.
3. Students will visually inspect the test tubes to see if the amounts dispensed were similar from tube to tube.
4. Using tracer I-125 from any R.I.A. test kit, have students set-up at least 2 different sets of test tubes and number them 1-10 and 11-20 respectively.
5. Students will choose any two pipettes from the variety available size 10 ul - 200 ul and attempt to pipette equal amounts into each set of ten then change pipette and dispense into next tubes 11-20.
6. Calculate C.V. for tubes 1-10.
7. Calculate C.V. for tubes 11-20.
8. Repeat until C.V. values are less than +/- 10%.

Ex: Using pipettes 20 ul and I-125 tracer and counting tubes in Gamma well counter the follows counts are recorded;

- | | | |
|-----|--------|---|
| 1. | 10,551 | <u>coefficient of variance</u> |
| 2. | 10,392 | $\%C.V. = \frac{SD}{\bar{X}} \times 100$ |
| 3. | 11,001 | \bar{X} |
| 4. | 10,845 | $S.D. = \frac{(X_i - \bar{X})^2}{n - 1}$ |
| 5. | 10,782 | $n - 1$ |
| 6. | 10,615 | $n = \text{total number of tubes used}$ |
| 7. | 10,432 | |
| 8. | 10,444 | $\bar{X} = \frac{\sum X}{n} = \frac{106,275}{10} = 10,627.5 \text{ round to } 10,628$ |
| 9. | 10,681 | $n \quad 10$ |
| 10. | 10,532 | |

$$(X_i - \bar{X})^2$$

$$10,551 - 10,628 = (-77)^2 = 5929$$

$$10,392 - 10,628 = (-236)^2 = 55,696$$

$$11,001 - 10,628 = (373)^2 = 138,384$$

$$10,845 - 10,628 = (217)^2 = 47,084$$

$$10,782 - 10,628 = (154)^2 = 23,716$$

$$10,615 - 10,628 = (-13)^2 = 169$$

$$10,432 - 10,628 = (-196)^2 = 38,416$$

$$10,444 - 10,628 = (-184)^2 = 33,856$$

$$10,681 - 10,628 = (53)^2 = 2,809$$

$$10,532 - 10,628 = (-96)^2 = \underline{9216}$$

355,275

$$\text{S.D.} = \frac{355,275}{9} = 198.6$$

9

$$\text{Calculate C.V. \%} = \frac{198.6}{10,628} \times 100 = 1.86\%$$

This is the percent variance or deviation for all ten test tubes that were pipetted.

Student Worksheet

1. After pipetting a known volume into the first ten tubes 1-10, count in gamma counter and record results.

- 1.
- 2.
- 3.
- 4.
- 5.
- 6.
- 7.
- 8.
- 9.
- 10.

2. Calculate $\bar{X} = \frac{\sum X}{n}$

3. Calculate $\sum (X_i - \bar{X})^2$

- | | | | | | |
|-----|-------|---|-------|-----|------|
| 1. | _____ | - | _____ | = (|)² = |
| 2. | _____ | - | _____ | = (|)² = |
| 3. | _____ | - | _____ | = (|)² = |
| 4. | _____ | - | _____ | = (|)² = |
| 5. | _____ | - | _____ | = (|)² = |
| 6. | _____ | - | _____ | = (|)² = |
| 7. | _____ | - | _____ | = (|)² = |
| 8. | _____ | - | _____ | = (|)² = |
| 9. | _____ | - | _____ | = (|)² = |
| 10. | _____ | - | _____ | = (|)² = |

4. Calculate S.D. = $\sqrt{\frac{(X_i - \bar{X})^2}{n - 1}}$

5. Calculate % C.V. = $\frac{\text{S.D.}}{\bar{X}} \times 100$

Laboratory 2

Normal Distribution , Confidence Intervals and Percent Error Laboratory

Data Given: 20 individual counts

- | | | | |
|-----|--------|-----|--------|
| 1. | 15,505 | 11. | 17,981 |
| 2. | 14,901 | 12. | 18,002 |
| 3. | 13,876 | 13. | 16,544 |
| 4. | 19,351 | 14. | 16,994 |
| 5. | 18,411 | 15. | 15,456 |
| 6. | 18,302 | 16. | 17,893 |
| 7. | 16,451 | 17. | 16,543 |
| 8. | 17,339 | 18. | 15,988 |
| 9. | 16,272 | 19. | 16,901 |
| 10. | 14,981 | 20. | 17,042 |

1. Calculate $\bar{X} = \frac{\sum X}{n} = \underline{\hspace{2cm}}$

2. Calculate ± 1 S.D. =

What is the range in counts for 68% confidence ?

3. Calculate ± 2 S.D. =

What is the range in counts for 95% confidence ?

4. Calculate ± 3 S.D. = _____
What is the range in counts for 99% confidence _____?
5. Is ct. number (3) 13.876 outside 3 S.D. = _____
6. Is ct. number (4) 19,351 outside 3 S.D. = _____
Why or why not? _____
7. How many cts. would be needed to be 99% confident and $\pm 2\%$ error?
Show work _____.
8. If a source is counted provides 100 ct/sec, how long will you need to count this source to be within 95% confidence and $\pm 8\%$ error?
Show work _____.
9. A source is counted in a nuclear medicine department during a routine liver scan gives us 17,500 counts what is the % error?
Show all work _____.
10. What percent confidence is necessary for research?

Laboratory 3

Examining Principles and Procedures for Reagents

The purpose of this laboratory exercise is to have students become familiar with the component parts of a typical R.I.A. booklet used in Nuclear Medicine.

