5-1-2020

Oral Localization of Scardovia Wiggsiae

Graydon Ramos Carr

ORAL LOCALIZATION OF SCARDOVIA WIGGSIAE

By

Graydon Carr

Bachelor of Science – Cell and Developmental Biology
University of California, at Santa Barbara
2012

Doctor of Dental Surgery
University of the Pacific
2017

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science – Oral Biology

School of Dental Medicine
The Graduate College

University of Nevada, Las Vegas
May 2020

UNLV | GRADUATE COLLEGE

Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

February 25, 2020

This thesis prepared by

Graydon Carr

entitled

Oral Localization of Scardovia Wiggsiae

is approved in partial fulfillment of the requirements for the degree of

Master of Science – Oral Biology
School of Dental Medicine

Karl Kingsley, Ph.D.
Examination Committee Chair

Kathryn Hausbeck Korgan, Ph.D.
Graduate College Dean

Katherine Howard, Ph.D.
Examination Committee Member

Brian Chrzan, Ph.D.
Examination Committee Member

Jennifer Pharr, Ph.D.
Graduate College Faculty Representative

Abstract

ORAL LOCALIZATION OF SCARDOVIA WIGGSIAE

By

Graydon Carr

Dr. Karl Kingsley, Examination Committee Chair
Professor of Biomedical Sciences
Director of Student Research
University of Nevada, Las Vegas
School of Dental Medicine

Early childhood caries is one of the most prevalent diseases in the United States among children. The formation of caries is a complex, multifactorial process that is still being studied. Researchers have thought for years that *Streptococcus mutans* was the primary causative agent of early childhood caries. The recent discovery of a novel cariogenic pathogen, *Scardovia wiggsiae* and its significant contribution to the etiology of early childhood caries has led oral health researchers to re-evaluate this microorganism and its link to this disease. While there have been several projects undertaken within the University of Nevada, Las Vegas School of Dental Medicine (UNLV SDM), the majority of them have analyzed the overall prevalence in saliva amongst different categories of our clinic population.

To date, no studies at UNLV SDM have sought to determine the precise location of *S. wiggsiae* in the oral cavity amongst those patients harboring this bacterium. The purpose of this study was to determine where in the oral cavity, if any, *S. wiggiae* primarily resides. This may shed light on the best prophylactic means of reducing the risk for *S. wiggiae* induced ECC. Sample collection during this study was performed using paper points in multiple sites within the oral cavity, evaluating both hard and soft tissues. A DNA quality and quantitative evaluation was also performed on all samples collected, to determine the efficacy of paper point sample collection in our dental clinic in hopes to pave way for this technique to be used in future studies within the UNLV SDM.

Acknowledgments

A very big thank you to Dr. Karl Kingsley for allowing me to work with him throughout this research project. He was nothing but helpful and a joy to work with. His support has been invaluable. I would also like to thank my committee members, Dr. Katherine Howard, Dr. Brian Chrzan, and Dr. Jennifer Pharr for their assistance and support.

Table of Contents

List of Tables

Chapter 3

List of Figures

Chapter 2

Chapter 3

Chapter 1: Introduction

Background and Significance

Early childhood caries (ECC) is considered one of the most prevalent childhood diseases, affecting children globally. The American Dental Association identifies this disease as a significant global health issue that should and needs to be investigated further (Tanner, Kent, et al., 2011). In the United States, prevalence has been shown to be as high as 28%, with a higher prevalence associated with children of low socio-economic status. The etiology of early childhood caries includes local bacteria, host susceptibility, and dietary factors. One of the most common causes of ECC is believed to be *S. mutans*, that is however, until the bacterium *S. wiggsiae* has been shown to have a causative link as well (Chen et al., 2019; Tanner, Kent, et al., 2011). Determining the full gamut of etiological factors for ECC would provide doctors and clinicians the best possible means to solving, or at least mitigating, this rampant disease.

S. wiggsiae, originally classified to be an unidentified *Bifidobacterium* species, is a Gram-positive bacillus from the *Bifidobacteriales* family (Becker et al., 2002). Prior to discovery of *S. wiggsiae*, it was thought that *S. mutans* was the primary pathogen responsible for ECC, however, recent literature has found *S. wiggsiae* to be one of the major species detected in children with ECC. Studies in both animal models and human trials have shown *S. wiggsiae* cultured in children suffering from early childhood caries with no presence of *S. mutans* (Kressirer et al., 2017; Tanner, Mathney, et al., 2011). In addition, it has been postulated that the combination of both *S. mutans* and *S. wiggsiae* together can increase the susceptibility for caries.

Understanding the complete cause of dental caries and the pathogenicity of *S. wiggsiae* can provide orthodontists and dental professionals insight into how to create a healthy oral environment for our patients. Within the UNLV School of Dental Medicine (SDM), studies have been analyzing the prevalence of *S. wiggsiae* amongst the clinic's patient population. These populations have included both pediatric and adult patients, and patients undergoing orthodontic therapy (BJ, 2015; Milne et al., 2018; Row et al., 2016). Most of these studies, however, test whole saliva samples leaving the question as to where specifically in the oral cavity *S. wiggsiae* resides, unanswered.

The primary focus of this study is to localize and identify the specific regions in the oral cavity where *S. wiggsiae* resides. Our hypothesis is that we will find *S. wiggsiae* isolated to dental plaque on tooth structure only, and not in the soft tissue or gingival crevicular fluid. Both adult and child patients with orthodontic appliances will be analyzed.

Research Question

1. Can paper point sampling adequately extract DNA from specific oral sites in patients to pass DNA purity standards?
    - H_0: No, paper point sampling cannot adequately extract DNA from specific oral sites in patients to pass DNA purity standards
    - H_A: Yes, paper point sampling can adequately extract DNA from specific oral sites in patients to pass DNA purity standards
2. Can *Scardovia wiggsiae* be localized to hard tissue dental plaque in patients undergoing orthodontic treatment?

- H_0: No, *Scardovia wiggsiae* cannot be localized to hard tissue dental plaque in patients undergoing orthodontic treatment
- H_A: Yes, *Scardovia wiggsiae* can be localized to hard tissue dental plaque in patients undergoing orthodontic treatment

Approval

The Office for the Protection of Research Subjects (OPRS) and the Institutional Review Board (IRB) of the University of Nevada, Las Vegas (UNLV) reviewed and approved the original protocol for collection of saliva and oral samples under "The Prevalence of Oral Microbes in Saliva from the University of Nevada, Las Vegas – School of Dental Medicine pediatric and adult clinical population (#1502-506M). Patients provided Informed Consent; Pediatric patients also provided Pediatric Assent.

Research Design

The primary research design of this study is prospective and experimental. Subjects will be randomly recruited by members of the UNLV-SDM clinic during their dental visits between December 2017 to 2018. Informed Consent will be required and conducted onsite. Inclusion criteria: adolescent subjects will have to be between the ages of eight (8) and seventeen (17), and adult patients will be from eighteen (18) to sixty-five (65). All subjects must also be currently undergoing comprehensive orthodontic treatment, with both brackets and/or bands bonded to the teeth. Exclusion criteria: any subject younger than eight (8) or over the age of sixty-five (65), and any subject that refuses to participate in the study.

Five local samples from each subject will be taken and analyzed from: Buccal mucosae, supragingival plaque from upper first molar, supragingival plaque from a lower incisor, and the tongue using paper point sampling. Finally, a whole saliva sample will be also be retrieved for analysis.

DNA isolation and purity testing were carried out using a DNA extraction kit. PCR screening analyzed the presence of *S. wiggsiae* using a *Scardovia wiggsiae* primer set where results were reviewed for statistical analysis.

References

Becker, M. R., Paster, B. J., Leys, E. J., Moeschberger, M. L., Kenyon, S. G., Galvin, J. L., Boches, S. K., Dewhirst, F. E., & Griffen, A. L. (2002). Molecular analysis of bacterial species associated with childhood caries. *Journal of Clinical Microbiology*, *40*(3), 1001–1009. https://doi.org/10.1128/JCM.40.3.1001-1009.2002

BJ, S. (2015). Screening and Prevalence of the Novel Cariogenic Pathogen Scardovia wiggsiae among Adult Orthodontic and Non-Orthodontic Patient Saliva Samples. *International Journal of Dentistry and Oral Health*, *1*(6). https://doi.org/10.16966/2378-7090.159

Chen, K. J., Gao, S. S., Duangthip, D., Lo, E. C. M., & Chu, C. H. (2019). Prevalence of early childhood caries among 5-year-old children: A systematic review. *Journal of Investigative and Clinical Dentistry*, *10*(1), e12376. https://doi.org/10.1111/jicd.12376

Kressirer, C. A., Smith, D. J., King, W. F., Dobeck, J. M., Starr, J. R., & Tanner, A. C. R. (2017). Scardovia wiggsiae and its potential role as a caries pathogen. *Journal of Oral Biosciences*, *59*(3), 135–141. https://doi.org/10.1016/j.job.2017.05.002