In this laboratory, the student will use a booklet on an R.I.A. procedure commonly performed in your lab (ex.: T4, T3, digoxin, Haa, Cortisol, etc.).

1. What is the intended use of this kit?
2. Why is it important to know the level (quantity) in samples?
3. How does this blood level (T4, Digoxin, CEA, FSH, etc.) function in the body?
4. What is the basic principle of this assay? Competitive or non-competitive?
5. List and describe each component in this test.
6. What supplies and materials are needed?
7. How do you prepare reagents?
8. Explain and describe the protocol used in this test?
9. What precautions (if any) need to be noted?
10. What are the quality control measures?
11. Limitations of test?
12. What is the (Ag), (Ab), standards, sample in test?

Procedure 2

Set up a worksheet explaining which tubes correspond to which item or component, allowing space for values obtained after running the test.

For example:

Test tube #'s	Component
1,2	total counts (Ag* only)
3,4	NSB (non-specific binder from manufacturer plus standard "0")
5,6	standard - "0" concentration
7,8	standard - 2.5 concentration
9,10	standard - 5.0 concentration
11,12	standard - 10.0 concentration
13,14	standard - 15.0 concentration
15,16	standard - 30.0 concentration
17,18	patient unknown (Mary Smith)
19,20	control A (high count rates)
21,22	control B - (low count rates)

Resultant values:

The purpose of any R.I.A. test is to ascertain the value of the unknown or patient, or Mary Smith in this case.

Procedure 3

Adding appropriate reagents: assume we are testing Mary Smith for a T₄ blood concentration level.

Pipette into test tubes 1 and 2 the appropriate amount of total counts from the kit, let's say 20ul.

Follow your worksheet and add the specified amounts of each standard (20ul) to their corresponding tubes.

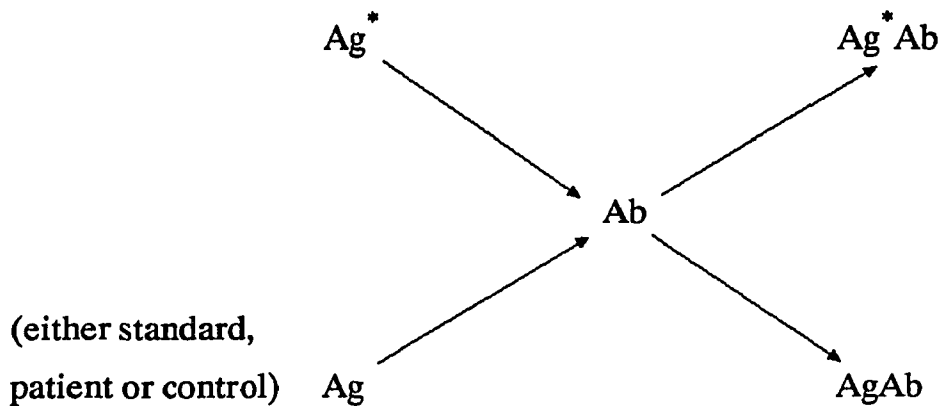
Add patient serum (20ul) to the appropriate tubes (15 and 16).

Add control A to tubes 17 and 18, and add control B to tubes 19 and 20.

Pipette Ag* (radioactive antigen) to each test tube (1-20) in a given amount (100 ul for our purposes).

Now, pipette the Ab into each test tube (1-20) in a given amount (let's say 200ul).

At this point, competition for binding sites on the Ab begins between either non-radioactive Ag, the NSB, standards, patient unknown, control and the radioactive (usually I-125), labeled ligand (Ag*).



The higher the concentration of NSB, standards, patient or control, the less the binding of I-125 to the Ab binding sites. Thus, when counting the radioactivity, there is an inverse relationship between counts and concentration, which will be demonstrated shortly.

Procedure 4

Incubation of reagents: allow the reaction to reach a state of equilibrium ($K_1 - K_2$). Depending on the type of test performed, this incubation may be at room temperature or may require heating in water baths from, typically, 10 minutes to 2 hours.

Procedure 5

Centrifugation: this allows for binding to be completed and aids in separation.

Procedure 6

Separation of bound from free: the results of the test are obtained from counting the bound portion only. This is typically done by decanting the reagents nor bound into a sink or toilet.

Procedure 7

Count tubes in gamma well counter.

Procedure 8

Analyze resultant values: refer back to Mary Smith's T₄ test.

The manufacturer has sent known concentrations on components referred to as the standards at various values and have also supplied us with controls of known values.

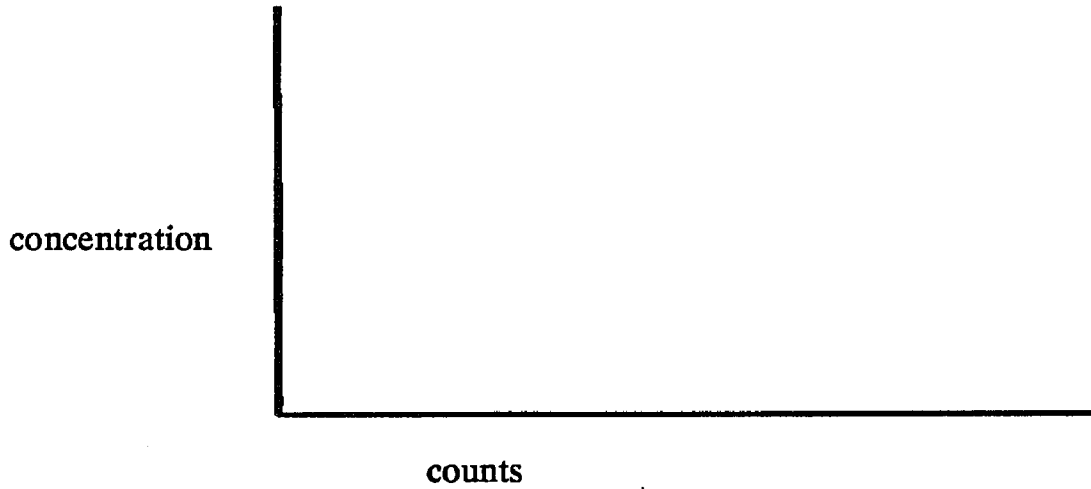
If the normal values for a T₄ patient is known, let's say 5-20ug/dl, we can analyze the results. After running the test,

assume the following data were obtained:

<u>TEST TUBE</u>	<u>COUNTS</u>	<u>COMPONENTS</u>
1	25,404	total counts
2	24,355	"
3	21,456	NSB
4	20,596	"
5	19,347	"0" concentration
6	19,562	"
7	16,322	2.5 concentration
8	16,848	"
9	13,471	5.0 concentration
10	13,484	"
11	10,958	10.0 concentration
12	11,651	"
13	8,945	15.0 concentration
14	8,886	"
15	6,842	30.0 concentration
16	6,731	"
17	1,785	patient-Mary Smith
18	1,698	"
19	15,421	Control A (low)
20	15,822	"

21	7,345	Control B (high)
22	7,269	"

Plot the standard curve for the above:



Interpolate the values for Mary Smith and controls A and B.

Laboratory 5

R.I.A. LABORATORY

Running and Analyzing Resultant Values for Common R.I.A. Tests

This lab should be run at least 2 different times by students.

1. **Set up worksheet including all components in assay listed in packet insert, such as: (see typical R.I.A. testing LAB for example)**

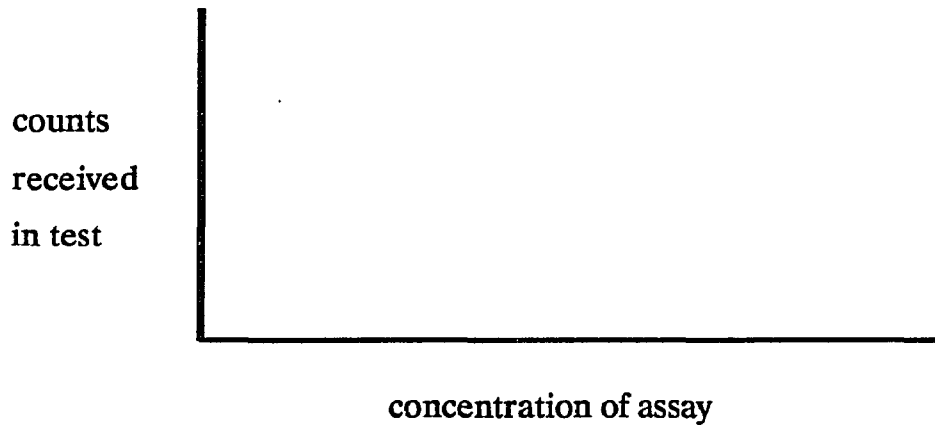
total counts, NSB, B₀, all standards, controls and patient unknowns

Do all test tubes in duplicates. For example:

COMPONENT	TEST TUBE #
total counts	1,2
NSB	3,4
B ₀	5,6
standard 1	7,8
etc ...	

2. **Perform test as described in packet insert in test kit.**
3. **Count tubes in gamma well counter.**

4. Plot counts received vs. known concentration of standard on graph paper.



5. Interpolate resultant values for controls and patient unknowns in graph on original worksheet.

WORKSHEET

TUBE #	COMPONENT	COUNTS	INTERPOLATED RESULTS
---------------	------------------	---------------	-----------------------------

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

A Typical R.I.A. Procedure Protocol

THC used as an example

Details of the Procedure

A standard curve must be constructed for each set of unknowns assayed. Follow the order of the steps exactly.

1. Label and arrange the assay tubes according to the assay protocol shown in Table 2.
2. Dilute the ^{125}I -THC derivative with BSA buffer (1:50) according to the instructions for dilution of tracer. Add 200ul working tracer to the total count tubes, stopper and set them aside for counting at the completion of the assay.
3. Add 50ul cannabinoid standard to the correspondingly numbered tubes. Note that the 0ng/ml standard is dispensed into the NSB and zero standard tubes.
4. Add 50ul of the urine low control and high control to the correspondingly numbered tubes.
5. Add 50ul of the urine samples to their correspondingly numbered tubes.
6. Add 100ul of the BSA buffer to the NSB tubes.
7. Add 100ul of the THC antibody to all assay tubes (do not add antibody to NSB tubes).
8. To each assay tube add 200ul of the working tracer solution.
9. Add 200ul of second antibody complex to all tubes (mix the second antibody well before use).
10. Gently vortex mix all tubes and incubate the entire assay for 15 minutes at room temperature (25°C). The assay rack may be covered with Parafilm (optional). Avoid incubating in direct sunlight.
11. Centrifuge the assay tubes at 2500g for 15 minutes.
12. Replace the assay tubes in their rack. Aspirate the supernatant fluid taking care not to disturb the pellet (See Note 3). Count all assay tubes in a gamma counter for at least one minute per tube.