Milne, W., Rezaei, G., Whiteley, A., & Kingsley, K. (2018). Cariogenic Pathogen Scardovia Wiggsiae Screening Among Pediatric Orthodontic Patients: A Pilot Study . *Current Research in Dentistry*, *9*(1), 1–5. https://doi.org/10.3844/crdsp.2018.1.5

Row, L., Repp, M. R., & Kingsley, K. (2016). Screening of a Pediatric and Adult Clinic Population for Caries Pathogen Scardovia Wiggsiae. *The Journal of Clinical Pediatric Dentistry*, *40*(6), 438–444. https://doi.org/10.17796/1053-4628-40.6.438

Tanner, A. C. R., Kent, R. L., Holgerson, P. L., Hughes, C. V., Loo, C. Y., Kanasi, E., Chalmers, N. I., & Johansson, I. (2011). Microbiota of severe early childhood caries before and after therapy. *Journal of Dental Research*, *90*(11), 1298–1305.

https://doi.org/10.1177/0022034511421201

Tanner, A. C. R., Mathney, J. M. J., Kent, R. L., Chalmers, N. I., Hughes, C. V., Loo, C. Y., Pradhan, N., Kanasi, E., Hwang, J., Dahlan, M. A., Papadopolou, E., & Dewhirst, F. E. (2011). Cultivable anaerobic microbiota of severe early childhood caries. *Journal of Clinical Microbiology*, *49*(4), 1464–1474. https://doi.org/10.1128/JCM.02427-10

Chapter 2

Quantitative Comparison of Oral Site-Specific DNA Isolates Reveals Differential Outcomes

This chapter has been published in the Journal of Advances in Biology & Biotechnology and is presented in the style of that Journal. The complete Citation is:

Carr G, Alexander A, Dionisio D, Kingsley K. Quantitative Comparison of Oral Site-Specific DNA Isolates Reveals Differential Outcomes. Journal of Advances in Biology & Biotechnology.

Role of Authors:
This work was carried out in collaboration between all authors. Authors Graydon Carr, Arvin Alexander, and Kevin Dionisio were responsible for sample processing. Authors Dr. Karl Kingsley and Dr. Graydon Carr were responsible for project design, funding and manuscript preparation. All authors read and approved the final manuscript.

Abstract

Introduction: More and more evidence has accumulated that suggests salivary sampling may provide direct analysis or oral conditions and microbial constituents, but may also be useful in the diagnosis and early detection of other chronic diseases. Although multiple methods of oral sampling currently exist, some methods are prohibitively expensive or based upon technologies not ubiquitously available at public health centers or state-funded colleges. This study provides a comparative analysis of DNA concentrations and quality from five specific oral sites derived using sterile paper points, including the gingival crevice between the upper central incisors, biofilm of the upper first molar, lingual incisor, and the dorsum of the tongue for comparison with unstimulated saliva collection.

Methods: This study analyzed previously collected unstimulated saliva and paper point samples. In brief, DNA was isolated from each using TRIzol (phenol:chloroform) extraction and DNA quantification and quality was measured using a NanoDrop spectrophotometer at 260 and 280 nm.

Results**:** Analysis of Paper Point (PP) biofilm sampling sites from upper first molar, lower incisor, and dorsum of the tongue revealed similar average DNA concentrations, ranging between 14,342 ng and 14,402 ng (p=0.9851). Although variations were observed between different patients, samples from different oral sites within the same patient were strikingly similar, R=0.8355. Comparison of DNA isolated from fluids, gingival crevicular fluid (GCF) and unstimulated saliva revealed average DNA concentrations that were similar to the biofilm sampling sites (14,686 ng and 13,743 ng, respectively), which were not significantly different from one another (p=0.7893). DNA concentrations ranged considerably between patients (low = 4,410 ng; high = 48,783 ng), but were most similar with different samples (GCF, saliva) from the same patient (Pearson's R=0.6979). In addition, DNA purity measured by A260:A280 nm absorbance did not reveal any significant difference among sampling sites (range 1.62 – 1.70; p=0.427).

Discussion**:** Although many methods are available to provide oral sampling, simple and low-cost methods such as paper point sampling, unstimulated saliva collection and buccal swabs may represent tools that provide sufficient DNA quality and quantity for molecular screening. In addition, although heterogeneity between patient samples will always be present – samples from various oral sites within the same patient may provide roughly equivalent DNA samples for further screening and molecular analysis.

Keywords: Saliva sampling; paper points; DNA concentration; DNA purity.

Introduction

More and more evidence has accumulated that suggests salivary sampling may provide direct analysis of oral conditions and microbial constituents, but may also be useful in the diagnosis and early detection of other chronic diseases [1,2]. For example, new studies have demonstrated that significant detectable changes in the subgingival microbial flora in patients with periodontitis may not only predict prognosis and treatment success, but may also correlate with and predict systemic changes to type 2 diabetes mellitus or cancer [3-5]. Despite these advances, there have been relatively few studies comparing site-specific oral sampling with bacterial DNA yields and other microbial screening outcomes [6].

Although multiple methods of oral sampling currently exist, some methods such as fluorescence in situ hybridization (FISH) "lab-on-a-chip" or point-of-care (PoC) immunoflow assays are prohibitively expensive or based upon technologies not ubiquitously available to oral health researchers at public health centers or state-funded colleges [6,7]. The remaining low-cost and easily accessible methods for microbial detection (including unstimulated saliva collection, sterile paper point sampling) have relatively few studies providing both qualitative and quantitative DNA analysis [8,9]. Quantitative and qualitative comparisons of DNA isolated using these low-cost and ubiquitous sampling methods may provide valuable analysis to determine if these methods result in widely varying measures and outcomes [10].

The objective of the current study is to provide a comparative analysis of DNA concentrations and quality from five specific oral sites derived using sterile paper points, including the gingival crevice between the upper central incisors, biofilm of the upper first molar and lingual incisor, as

well as the dorsum of the tongue. In addition, comparisons can be made with unstimulated saliva, which was also concurrently collected from each patient at the time of the original sample collection. This analysis may provide significant insights into the comparative heterogeneity and sampling outcomes associated with site-specific oral sampling methods.

Methodology

2.1 Study Approval

The Office for the Protection of Research Subjects (OPRS) and the Institutional Review Board (IRB) of the University of Nevada, Las Vegas (UNLV) reviewed and approved the original protocol for collection of saliva and oral samples under "The Prevalence of Oral Microbes in Saliva from the UNLV – School of Dental Medicine pediatric and adult clinical population (#1502-506M). In brief, patients (and parents or guardians if under 18 years of age) were asked for voluntary participation. All patients that declined participation were excluded. Any patient (with consent of parent or guardian if needed) that volunteered to participate was asked to provide Informed Consent and/or Pediatric Assent for those under 18 years of age. No remuneration was given to any subject.

2.2 Sample Collection

In brief, all patients were given a sterile saliva collection tube and subsequently asked to provide up to 5 mL of unstimulated saliva. In addition, sterile paper points were used to acquire samples from the dorsum of the tongue, buccal surface of the maxillary first molar (tooth #3), lingual surface of the mandibular central incisor (tooth #25) and the buccal gingival crevice of the maxillary central incisor (tooth #9), which were each placed in individual sterile collection tubes. All samples were stored on ice and transferred to a biomedical biosafety level 2 (BSL-2) laboratory for long-term storage and processing.

2.3 DNA Isolation

As previously described, DNA isolation from each of the saliva samples was performed using the Invitrogen TRIzol reagent, which involves a sequential precipitation of DNA from a single sample suitable for obtaining polymerase chain reaction (PCR) quality DNA [11,12]. In brief, 100 uL of sterile filtered 1X phosphate buffered saline (PBS) was added to each of the paper point containing collection tubes and vortexed for 20 seconds to elute any attached bacteria [13]. 100 uL of saliva or the 1X PBS-eluted samples was added to 300 uL of TRIzol reagent and triturated prior to incubation for five minutes at room temperature. To this mixture 200 uL of chloroform was added and mixed and then incubated for an additional two to three minutes. The samples were then centrifuged at 4C at 12,000 g or relative centrifugal force (RCF) for 15 minutes. The DNA-containing interphase was transferred to a new sterile microcentrifuge tube with the addition of 300 uL of 100% ethanol, which was mixed by inverting each sample prior to incubation for two to three minutes at room temperature. Each sample was then centrifuged for an additional five minutes at 2,000 g or RCF to pellet the DNA. The ethanol was aspirated, and each DNA pellet was resuspended in 100 uL of sterile DNA rehydration solution for analysis and comparison.

2.4 DNA Analysis

The quality and quantity of DNA was assessed by spectrophotometric absorbance readings at 260 and 280 nm (A260:A280) using a NanoDrop spectrophotometer from ThermoFisher. DNA concentration is generally estimated by this method by measuring A260 nm absorbance, adjusting this measurement for turbidity at A320 nm) and the dilution factor. High-quality DNA will have an A260:A280 ratio of approximately 1.7 – 2.0.

2.5 Statistical Analysis

Statistical differences between DNA concentrations (ng/uL) were measured using two-tailed Students t-tests, which are appropriate for parametric data [14]. Analysis of DNA concentrations within the same patient were assessed using Pearson's correlation or R, which will reveal the association between different sites within the same patient and are also appropriate for this type of parametric data.