Laboratory 6

Equipment Performance Evaluation Laboratory

There are three basic ways of analyzing your gamma counter's effectiveness and unbiasedness of results.

1. Chi-square test (checks for reliability of counter)
2. Efficiency (checks for sensitivity of instrument)
3. Background check (rules out contamination)

A test that is performed to ensure that variation from a series of individual counts are likely to be due to random nature of radioactive decay and not by other influences.

Part 1

Using a single source (T-1/2 many wks) I-125

1. count a series of 1 min cts
2. count this same sample 20X
3. Place values in appropriate spaces
4. Calculate Chi-square value
5. Compare to tabulated results

Is instrument reliable?

Why or why not?

This set of data of different counts are compared to the theoretical variation that one would expect from random sampling.

$$\text{Chi-square test } \chi^2 = \varepsilon \frac{(X - \bar{X})^2}{\bar{X}}$$

compared calculated result to tabulated (P) values

(P) value should be around (.5) must fall between .1-.9 or equipment is considered faulty.

DATA FROM COUNTING I-123 SOURCE 20 TIMES

1.	11.
2.	12.
3.	13.
4.	14.
5.	15.
6.	16.
7.	17.
8.	18.
9.	19.
10.	20.

Part 2

Another way to analyze equipment is by calculating the efficiency.

This is performed by comparing the number of counts registered to the number of potentially effective emissions from the source.

$$\frac{\text{CPM}}{\text{DPM}} = \frac{\text{Counts per/min}}{\text{disintegrations per/min}}$$

1. Take a known NBS standard source CS-137, 50-60, calculate the DPM based on its decay.

$$1 \text{ curie} = 3.7 \times 10^{10} \text{ dps}$$

$$1 \text{ curie} = 22 \times 10^{12} \text{ dpm}$$

show all work

2. Count the source in the well-counter 10 times for 1 min each and record data.

- | | |
|----|-----|
| 1. | 6. |
| 2. | 7. |
| 3. | 8. |
| 4. | 9. |
| 5. | 10. |

3. Based on the average \bar{X} of these count rates/min calculate:

$\frac{\text{CPM}}{\text{DPM}}$ for this gamma well counter

Laboratory 7

Laboratory Worksheet for Indium-111 Labeled for Leukocytes

I.

Patient information: (Patient should not be dehydrated)

Date: _____

Hospital: _____

Patient Name: _____

Indium Rx #:

WBC Count:

II.

Radiopharmaceutical preparation: Check off each step as completed.

- ___ 1. Time blood was drawn: _____ Volume of blood drawn: _____ mls 50ml.
- ___ 2. Plasma volume after separation: _____ mls (Approx. 25ml, Need at least 15ml)(10cc HESPAN if needed after 30min, Doctors Cons.)
- ___ 3. Centrifugation for 5 minutes at 400-450g (setting 70) and separate WBC button from supernate.
- ___ 4. Draw off platelet-rich plasma (PRP) and save.
- ___ 5. Saline wash: Wash button with 3mls of saline. Centrifuge for approximately 5 minutes at 400-450g. Draw off wash and discard.

- ___ 6. Reconstitute the WBC button using approximately 3mls of saline.
Break up button with needle.
- ___ 7. Activity added to button: ___ uci Assay time ___
- ___ 8. Total incubation time: _____
- ___ 9. Centrifuge PRP for 10 minutes at 1000g (setting _____ 90) Yielding
PPP top layer.
- ___ 10. Plasma wash 2-5mls of PPP from top layer. Let stand for 5 minutes.
- ___ 11. Centrifugation at 400-450g for five minutes. Draw off hot supernate
and save.
- ___ 12. Resuspend button with 4mls of plasma (PPP top).
- ___ 13. Assay of hot supernate and discard: _____ uci.
- ___ 14. Assay of WBC button: _____ uci.
- ___ 15. Labeling efficiency of In-111 labeled WBC:

$$(14) \frac{\text{_____}}{\text{_____}} / (7) \frac{\text{_____}}{\text{_____}} \times 100 = \frac{\text{_____}}{\text{_____}}$$

_____ time
 _____ uci
 _____ ml

Laboratory 8

Linearity Testing in R.I.A. Procedures

RIA/THC LAB

* note this lab exercise utilizes a THC kit, which is commonly used in RIA labs, but any other kit can be substituted.

Introduction

The basic purpose of this lab is to study serial dilution linearity of natural human urine containing cannabinoid metabolites (primarily 11-NOR-D⁹-THC-9-CARBOXYLIC ACID). There is evidence showing that some urine specimens containing cannabinoid metabolites do not maintain a linear response to RIA activity upon serial dilution, while others do. The reason for this is unclear. Possible explanations include solubility changes influenced by the amount of protein present, or the presence of an interfering substance which becomes more pronounced with decreasing concentrations of antigen. As this lab develops, depending on results, these, and other possible influences, will be explored.

Basic Procedure

Dilute a urine specimen containing between 100 and 200 ng/ml THC metabolite:

	<u>URINE</u>	<u>DILUTANT</u>	<u>TOTAL VOLUME</u>
1:2	200ul	200ul	400ul
1:4	100ul	300ul	400ul
1:6	100ul	500ul	600ul
1:9	50ul	400ul	450ul
1:11	50ul	500ul	550ul
1:16	20ul	300ul	320ul
1:21	20ul	400ul	420ul

Using (1) water, (2) negative natural urine, (3) negative synthetic urine. Compare results. This needs to be preformed on several different urine samples.

For values between 200 and 400 ng/ml

	<u>URINE</u>	<u>DILUTANT</u>	<u>TOTAL VOLUME</u>
1:4			
1:6			
1:9			
1:11			
1:16			
1:21			
1:31	20ul	600ul	620ul

For values between 400 and 800 ng/ml

1:9		
1:11		
1:16		
1:21		
1:31		
1:42	dilute	1:21, 1:2
1:62	dilute	1:31, 1:2

Construct curves to determine linearity. In particular, point where linearity begins to deviate should be noted. In addition, any consistency in holding to, or deviating from linearity should be recorded.

Laboratory 9

Effects of Time on R.I.A. Testing Procedures

RIA/THC LAB II

Introduction

It is well known in RIA testing that time is a critical parameter in the outcome of the analysis. This fact becomes crucial when considering the workup itself involving adding reagents in a sequential fashion. The purpose of this lab is to determine the total effect of time difference between the front and end of the assay on the final results. Only the calibrators and controls contained in the THC kit are needed.

Basic Procedure

The high and low controls are to be compared by running two sets (everything in duplicate including calibrators, therefore one set of high and low controls equals 4 tubes) per assay - one before and one after the calibrators. Since there are seven calibrators, each assay will have 18 tubes (19 with total tube). One high and low control set will assume the positions for unknown samples during counting.

Following package insert, add all reagents as readily as possible recording time. Repeat, but doubling the time for adding reagents. Repeat again doubling once more. Compare values calculated for the high and low controls at the beginning and end of the assay.

Repeat the above procedure adding isotope first, 2nd antibody second, and 1st antibody last. Compare.

Laboratory 10

Altering the Standard Curve

RIA/THC LAB III

Introduction

The proportions of reagents added in the THC assay according to the package insert are calculated to provide the most beneficial curve between the highest (100) and lowest (0) calibrators. It is possible, however, to alter the curve by changing reagent and sample ratios. Occasionally, it is desirable to make such an alteration. For instance, when assaying a sample containing THC metabolite above the range of the curve, you could dilute the sample to fit the curve, or you could alter the curve to fit the sample.

Basic Procedure

Using calibrators only, using the package insert proportions as a base, run the following assays with the indicated changes in reagents or calibrators. You should have already run a normal curve for comparison. Everything in duplicate. P = package insert.

<u>CALIBRATOR</u>	<u>1ST ANTIBODY</u>	<u>ISOTOPE</u>	<u>2ND ANTIBODY</u>
X2	P	P	P
X4	P	P	P
P	X2	P	P
P	X2	X2	P
P	P	X2	P
P	X4	X4	P
P	P	P	X2
P	X4	X4	X4

Additional changes may be tried as stimulated by above results.

Compare and explain variations of curves. Which assay could be used to determine a higher level which normally would have to be diluted? Which assay would be more sensitive to low levels?

Laboratory 11

Plasma Volume

Purpose

Plasma volume studies are a great asset in treating patients suffering from congestive heart failure, nephrosis, cirrhosis, polycythemia, and the anemias. It is especially useful when doing serial studies on patients in whom large blood losses and blood replacements are apt to occur.

Rationale

The radioiodinated human serum albumin being a protein, travels solely with the proteins in the plasma.

Dosage and Radiation

The dosage of 10 c of radioiodinated human serum albumin is so small that the total radiation dose is approximately 0.2 rads. In the case of a daily serial study, the doses run 10 uc on the first day, with 20 uc on the second day, and 40 uc on the third day. The increased dosage is necessary because it is essential that the patient's sample count be at least twice background. This dosage increase insures adequate counting statistics.

Patient Preparation

None.

Material and Equipment

1. Human serum albumin ^{131}I (IHSA) – 15 microcuries
2. Syringes
 - a. = 20ml. (1)
 - b. 10ml. (3)
3. Needles - 20 gauge
4. Timer
5. Microhematocrit and capillary tubes
6. Centrifuge - capable of spinning 10 ml. samples at 2000 rpm.
7. Pipettes - 3 ml. (3)
8. Test Tubes
 - a. glass (3) 13 mm
 - b. plastic (3) 13 mm
9. Scintillation well counter

PROCEDURE

1. Obtain patient's weight and height.
2. Dilute 15 uc ^{131}I IHSA in a 30 ml. saline vial to which has been added 1 ml. or 0.5 mg. human serum albumin.
3. Withdraw 20 ml. and count the activity in the syringe at a fixed distance above the well with the analyzer set at 150 Kev to infinity.
4. If the patient had any previous isotope procedure, draw 10 ml. of whole blood in a heparinized tube as a control. Inject 20 ml. of diluted IHSA into the patient.
5. Withdraw 10 ml. of venous blood at 10 and 15 minutes post injection from opposite arm not receiving transfusion.
6. Count the empty ^{131}I IHSA syringe.
7. Do hematocrit on both patient samples.
8. Centrifuge the samples for 15 minutes at 2000 rpm.
9. Pipette 3 ml. of plasma from both 10 and 15 minute samples and count the radioactivity in the well using setting in step. Record the background.

CALCULATIONS

Total plasma activity is equal to counts before injection minus counts after injection times the distance factor. NOTE: (Distance factor utilizes the inverse square law whereby the syringe is counted a certain distance from the crystal and the counts are decreased by a fixed and known amount). If a distance factor is not established, simply dilute 1 ml. of the remaining 10 ml. saline solution mixture to 100 ml. Pipette 1 ml. of the dilution and count in the well.

$$\text{Plasma volume} = \frac{\text{Net Standard Count} \times \text{Dilution}}{\text{Net Sample Count/ml}}$$

$$\text{Whole Blood volume} = \frac{\text{Plasma Volume}}{1 - (\text{Hct} \times 0.91)}$$

Red cell mass - Whole Blood volume - Plasma volume

Express all values as cc per wt. in kg.