Results

A total of n=105 patient samples were available for DNA analysis and comparison in this study. Analysis of the samples collected using paper points (PP) revealed average DNA concentrations at all three biofilm sampling sites were similar; maxillary first molar (buccal), mandibular central incisor (lingual), and dorsum of tongue (14,324 ng, 14,402 ng, 14,341 ng, respectively; p=0.9851). Although the DNA concentration ranged quite significantly between patients (low = 4,065 ng; high = 48, 676 ng), these were most similar among different oral sampling sites within the same patient (Pearson's R=0.8355).

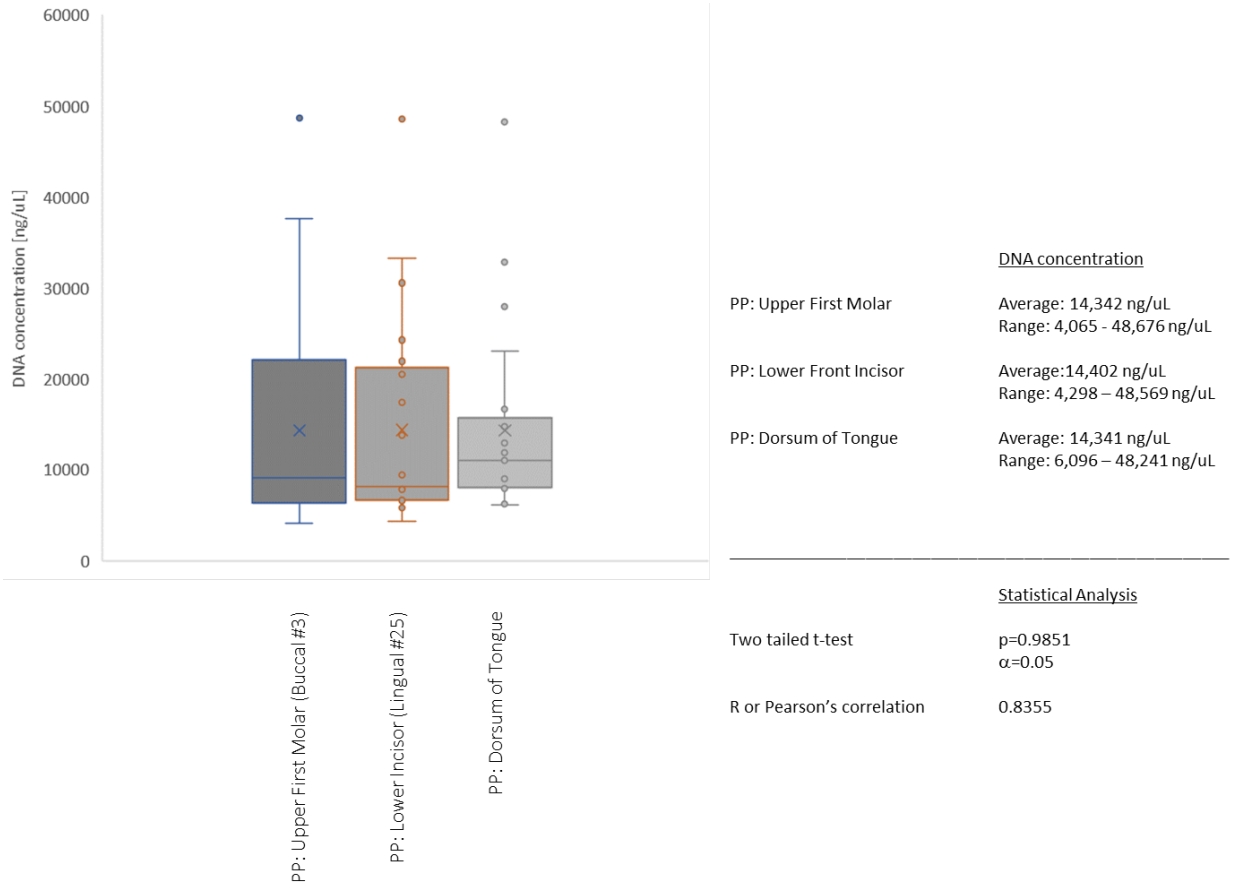


Figure 1. Analysis of Paper Point (PP) biofilm sampling sites. Comparison of DNA isolated from upper first molar (buccal #3), lower incisor (lingual #25) and dorsum of the tongue revealed similar DNA concentrations, ranging between 14,342 ng and 14,402 ng, p=0.9851. Although variations were primarily observed between different patients, samples from different oral sites within the same patient were strikingly similar, R=0.8355.

Analysis of the samples collected using liquid or aqueous components revealed average DNA concentrations that were slightly higher among the paper point (PP) samples of gingival crevicular fluid (GCF) than unstimulated saliva (14,686 ng and 13,743 ng, respectively), although this was not statistically significant, p=0.7893 (Figure 2). Although DNA

concentrations ranged quite significantly between patients (low = 4,410 ng; high = 48,783 ng), these were most similar with different samples (GCF, saliva) from the same patient (Pearson's R=0.6979).

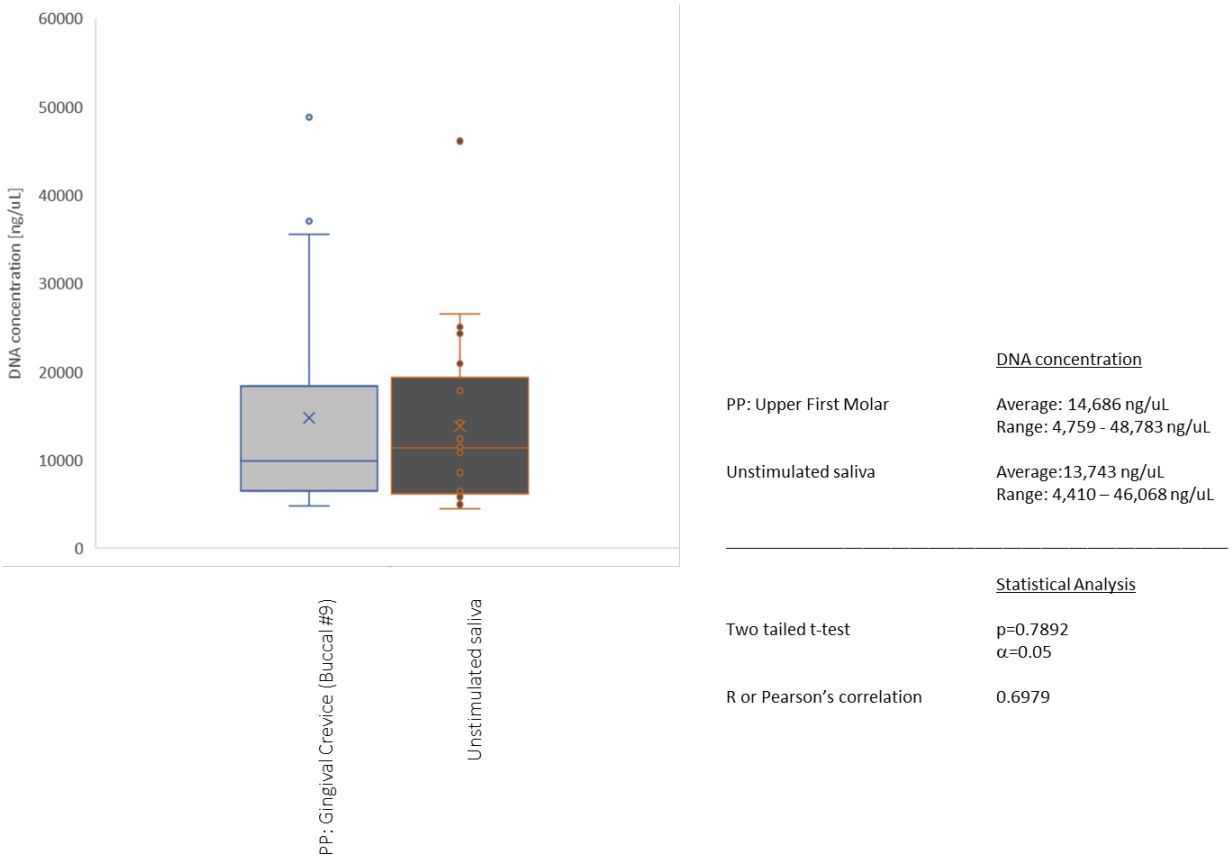
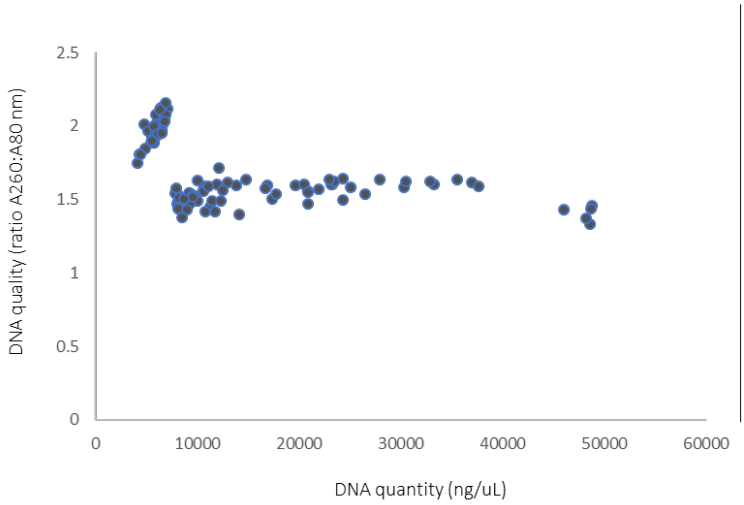


Figure 2. Analysis of Gingival Crevicular Fluid (GCF) and unstimulated saliva sampling. Comparison of DNA isolated from GCF at the buccal interface of tooth #9 using PP and unstimulated saliva revealed similar DNA concentrations (14,686 ng and 13,743 ng, respectively), which were not statistically significant, p=0.7893. DNA concentrations ranged significantly between patients (low = 4,410 ng; high = 48,783 ng), but were most similar with different samples (GCF, saliva) from the same patient (Pearson's R=0.6979).

To determine if the overall quantity of DNA isolated from any given oral sampling site was correlated with the overall quality of DNA, absorbance readings at 260 and 280 nm were taken to provide an estimate of DNA purity (Fig. 3). These data clearly indicate that no statistically significant relationship between DNA concentration and DNA purity were observed (R=0.2175). Although a small subset of samples at the very lowest concentrations were found to have slightly higher DNA purity, the vast majority of samples did not vary significantly in DNA purity, with average DNA concentrations ranging between 1.62 and 1.70 (p=0.427).



Sampling site	A260:A80 ratio	
Gingival Crevice	Average:	1.70
	Range:	1.46 – 2.12
Upper First Molar	Average:	1.69
	Range:	1.44 – 2.11
Lingual Incisor	Average:	1.67
	Range:	1.33 – 2.16
Dorsal Tongue	Average:	1.64
	Range:	1.37 – 2.11
Unstimulated Saliva	Average:	1.62
	Range:	1.38 – 2.03

Figure 3. Analysis of DNA quality (A260:A80 nm) compared with DNA quantity (ng/uL). The comparison of DNA quantity with DNA quality did not reveal any significant association, R=0.2175. The DNA concentration averages for each oral sampling site were comparable and not significantly different from one another, ranging between 1.62 and 1.70, p=0.427.

Discussion

The objective of the current study was to provide a comparative analysis of DNA concentrations and quality from five specific oral sites derived using sterile paper points, including the gingival crevice between the upper central incisors, biofilm of the maxillary first molar and mandibular central incisor, as well as the dorsum of the tongue and unstimulated saliva. The results of this analysis demonstrated that paper point sampling of biofilm directly from the tooth or tongue surface revealed strikingly similar average DNA concentrations. This may be among the first studies to specifically assess these parameters, although some previous work has compared DNA quantity with various acquisition methods (buccal swab, unstimulated saliva) [15,16].

In addition, these data demonstrated that no significant or specific relationships appeared to exist between the overall quantity of DNA obtained and the assessment of DNA quality. This may be another significant finding, as few previous studies have specifically assessed these parameters when evaluating DNA recovery from various sites within the oral cavity [17,18]. This may represent an important clinical finding, as many institutions and public health facilities may not have access to both salivary collection tubes and site-specific sampling tools.

This study does have some inherent limitations, which must also be considered when evaluating these results. First, there were financial and time constraints on the number of samples that could be analyzed and screened. This may be a common limitation to many clinical and epidemiologic studies, but it is hoped that the larger sample size in this study (n=105) may reduce any bias that could be evident in smaller samples [14]. In addition, not all samples were collected or processed on the same day – therefore, it is always possible that other factors not

directly associated with the parameters measured may have influenced the outcomes. This is also an inherent risk in any type of biomedical study and every effort was made to ensure that samples were measured in duplicate or triplicate and all results were averaged to minimize any potential bias.

Conclusions

Although many methods are available to provide oral sampling, simple and low-cost methods such as paper point sampling, unstimulated saliva collection and buccal swabs may represent tools that provide sufficient DNA quality and quantity for molecular screening. In addition, although heterogeneity between patient samples will always be present – samples from various oral sites within the same patient may provide roughly equivalent DNA samples for further screening and molecular analysis.

Acknowledgements

The authors would like to thank Dr. James Mah and the Department of Advanced Education, Orthodontic program as well as Dr. Jeffrey Ebersole and the Office of Research at the University of Nevada, Las Vegas – School of Dental Medicine for the funding and support to complete this project.

Competing Interests

The authors have declared that no bias or conflicts of interest exist.

Author Contributions

GC, AA and KD were involved in data collection and sample preparation. GC and KK were responsible for overall study design and data analysis. All authors participated in manuscript preparation.

References

1. Mohajeri N, Imani M, Akbarzadeh A, Sadighi A, Zarghami N. An update on advances in new developing DNA conjugation diagnostics and ultra-resolution imaging technologies: Possible applications in medical and biotechnological utilities. Biosens Bioelectron. 2019 Aug 28;144:111633. doi: 10.1016/j.bios.2019.111633. [Epub ahead of print] Review. PMID: 31499388

2. Shah S. Salivaomics: The current scenario. J Oral Maxillofac Pathol. 2018 Sep-Dec;22(3):375-381. doi: 10.4103/jomfp.JOMFP_171_18. Review. PMID: 30651683

3. Liu LS, Gkranias N, Farias B, Spratt D, Donos N. Differences in the subgingival microbial population of chronic periodontitis in subjects with and without type 2 diabetes mellitus-a systematic review. Clin Oral Investig. 2018 Nov;22(8):2743-2762. doi: 10.1007/s00784-018-2660-2. Epub 2018 Oct 10. PMID: 30306333

4. Dentino A, Lee S, Mailhot J, Hefti AF. Principles of periodontology. Periodontol 2000. 2013 Feb;61(1):16-53. doi: 10.1111/j.1600-0757.2011.00397.x. Review. PMID: 23240942

5. Rajpoot M, Sharma AK, Sharma A, Gupta GK. Understanding the microbiome: Emerging biomarkers for exploiting the microbiota for personalized medicine against cancer. Semin Cancer Biol. 2018 Oct;52(Pt 1):1-8. doi: 10.1016/j.semcancer.2018.02.003. Epub 2018 Feb 6. Review. PMID: 29425888

6. Alassiri S, Parnanen P, Rathnayake N, Johannsen G, Heikkinen AM, Lazzara R, van der Schoor P, van der Schoor JG, Tervahartiala T, Gieselmann D, Sorsa T. The Ability of Quantitative, Specific, and Sensitive Point-of-Care/Chair-Side Oral Fluid Immunotests for aMMP-8 to Detect Periodontal and Peri-Implant Diseases. Dis Markers. 2018 Aug 5;2018:1306396. doi: 10.1155/2018/1306396. eCollection 2018. Review. PMID: 30154936

7. Frickmann H, Zautner AE, Moter A, Kikhney J, Hagen RM, Stender H, Poppert S. Fluorescence in situ hybridization (FISH) in the microbiological diagnostic routine laboratory: a review. Crit Rev Microbiol. 2017 May;43(3):263-293. doi: 10.3109/1040841X.2016.1169990. Epub 2017 Jan 27. Review. PMID: 28129707

8. Padial-Molina M, López-Martínez J, O'Valle F, Galindo-Moreno P. Microbial Profiles and Detection Techniques in Peri-Implant Diseases: a Systematic Review. J Oral Maxillofac Res. 2016 Sep 9;7(3):e10. eCollection 2016 Jul-Sep. Review. PMID: 27833735

9. Rakic M, Grusovin MG, Canullo L. The Microbiologic Profile Associated with Peri-Implantitis in Humans: A Systematic Review. Int J Oral Maxillofac Implants. 2016 Mar-Apr;31(2):359-68. doi: 10.11607/jomi.4150. Epub 2015 Oct 6. Review. PMID: 26478978

10. Göhler A, Samietz S, Oliver Schmidt C, Kocher T, Steinmetz I, Holtfreter B. Comparison of Oral Microbe Quantities from Tongue Samples and Subgingival Pockets. International Journal of Dentistry. 2018, 1-11; Article ID 2048390. https://doi.org/10.1155/2018/2048390.