Laboratory 11

^{51}Cr RED CELL MASS

PURPOSE

Blood volume studies are a great asset in treating patients suffering from congestive heart failure, nephrosis, cirrhosis, polycythemia and anemias. It is also used to follow the status of the patient who has had open heart surgery and other major surgery with associated large blood loss and replacement.

RATIONALE

^{51}Cr as sodium chromate is present in the hexavalent (+6) state and readily penetrates the red cell to attach to the hemoglobin where it is reduced to the trivalent (+3) state.

MATERIAL AND EQUIPMENT

1. A-C-D (Strumia formula) solution
2. Sodium chromate (^{51}Cr) - 60 microcuries
3. Syringes - 50 cc (2), 10 cc (2)
4. Needles - 18 gauge, 20 gauge
5. Hematocrit tubes
6. Test tubes (8)
7. Volumetric flasks - 100 cc (2)
8. Heparin solution - 1000 USP units per cc
9. Timer
10. Centrifuge
11. Tourniquet

12. Scintillation well counter

PROCEDURE

1. Obtain patient's weight and height.
2. Withdraw the 10 ml. of Strumia formula ACD solution into a 50 ml. syringe.
3. Into the same syringe, draw 40 ml. of whole blood to make a total volume of 50 ml.
4. Expel the entire syringe contents back into the tagging vial. Use an 18 gauge needle to avoid hemolysis and have a 20 gauge needle for an air-way.
5. Add 60 uc $\text{Na}_2^{51}\text{CrO}_4$ to the vial and let stand at room temperature for 15 minutes with occasional mixing every 2-3 minutes.
6. Withdraw 40 ml. from the mixture and weigh the capped syringe. The remaining 10 ml. will be used as the standard.
7. If the patient had any previous isotope procedure, withdraw 15 ml. of whole blood as a background control.
8. Have physician inject the tagged blood back into the patient.
9. At 10 and 15 minutes post injection, withdraw 15 ml. whole blood from the contralateral arm into a heparinized syringe.
10. Do hematocrit on both patient and sample and on standard.
11. Pipette 3 ml. whole blood from each patient sample.
12. Pipette 1 ml. of whole blood standard and dilute to 100 ml. with distilled water. Pipette 3 ml. of this dilution for counting.
13. Centrifuge standard and both patient's samples for 15 minutes at 2000 rpm.
14. Pipette 3 ml. plasma from each patient sample.
15. Pipette 1 ml. of plasma standard and dilute to 100 ml. with distilled water. Pipette 3 ml. of this dilution for counting.
16. Count all samples in a scintillation well-type counter and record the background.

CALCULATIONS

Red Cell Mass =

Where:

- a. Standard whole blood count
- b. Standard plasma count
- c. Hematocrit of the standard
- d. Patient's whole blood count

- e. Patient's plasma count
- f. Patient's hematocrit

Vwbi. Volume of whole blood injected

$$\text{Whole Blood Volume} = \frac{\text{Red Cell Mass}}{\text{Pt. hct} \times 0.918}$$

$$\text{Plasma volume} = \text{Whole Blood volume} - \text{Red Cell Mass.}$$

Laboratory 11

Principle of Dilution Laboratory Exercise

Formulas

$$Q = V \times C \text{ or } V = Q/C$$

Q = total quantity of tracer

C = tracer concentration in diluting fluid

V = diluting volume

$$V_1C_1 = V_2C_2$$

In calculating blood volumes if either plasma volume or red cell volume is determined, the total blood volume can be calculated with the aid of hematocrit.

$$\text{PLASMA VOLUME} = \frac{\text{Net standard counts} \times \text{dilution}}{\text{Net sample counts/ml}}$$

$$\text{WHOLE BLOOD VOLUME} = \frac{\text{Plasma volume}}{(1-\text{Hct.} \times 0.91)}$$

OR

$$\frac{\text{Red blood cell volume}}{(\text{hematocrit} \times 0.91)}$$

EXAMPLE: 15uCi of I-125 HSA in a 10ml syringe = $\frac{15\text{uCi}}{10\text{ml}}$

This material is then tagged to patient's plasma and reinjected into the individual's arm. Fifteen minutes later, 10ml of blood is withdrawn from patient. Activity is calculated as:

$$\frac{0.023\text{uCi}}{10\text{ml}}$$

Substitute known values into dilution equation:

$$V_1C_1 = V_2C_2$$

$$\frac{15\text{uCi}}{(10\text{ml})(10\text{ml})} = \frac{0.023\text{uCi}}{V_2(10\text{ml})}$$

$$15\text{uCi} = 0.0023V_2$$

So, patient's plasma volume = 6521ml (this is V_2)

Normal plasma volum = 43 ml/kilogram of body weight

Calculated hematocrit = 49%

Whole blood volume = 6.5 liters = 11.81 liters of blood
(1 - .49 x 0.91)

Students will calculate blood volumes on the following patients:

1. patient's weight = 92kg

$$V_1 = 15\text{ml}$$

$$C_1 = \frac{10\text{uCi}}{15\text{ml}} \text{ (use I-131 HSA for plasma determination)}$$

$$C_2 = \frac{0.035\text{uCi}}{15\text{ml}}$$

$$\text{hematocrit} = 0.41$$

PATIENT'S BLOOD VOLUME _____ Is patient normal?

Why or why not? SHOW ALL WORK ...

2. patient's weight = 117kg

$$V_1 = 10\text{ml}$$

$$C1 = \frac{10\text{uCi}}{10\text{ml}}$$

$$C2 = \frac{0.078\text{uCi}}{10\text{ml}}$$

$$\text{hematocrit} = 0.56$$

PATIENT'S BLOOD VOLUME _____ Is patient normal?

Why or why not? SHOW ALL WORK ...

Laboratory 12

SCHILLING

Purpose

To determine the ability to absorb Vitamin B₁₂.

Rationale

Oral Vitamin B₁₂ combines with a small polypeptide in the gastric juice. This polypeptide is secreted by the parietal cells of the gastric mucosa, and usually is referred to as Intrinsic Factor. The Intrinsic Factor combines with Vitamin B₁₂, the complex is later absorbed as it passes into the ileum portion of the small intestine. Diseases such as Pernicious Anemia, total Gastrectomy, and even some subtotal gastrectomized patients have a deficient Intrinsic Factor secretion and will fail to absorb Vitamin B₁₂ in the ileum. Other gastrointestinal disease such as sprue, tuberculosis of the ileum, regional ileitis, lymphomatous infiltration of the ileum, may also show failure to absorb Vitamin B₁₂. Blind loops caused by Billroth I procedure or multiple small intestinal diverticuli absorb Vitamin B₁₂ because of preutilization by resident blind loop bacteria. Administration of broad spectrum antibiotics will kill the bacteria and correct this condition thereby causing normal Vitamin B₁₂ absorption. Short circuiting procedures or fistula formation causing bypassing of the ileum as well as severe diarrheal states will also have defective Vitamin B₁₂ absorption.

Oral Vitamin B₁₂ Absorption

State	B ₁₂ alone	B ₁₂ with Int. Factor	After Antibiotics
Normal	+		
P.A.; Gastrectomy	-	+	
Blind Loop	-	-	+
Disease of Ileum	-	-	-
Sprue	+/-		
Gastro-colic Fistula	-	-	-

Patient Preparation

The patient is to be in an overnight fasting state from solid food, liquids are allowable. He is then instructed to abstain from solid foods for the next four hours, coffee and water are permissible. In the case of diabetics, coffee, toast, and jelly are allowable one hour after the onset of the procedure.

Material and Equipment

1. Cyanocobalamin Injection (Vitamin B₁₂) – 1000 ugm/cc
2. Cyanocobalamin (⁵⁷Co or ⁶⁰Co) – 0.5 microcuries
3. Reference Standard (⁵⁷Co or ⁶⁰Co) – 1:50 dilution of 0.5 microcuries.
4. Gallon jug

Procedure

1. Administer the cobalt-labeled cyanocobalamin capsule to the fasting patient with water.
2. Inject 1 cc. (1000 micrograms) of non-radioactive Vitamin B₁₂ intramuscularly immediately following the ingestion of the capsule.
3. Instruct the patient to remain fasting for four hours and to collect all of the urine excreted during the following 24 hours.

Urine Calculations

1. Measure the 24-hour urine volume and pipette a 20 cc. aliquot for counting.
2. Pipette 1 cc. of standard and dilute to 20 cc. This is equal to 2% of the administered dose, or 1/50 of the standard.
3. Count the urine aliquot and standard in the well for three minutes. Determine a background count.

$$\% = \frac{(\text{cpm in patient's sample} - \text{Bkgd}) \times \frac{\text{Total volume}}{20}}{(\text{cpm in standard sample} - \text{Bkgd}) \times 50} \times 100$$

SCHILLINGS TEST FOR VITAMIN B-12 EXCRETION

Using the formula:

$$\text{Vitamin B-12 \% uptake} = \frac{\text{CPM in pt.} - \text{bkgd.} \times \frac{\text{total vol.}}{20}}{\text{CPM in standard sample} - \text{bkgd.} \times 50} \times 100$$

Normal value = greater than 7%

Example:

CPM in pt. = 65,360
 Bkgd. = 9,520
 Total volume = 1600 cc.
 CPM in standard
 sample = 62,335

$$\frac{\text{CPM in pt. } 65,360 - \text{bkgd. } 9,521 \times \frac{\text{total volume } 1600 \text{ cc.}}{20}}{\text{CPM in std. } 62,335 - \text{bkgd. } 9,526 \times 50} \times 100$$

% Excretion = 14.5%

Pt. is normal for B-12

LABORATORY EXERCISE FOR SCHILLING TEST EVALUATION

1. Each student will properly calculate the % of Vitamin B-12 from the two (2) sets of data given.
2. Each student will then provide a diagnosis based on values obtained from each pt's data.

Pt. #1

Phase II

Data collected using Intrinsic Factor

$$\text{Vitamin B-12 \% uptake} = \frac{\text{CPM in pt.} - \text{bkgd.} \times \frac{\text{total vol.}}{20}}{\frac{\text{CPM in standard} - \text{bkgd.} \times 50}{\text{sample}}} \times 100$$

CPM in pt. 1 = 10,351
 Bkgd. = 105
 Total volume = 1200 cc.
 CPM in std. = 140052

% of vitamin B-12 is =

Diagnosis =

Pt. #2**Data collected in Phase I using Intrinsic Factor**

CPM in pt. 2 = 1,005

Total volume = 1800 cc.

CPM in std. = 37,562 cc.