11. Tiku V, Todd CJ, Kingsley K. Assessment of Oral Human Papillomavirus Prevalence in a Multi-ethnic Pediatric Clinic Population. Compend Contin Educ Dent. 2016 Nov/Dec;37(10):e1-e4. PMID: 27875050

12. Row L, Repp MR, Kingsley K. Screening of a Pediatric and Adult Clinic Population for Caries Pathogen Scardovia Wiggsiae. J Clin Pediatr Dent. 2016;40(6):438-444. PMID: 27805882

13. Hartroth B, Seyfahrt I, Conrads G. Sampling of periodontal pathogens by paper points: evaluation of basic parameters. Oral Microbiol Immunol. 1999 Oct; 14(5): 326-30. PMID: 10551161

14. Hays WL. Statistics. 5th Ed. Harcourt Press. Inferences about population means. 1994: 311-342.

15. Turinsky AL, Butcher DT, Choufani S, Weksberg R, Brudno M. Don't brush off buccal data heterogeneity. Epigenetics. 2019 Feb;14(2):109-117. doi: 10.1080/15592294.2019.1581592. Epub 2019 Mar 1. PMID: 30821575

16. Theda C, Hwang SH, Czajko A, Loke YJ, Leong P, Craig JM. Quantitation of the cellular content of saliva and buccal swab samples. Sci Rep. 2018 May 2;8(1):6944. doi: 10.1038/s41598-018-25311-0. PMID: 29720614

17. Mättö J, Saarela M, von Troil-Lindén B, Alaluusua S, Jousimies-Somer H, Asikainen S. Similarity of salivary and subgingival Prevotella intermedia and Prevotella nigrescens isolates by arbitrarily primed polymerase chain reaction. Oral Microbiol Immunol. 1996 Dec;11(6):395-401. PMID: 9467372

18. Müller HP, Lange DE, Müller RF. Actinobacillus actinomycetemcomitans recovery from extracrevicular locations of the mouth. Oral Microbiol Immunol. 1993 Dec;8(6):344-8. PMID: 8152834

Chapter 3

Oral Site-Specific Sampling Reveals Differential Location for Scardovia wiggsiae

This chapter has been published in the Microbial Research Journal International and is presented in the style of that Journal. The complete Citation is:

Carr G, Alexander A, Nguyen L, Kingsley K. Oral Site-Specific Sampling Reveals Differential Location for *Scardovia wiggsiae*. Microbial Research Journal International. 2020

Role of Authors:
This work was carried out in collaboration between all authors. Authors Dr. Karl Kingsley and Dr. Graydon Carr were responsible for project design, funding and manuscript preparation.

Abstract

Introduction: The newly discovered cariogenic pathogen *Scardovia wiggsiae* has been the subject of intense scientific interest due to the role it may play in the development or progression of caries and oral disease. The primary objective of this study was to perform DNA microbial screening from five specific oral sites, including the gingival crevice between the upper central incisors, biofilm of the upper first molar and lingual incisor, as well as the dorsum of the tongue – for comparison with unstimulated saliva. These data may provide significant insights into site-specific oral locations that harbor *S. wiggsiae.*

Methods: More than one hundred previously collected clinical samples (n=105) were identified for inclusion in this study. DNA isolates were screened using a NanoDrop spectrophotometer to determine overall DNA quantity and quality. Samples with sufficient quality and quantity were screened for the presence of *S. wiggsiae* using validated PCR primers.

Results: More than one hundred patient samples (n=105) were identified, which were comprised of mostly female (57%) versus male (43%) and minority (71%) versus White (29%). The average DNA concentrations ranged between 13.74 and 14.69 ug/uL, with A260:A280 ratios ranging between 1.62 – 1.70. Results of molecular screening using *S. wiggsiae* specific primers

demonstrated only a small percentage of pooled samples (7.6%) harbored this DNA, which was highly concentrated among the samples from tooth surfaces (Upper First Molar, Lingual Incisor) and saliva compared with the gingival crevice and dorsum of the tongue.

Discussion: These data provide novel information regarding specific oral locations, including tooth surfaces that harbor *S. wiggsiae*. In addition, these sites also provide new information regarding oral sites that do not appear to harbor this organism, including the gingival crevice and dorsum of the tongue. This information may be particularly useful to oral health researchers as they strive to limit and reduce the cariogenic microbiome among high-risk populations.

Key words: *Scardovia wiggsiae*, caries, pathogen, screening.

Abbreviations: Severe early childhood caries (SECC), early childhood caries (ECC), Institutional Review Board (IRB), Office for the Protection of Research Subjects (OPRS), University of Nevada, Las Vegas (UNLV), School of Dental Medicine (SDM), paper points (PP), phosphate buffered saline (PBS biosafety level (BSL-2), relative centrifugal force (RCF), melting temperatures (Tm),

Introduction

The oral microbiome is comprised of a rich and complex network of organisms that play significant roles in the maintenance of good oral health but also in the development of oral disease, such as dental caries [1,2]. The newly discovered cariogenic pathogen *S. wiggsiae* has been the subject of intense scientific interest due to the role it may play in the development or progression of caries and oral disease [3,4]. *Scardovia* was of interest in oral health research for the significant contributions to severe early childhood caries (SECC), both in the presence and absence of canonical cariogenic organisms – such as *S. mutans* [5]. This gram-positive anaerobic

bacillus is both acidogenic and acid tolerant, which are known to be the most significant contributing virulence factors towards the development of dental caries [6].

More evidence is now emerging regarding the prevalence and epidemiology of S*cardovia wiggsiae*, particularly among children with early childhood caries (ECC) [7,8]. These studies clearly describe the potential for caries development and pathology among children and teenagers both in the presence and absence of other clearly defined cariogenic organisms, such as *Streptococcus mutans* [9-11]. However, less is known about the prevalence and epidemiology of this organism among adult populations and populations without significant caries experience [12,13].

Recent studies from this group have begun to elucidate the prevalence and epidemiology of *Scardovia* among both pediatric and adult populations, with and without caries experience [14-16]. Although these data have provided evidence of *Scardovia* in both pediatric and adult populations with and without caries experience, more detailed epidemiology of this organism among high-risk groups including Orthodontic patients is continuing [17-19]. Many of these studies have screened unstimulated saliva using highly specific molecular techniques, but few studies to date evaluated the presence of this organism at specific sites within the oral cavity [11,20].

If recommendations are to be made in order to improve oral health and reduce risk for disease caused by this organism, a more specific oral microbial sampling must be completed to determine if methods such as flossing (specific to improve gingival health and focused on the gingival crevice) or brushing (more targeted towards supragingival plaque and biofilm) might be more effective at disease prevention [13]. The primary objective of this study was to perform DNA microbial screening from five specific oral sites that were previously derived using sterile

paper points, including the gingival crevice between the upper central incisors, biofilm of the upper first molar and lingual incisor, as well as the dorsum of the tongue – for comparison with unstimulated saliva. These data may provide significant insights into site-specific oral locations that harbor *S. wiggsiae*.

Material and Methods

2.1 Human subjects

The original protocol for sample collection was reviewed and approved by the Institutional Review Board (IRB) in the Office for the Protection of Research Subjects (OPRS) at the University of Nevada, Las Vegas (UNLV) titled "The Prevalence of Oral Microbes in Saliva from the University of Nevada, Las Vegas – School of Dental Medicine pediatric and adult clinical population" (OPRS#1502-506M). Briefly, inclusion criteria were any UNLV School of Dental Medicine (SDM) clinic patient that agreed to participate. Exclusion criteria were any UNLV-SDM patient (or parent / guardian of patients under the age of 18 years) that declined to participate and any person not a patient at a UNLV-SDM clinic. No patients received money or services in exchange for participation. All patients that volunteered for the original study asked provided Informed Consent (and Pediatric Assent if under the age of 18 years old).

2.2 Clinical samples

In brief, saliva collection from the original protocol was facilitated using a sterile sample collection container (50 mL conical centrifuge tube) with patients providing up to 5.0 mL of unstimulated saliva. During the clinical oral exam, site-specific oral sampling was performed using sterile paper points (PP) to collect from the gingival crevice between the front incisor (Tooth 9), the buccal surface of an upper maxillary molar (Tooth 3), the lingual surface of a mandibular incisor (Tooth 25), as well as the dorsum of the tongue. Each paper point was placed

into isotonic 1X phosphate buffered saline (PBS) solution and stored on ice prior to transfer to a biomedical biosafety level (BSL-2) laboratory for analysis. Each patient sample was given a randomly generated, non-duplicated number for laboratory analysis, which was not linked to any patient information or other identifying information. Only patient age, sex and ethnicity were noted for subsequent demographic analysis.

2.3 Sample processing

All clinical samples were processed to isolate DNA using the Invitrogen TRIzol reagent and protocol, which has been approved to process liquid and viscous clinical samples (blood, semen, saliva, sputum) to obtain DNA of sufficient quality for polymerase chain reaction (PCR) screening [14,21]. Briefly, PP samples were vortexed for 20 – 30 seconds to remove any adsorbent bacteria. The TRIzol reagent was added to 100 uL of the saliva or PP eluted samples and incubated prior to the addition of chloroform. Samples were then centrifuged at 12,000 g or relative centrifugal force (RCF) to isolate the nucleic acids (upper aqueous phase) from the solids and other proteins. Ethanol (100%) was added to each DNA isolate to facilitate precipitations and pellets were then centrifuged at 2,000 g or RCF and washed with 75% ethanol prior to resuspension in 100 uL of DNA rehydration solution. Quality and quantity of DNA was measured using a NanoDrop spectrophotometer at absorbance readings of A260 and A280.