Bkgd. = 501

% of vitamin B-12 is =

Diagnosis =

Answer Key to Questions

Chapter 1

<u>Section 1</u>	<u>Section 3</u>	<u>Section 4</u>
1. J	1. C	1. F
2. F	2. D	2. F
3. H	3. C	3. T
4. I	4. D	4. T
5. E	5. D	5. F
6. G	6. C	6. T
7. B	7. C	7. F
8. C	8. D	
9. D	9. D	
10. A	10. C	
	11. D	
	12. A	
	13. A	
	14. A	
	15. B	
	16. C	
	17. C	

Chapter 2

<u>Section 1</u>	<u>Section 2</u>
1. B	1. F
2. D	2. T
3. A	3. T
4. A	4. F
5. D	5. F
	6. T
	7. F

Chapter 3

<u>Section 2</u>	<u>Section 3</u>
8. T	1. C
9. F	2. D
10. T	3. C
11. F	4. E
12. F	5. D
13. F	6. D
14. F	7. B

Chapter 4

<u>Section 2</u>
1. C
2. B
3. 11.1 Sec
4. B
5. C

Chapter 5

<u>Section 2</u>	<u>Section 3</u>
1. D	1. F
2. E	2. F
3. C	3. T
4. D	4. F
	5. F

Chapter 6

1. F	7. F
2. E	8. F
3. B	9. F
4. T	10. F
5. T	11. T
6. T	

Chapter 7

1. B	6. C	11. B	16.C
2. D	7. B	12. C	17.A
3. B	8. C	13. A	18.D
4. B	9. D	14. B	19.C
5. C	10. D	15. C	20.A

Chapter 8**Section 1**

- | | | | | | | |
|-------|-------|-------|-------|-------|-------|------|
| 1. B | 1. B | 11. B | 21. B | 31. A | 41. B | 51.A |
| 2. A | 2. B | 12. A | 22. A | 32. B | 42. D | 52.D |
| 3. C | 3. A | 13. B | 23. D | 33. B | 43. A | 53.A |
| 4. B | 14. B | 24. B | 34. B | 44. D | 54.C | |
| 5. A | 15. B | 25. C | 35. C | 45. A | 55.A | |
| 6. A | 16. A | 26. B | 36. A | 46. D | 56.B | |
| 7. E | 17. B | 27. B | 37. C | 47. B | | |
| 8. E | 18. C | 28. C | 38. D | 48. A | | |
| 9. D | 19. C | 29. C | 39. C | 49. A | | |
| 10. A | 20. A | 30. D | 40. B | 50. B | | |

Section 2

- | | | |
|-------|-------|-------|
| 1. F | 11. T | 21. T |
| 2. T | 12. F | 22. T |
| 3. T | 13. T | |
| 4. F | 14. F | |
| 5. T | 15. T | |
| 6. F | 16. F | |
| 7. T | 17. T | |
| 8. T | 18. F | |
| 9. F | 19. T | |
| 10. T | 20. T | |

Chapter 9

1. D
2. A
3. A,B
4. B
5. D

BASIC RIA VOCABULARY LIST

ACCURACY	☒ Closeness to true or real value.
AFFINITY	☒ Strength (energy) of binding to the receptor.
ANALYTE	☒ The patient sample.
ANTIGEN (Ab)	☒ A protein formed as part of an immunologic response to foreign substance.
ANTIGEN (Ag)	☒ A substance capable of including formation of antibodies.
ANTISERUM	☒ Serum form an immune animal (contains various antibodies).
AVIDITY	☒ Measure of the strength of the bound Ag.
CONTROLS	☒ May be a standard or any known concentration of testing material that serves as a comparison between successive test runs.
CROSS REACTIVITY	☒ Similar structured Ag that interfere with analysis of a given assay.
EQUILIBRIUM SATURATION	☒ Competition between labeled and unlabeled ligand is allowed to occur simultaneously until equilibrium is reached between the ligand and the binder.
FREUD'S ADJUVANT	☒ An emulsifier that enhances the immune response and allows for slow absorption into an animals system when producing antiserum.
HAPTEN	☒ A substance that is not immunogenic in itself, but becomes immunogenic when complexed with another compound.
HEMATOCRIT	☒ <u>formed elements</u> whole blood
IMMUNOGEN	☒ A substance capable of inducing an immune response.
IMMUNO- GLOBULINS	☒ A group of serum proteins (antibodies) that specifically bind the antigens (Ag).

LIGAND	✕ The substance that is bound.
NON-SPECIFIC BINDING	✕ When labeled Ag and Ag free serum (without Ab's) are put into the same test tubes.
PRECISION	✕ Degree of agreement of repeated measurement of a quantity (usually expressed as coefficient of variance).
QUALITY CONTROL	✕ Ensure that the results of assays are <i>reliable</i> and representative of true concentrations.
RADIO-IMMUNOASSAY	✕ Radioligand assay in which the receptor is an antibody.
RECEPTOR	✕ A substance (protein) that specifically binds a certain compound (ligand).
SENSITIVITY	✕ Minimum quantity detectable.
SPECIFIC ACTIVITY	✕ <u>Activity</u> , usually expressed as $\frac{\text{uci}}{\text{mass}} \quad \frac{\text{g}}$
SPECIFIC GRAVITY	✕ Materials whose weights are being compared to that of water.
SPECIFICITY	✕ Capacity to discriminate antigens of similar structure.
STANDARD	✕ A substance that is added to certain reference tubes to serve as a yardstick for quantitation of the contents.
STANDARD CURVE	✕ A curve of dilutions of known amounts of substances to which the unknown may be compared.
TITER	✕ Measurement of antibody concentration - the dilution of antiserum that will bind 50% of added radioligand.