2.4 PCR screening

Molecular screening for the presence of *S. wiggsiae* was accomplished using PCR with the following reaction parameters: Initial incubation at 50C x 2 minutes, Denaturation at 95C x 10 minutes, and 30 cycles at the annealing (melting) temperatures (Tm) indicated below using primers synthesized from Eurofins MWG Operon:

Positive control

16s rRNA bacterial primer set

Forward 5'-ACG CGT CGA CAG ACT TTG ATC CTG GCT-3'; 27 nt; 56% GC; Tm: 76C

Reverse 5'- GGG ACT ACC AGG GTA TCT AAT-3'; 21 nt; 48%GC; Tm: 62C

16s rRNA Optimal temperature for primer set: Lower temperature – 5C = 58C

Scardovia wiggsiae primer set

Forward 5'- GTG GAC TTT ATG AAT AAG C-3'; 19 nt; 37% GC; Tm: 55C

Reverse 5'- CTA CCG TTA AGC AGT AAG-3'; 18 nt; 44% GC; Tm: 56C

Scardovia wiggsiae Optimal temperature for primer set: Lower temperature – 5C = 50C

Statistical analysis

Analysis of patient demographics were presented as simple descriptive statistics. Any differences between the study sample and the overall clinic demographics were assessed using Chi Square (χ^2), which were appropriate for non-parametric data analysis [22]. Analysis of screening results are also presented as descriptive statistics.

Results

More than one hundred patient samples (n=105) were identified for inclusion in this analysis (Table 1). The majority of these samples were originally derived from female patients (57%), which closely resembled the overall clinic population (60%), p=0.543. The racial and ethnic composition of the study samples was primarily from non-White minority patients (71.5%), which reflected the composition of the clinic population (75%), p=0.3556. In addition, roughly half of the samples were derived from pediatric patients (52%), which was also similar to the composition of the clinic from which they were derived (57%), p=0.3125.

Table 1. Demographic analysis of study sample.

	Study sample	Clinic population	Statistical analysis
Sex			
Female	57.1%	60.4%	$\chi 2=0.375$
Male	42.9%	39.6%	d.f.=1
			p=0.5403
Race / Ethnicity			
White	28.5%	24.7%	$\chi 2=0.853$
Minority	71.5%	75.3%	d.f.=1
			p=0.3556
Age			
Pediatric (<18 years)	52.3%	56.7%	$\chi 2=1.020$
Adult (>18 years)	47.7%	43.3%	d.f.=1
			p=0.3125

To evaluate whether the DNA isolated from these samples was appropriate for molecular screening, absorbance readings at A260 and A280 nm were combined to provide estimates of DNA quantity and quality (Table 2). The average DNA concentration from each of the oral sampling sites was not significantly different from the average DNA concentration obtained from whole, unstimulated saliva (13.74 ug/uL), p=0.7892. Although significant ranges in DNA concentration were observed between different patients, DNA concentrations from different oral sites within the same patient were not, p=0.6979. Measurement of DNA quality using the absorbance ratio A260:A280 demonstrated sufficient quality for all samples using the PCR screening (>1.55), ranging between 1.62 – 1.70.

Table 2. Analysis of DNA concentration and purity from study sample.

	DNA concentration	DNA quality (A260:A280)	Statistical analysis
Saliva (whole)	13.74 ug/uL (ave.)	1.62	
	4.41 – 46.1 ug/uL (range)	1.38 – 2.03	
Gingival crevice (PP)	14.69 ug/uL (ave.)	1.70	Two tailed t-test
	4.76 – 48.8 ug/uL (range)	1.46 – 2.12	p=0.7892
Dorsal tongue (PP)	14.34 ug/uL (ave.)	1.64	Two tailed t-test
	6.1 – 48.2 ug/uL (range)	1.37 – 2.11	p=0.8527
Lingual incisor (PP)	14.4 ug/uL (ave.)	1.67	Two tailed t-test
	4.3 – 48.6 ug/uL (range)	1.33 – 2.16	p=0.8458
Upper first molar (PP)	14.3 ug/uL (ave.)	1.69	Two tailed t-test
	4.1 – 48.7 ug/uL (range)	1.44 – 2.11	p=0.8608

Each sample was then screened using the positive control primers for bacterial DNA, 16S rRNA (Figure 1). These data demonstrated that all samples screened produced PCR bands with signal band intensity (SBI) greater than the limit of detection (LOD). Graphical analysis of PCR screening results demonstrated no specific patterns between 16S rRNA SBI and specific oral sites could be determined.

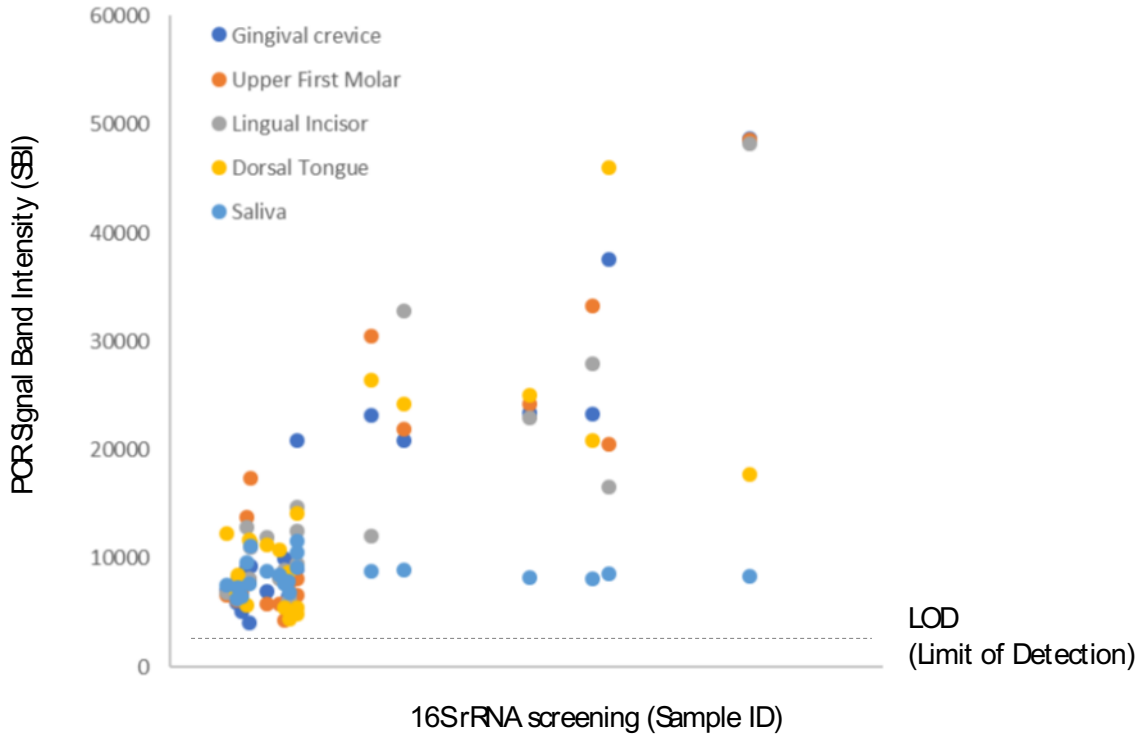


Figure 1. 16S rRNA screening of patient samples. Molecular screening of patient samples using PCR revealed 16S rRNA expression determined by signal band intensity (SBI) greater than the limit of detection (LOD) with no specific patterns observed between SBI and specific oral sites.

Due to the low prevalence of *Scardovia* observed in previous studies [14-19], more efficient screening was facilitated by pooling DNA isolates from each patient together (PP: Gingival crevice, PP: Upper first molar, PP: Lingual incisor, PP: Tongue dorsum; Saliva) (Figure 2). Using an equal volume of DNA from each site, the combined pooled samples were comprised of approximately 15-20 % of the total sample from each site (Fig. 2A). Screening of the pooled samples using the *S. wiggsiae* specific primers revealed only a small percentage of pooled samples (7.6%) generated positive PCR screening results (Fig. 2B)

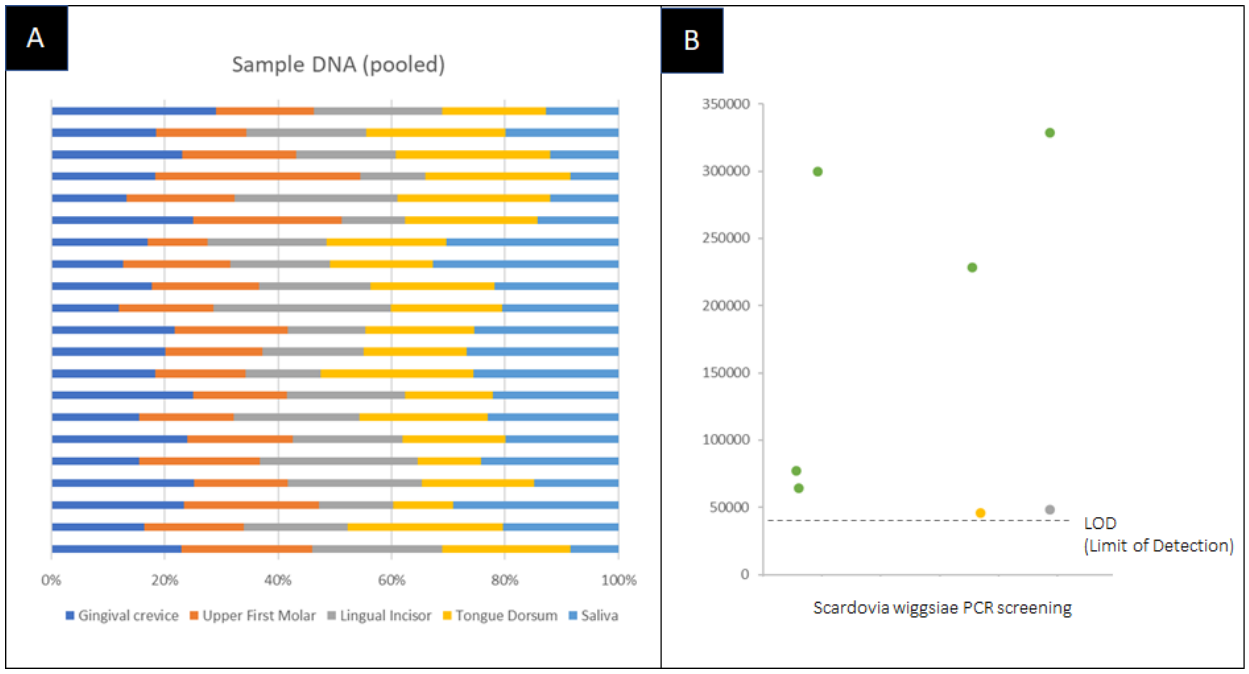


Figure 2. Pooled DNA from clinical samples screened for 16S rRNA. A) Pooling of DNA from each oral site (15-25%) for each individual patient created an efficient screening process. B) Results of molecular screening using *S. wiggsiae* specific primers demonstrated only a small percentage of pooled samples (7.6%) harbored this DNA.

Each of the corresponding site-specific samples that comprised the *Scardovia* PCR-positive pooled samples was then screened separately (Figure 3). This analysis revealed that only the upper first molar, lingual incisor, and saliva pooled samples were found to harbor *S. wiggsiae*, although much stronger signal band intensities were observed among the PP samples from supragingival plaque or biofilm from tooth surfaces (Upper First Molar, Lingual Incisor) and saliva.

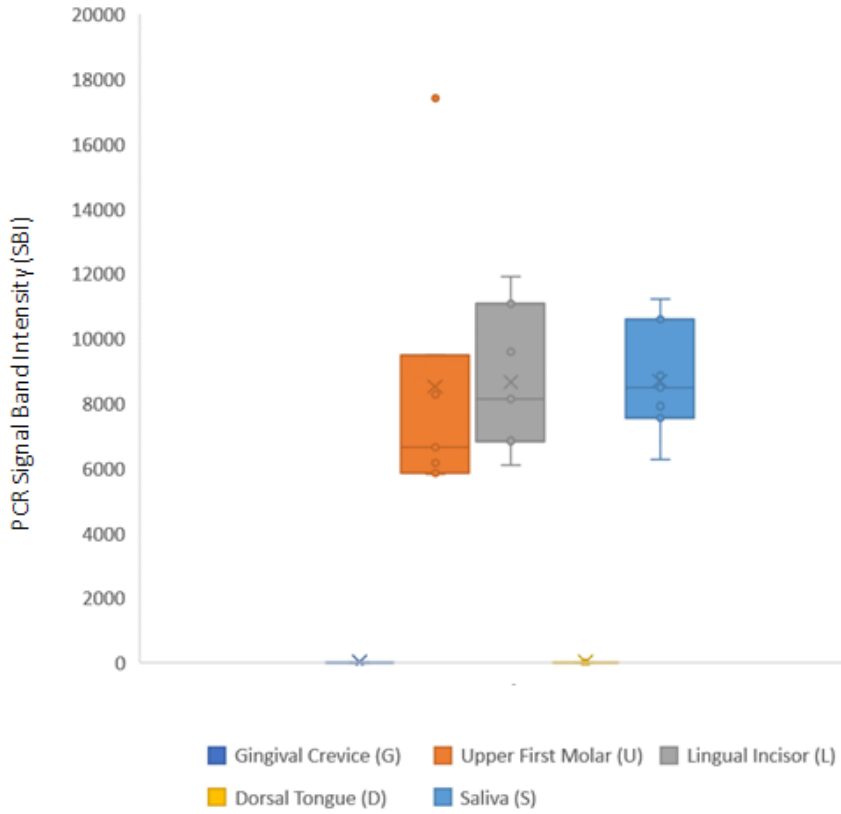


Figure 3. PCR screening of site-specific samples from corresponding *Scardovia* PCR-positive pooled samples. Each of oral sites from the pooled samples tested harbored *S. wiggsiae*, with stronger signal band intensities observed among the PP samples from tooth surfaces (Upper First Molar, Lingual Incisor) and saliva than the gingival crevice or dorsum of the tongue.

Discussion

The primary objective of this study was to perform DNA microbial screening from five specific oral sites including the gingival crevice between the upper central incisors, biofilm of the upper first molar and lingual incisor, as well as the dorsum of the tongue – for comparison with unstimulated saliva. These data have revealed significant insights into site-specific oral locations that harbored *S. wiggsiae*. For example, although previous studies have identified *Scardovia*

from caries-specific lesions and from whole saliva – this may be among the first studies to evaluate and screen for this pathogen from additional oral sites from patients without significant caries experience [3,5,8].

These data suggest that mandibular and maxillary surfaces from both anterior and posterior sites may be preferential oral locations, which may be significant as new evidence has now emerged that has suggested biomaterials and bioactive materials may selectively inhibit the virulence and modulate the microbial ecology of biofilms that include this organism [23,24]. Combining these agents with these data regarding oral location may be particularly useful for oral health researchers interested in selectively placing these agents among high risk populations, such as children with SECC [1,4,7]. These data may also be useful towards understanding the location and balance of organisms that comprise the caries microbiome in an effort to improve prevention and treatment strategies for children and young adults [25,26].

Despite the significance of these findings, some limitations inherent to this type of study should also be considered when evaluating these results. For instance, this study involved analysis of saliva samples from a predominantly low-income, minority-serving public dental school clinic [27-29]. This may suggest this sample set may have a lower health literacy and higher risk for caries than a random sampling of the overall population. This type of sampling bias could have influenced the findings and results of this study in ways that are not easy to predict. In addition, due to financial and other funding constraints, only a limited number of samples could be collected and analyzed for this project – which may also place some limitations on the overall generalizability of these results.

Conclusions

Although this study has some limitations due to the study population and sample size, these data provide novel information regarding specific oral locations, including tooth surfaces that harbor *S. wiggsiae*. In addition, these sites also provide new information regarding oral sites that do not appear to harbor this organism, including the gingival crevice and dorsum of the tongue. This information may be particularly useful to oral health researchers as they strive to limit and reduce the cariogenic microbiome among high-risk populations.

Competing Interests

The authors declare that there are no conflicts of interest.

References

1. Chandna P, Srivastava N, Sharma A, Sharma V, Gupta N, Adlakha VK. Isolation of Scardovia wiggsiae using real-time polymerase chain reaction from the saliva of children with early childhood caries. J Indian Soc Pedod Prev Dent. 2018 Jul-Sep;36(3):290-295. doi: 10.4103/JISPPD.JISPPD_225_17. PMID: 30246752

2. Tanner ACR, Kressirer CA, Rothmiller S, Johansson I, Chalmers NI. The Caries Microbiome: Implications for Reversing Dysbiosis. Adv Dent Res. 2018 Feb;29(1):78-85. doi: 10.1177/0022034517736496. Review. PMID: 29355414

3. Downes J, Mantzourani M, Beighton D, Hooper S, Wilson MJ, Nicholson A, Wade WG. Scardovia wiggsiae sp. nov., isolated from the human oral cavity and clinical material, and emended descriptions of the genus Scardovia and Scardovia inopinata. Int J Syst Evol Microbiol. 2011 Jan;61(Pt 1):25-9. doi: 10.1099/ijs.0.019752-0. Epub 2010 Feb 5. PMID: 20139283

4. Tanner AC, Mathney JM, Kent RL, Chalmers NI, Hughes CV, Loo CY, Pradhan N, Kanasi E, Hwang J, Dahlan MA, Papadopolou E, Dewhirst FE. Cultivable anaerobic microbiota of severe early childhood caries. J Clin Microbiol. 2011 Apr;49(4):1464-74. doi: 10.1128/JCM.02427-10. Epub 2011 Feb 2. PMID: 21289150

5. Tanner AC, Kent RL Jr, Holgerson PL, Hughes CV, Loo CY, Kanasi E, Chalmers NI, Johansson I. Microbiota of severe early childhood caries before and after therapy. J Dent Res. 2011 Nov;90(11):1298-305. doi: 10.1177/0022034511421201. Epub 2011 Aug 25. PMID: 21868693

6. Tanner AC. Anaerobic culture to detect periodontal and caries pathogens. J Oral Biosci. 2015 Feb;57(1):18-26. PMID: 25678835

7. Chandna P, Srivastava N, Sharma A, Sharma V, Gupta N, Adlakha VK. Isolation of Scardovia wiggsiae using real-time polymerase chain reaction from the saliva of children with early childhood caries. J Indian Soc Pedod Prev Dent. 2018 Jul-Sep;36(3):290-295. doi: 10.4103/JISPPD.JISPPD_225_17. PMID: 30246752

8. Kressirer CA, Chen T, Lake Harriman K, Frias-Lopez J, Dewhirst FE, Tavares MA, Tanner AC. Functional profiles of coronal and dentin caries in children. J Oral Microbiol. 2018 Jul 16;10(1):1495976. doi: 10.1080/20002297.2018.1495976. eCollection 2018. PMID: 30034639

9. Kressirer CA, Smith DJ, King WF, Dobeck JM, Starr JR, Tanner ACR. Scardovia wiggsiae and its potential role as a caries pathogen. J Oral Biosci. 2017 Aug;59(3):135-141. doi: 10.1016/j.job.2017.05.002. Epub 2017 May 24. PMID: 29104444

10. Eriksson L, Lif Holgerson P, Esberg A, Johansson I. Microbial Complexes and Caries in 17-Year-Olds with and without Streptococcus mutans. J Dent Res. 2018 Mar;97(3):275-282. doi: 10.1177/0022034517731758. Epub 2017 Sep 20. PMID: 28930642

11. Colombo NH, Kreling PF, Ribas LFF, Pereira JA, Kressirer CA, Klein MI, Tanner ACR, Duque C. Quantitative assessment of salivary oral bacteria according to the severity of dental caries in childhood. Arch Oral Biol. 2017 Nov;83:282-288. doi: 10.1016/j.archoralbio.2017.08.006. Epub 2017 Aug 16. PMID: 28858630

12. Eriksson L, Lif Holgerson P, Johansson I. Saliva and tooth biofilm bacterial microbiota in adolescents in a low caries community. Sci Rep. 2017 Jul 19;7(1):5861. doi: 10.1038/s41598-017-06221-z. PMID: 28724921

13. Richards VP, Alvarez AJ, Luce AR, Bedenbaugh M, Mitchell ML, Burne RA, Nascimento MM. Microbiomes of Site-Specific Dental Plaques from Children with Different Caries Status.

Infect Immun. 2017 Jul 19;85(8). pii: e00106-17. doi: 10.1128/IAI.00106-17. Print 2017 Aug. PMID: 28507066

14. Row L, Repp MR, Kingsley K. Screening of a Pediatric and Adult Clinic Population for Caries Pathogen Scardovia Wiggsiae. J Clin Pediatr Dent. 2016;40(6):438-444. PMID: 27805882

15. McDaniel J, McDaniel S, Tam A, Kingsley K, Howard KM. Screening a Saliva Repository for Scardovia wiggsiae and Streptococcus mutans: A Pilot Study. Journal of Advances in Microbiology 2017, (5) 1: 1-8. doi: 10.9734/JAMB/2017/36111

16. McDaniel S, McDaniel J, Tam A, Kingsley K. Howard KM. Oral Microbial Ecology of Selenemonas noxia and Scardovia wiggsiae. Microbiology Research Journal International 2017, 21(3) 1-8. doi: 10.9734/MRJI/2017/36110

17. Milne W, Rezaei G, Whiteley A, Kingsley K. Cariogenic pathogen Scardovia wiggsiae screening among pediatric orthodontic patients: a pilot Study. Current Research in Dentistry. 2018, 9: 1-5. doi: 10.3822/crdsp.2018.1.5

18. Reyes N, Pollock A, Whiteley A, Kingsley K, Howard KM. Prevalence of Scardovia wiggsiae among a pediatric Orthodontic patient population. EC Dental Science 2017; 13(5): 203-210.

19. Whiteley A, Kingsley K. Scardovia wiggsiae prevalence among adult and pediatric orthodontic and non-orthodontic patient populations. J Med Disc. 2017, 2(3): jmd17034. doi: 10.24262/jmd.2.3.17034.

20. Tanner AC, Sonis AL, Lif Holgerson P, Starr JR, Nunez Y, Kressirer CA, Paster BJ, Johansson I. White-spot lesions and gingivitis microbiotas in orthodontic patients. J Dent Res. 2012 Sep;91(9):853-8. doi: 10.1177/0022034512455031. Epub 2012 Jul 26. PMID: 22837552

21. Tiku V, Todd CJ, Kingsley K. Assessment of Oral Human Papillomavirus Prevalence in a Multi-ethnic Pediatric Clinic Population. Compend Contin Educ Dent. 2016 Nov/Dec;37(10):e1-e4. PMID: 27875050

22. . Hays WL. Statistics. 5th Ed. Harcourt Press. Inferences about population means. 1994: 311-342.

23. Philip N, Leishman SJ, Bandara HMHN, Walsh LJ. Casein Phosphopeptide-Amorphous Calcium Phosphate Attenuates Virulence and Modulates Microbial Ecology of Saliva-Derived Polymicrobial Biofilms. Caries Res. 2019;53(6):643-649. doi: 10.1159/000499869. Epub 2019 Jun 4. PMID: 31163430

24. Esberg A, Haworth S, Brunius C, Lif Holgerson P, Johansson I. Carbonic Anhydrase 6 Gene Variation influences Oral Microbiota Composition and Caries Risk in Swedish adolescents. Sci Rep. 2019 Jan 24;9(1):452. doi: 10.1038/s41598-018-36832-z. PMID: 30679524

25. Fakhruddin KS, Ngo HC, Samaranayake LP. Cariogenic microbiome and microbiota of the early primary dentition: A contemporary overview. Oral Dis. 2019 May;25(4):982-995. doi: 10.1111/odi.12932. Epub 2018 Sep 19. Review. PMID: 29969843

26. Zhan L. Rebalancing the Caries Microbiome Dysbiosis: Targeted Treatment and Sugar Alcohols. Adv Dent Res. 2018 Feb;29(1):110-116. doi: 10.1177/0022034517736498. Review. PMID: 29355418

27. Bowen D, Kingsley K. Screening for Oral High-risk Human Papillomavirus (HPV) among a Pediatric Patient Population. Journal of Advances in Medicine and Medical Research 2019, 29(9): 1-8: https://doi.org/10.9734/jammr/2019/v29i930124

28. Dhillon N, Kingsley K, Howard KM. Prevalence of Selenomonas noxia among Pediatric and Adult Orthodontic Patients. International Journal of Research and Reports in Dentistry. 2019, 2(1): 1-7.

29. Kingsley J, Kingsley K. Aggregatibacter actinomycetemcomitans and Fusobacterium nucleatum prevalence correlates with salivary microbial burden in Orthodontic patients. International Journal of Dental Research and Reviews, 2019, 2:9. doi: 10.28933/ijdrr-2019-01-2405

Chapter 4: Summary and Conclusions

The purpose of this study was to evaluate and define the precise location in the oral cavity that *S. wiggsiae* resides. Since this bacterium was so recently discovered, identified and specified in 2011 by Dr. Anne Tanner, research is limited and needs further exploration. To date there has been no studies focusing on its specific location in which it primarily resides. The majority of studies evaluate the prevalence in whole saliva studies. *S. wiggsiae*'s strong correlation to early childhood caries (ECC) opens the door for a new age of microbial research which can lead to the development of new antimicrobial agents able to target specific organisms and improving the treatment of ECC.

Chapter 2 of this document served as an important baseline for the following chapter, by providing a comparative analysis of DNA concentrations and quality from the five specific oral sites (n=105) using paper point sampling. Many methods to date are available for oral sampling. At UNLV SDM, and our limited funding and resources, it was essential to evaluate the efficacy of a simple and low-cost method of sample collection. The results of this study demonstrated that paper point sampling for sample collection provided sufficient DNA quality and quantity for molecular screening amongst.

Chapter 3 was a study using the same samples from chapter 2 (n=105) but sought to reveal significant insights into site-special oral locations harboring *S. wiggsiae*. Using PCR and gel electrophoresis for analysis, the results showed that *S. wiggsiae* primarily resides on the plaque biofilm on the maxillary and mandibular enamel surfaces. No patients were found to harbor this bacterium within the gingival crevice, or on the tongue surface.

Conclusions from both chapters 2 and 3 demonstrated that in our patient population few patients in orthodontic treatment were found to harbor *S. wiggsiae*, while simultaneously locating

its location to the bacterial biofilm situated on the enamel surface. Adequate DNA concentration and quality was established from our samples (n=105) which was demonstrated in chapter 2. None of our patient samples were of age to be diagnosed as having ECC. All of our patients were in mixed or permanent dentition at the time of sample collection. This can be explained further by our clinic population and may be the reason for a low prevalence of *S. wiggsiae*.

Based on the findings presented throughout this document, both alternative hypotheses can be accepted in regards to the original proposed research questions.

1. Can paper point sampling adequately extract DNA from specific oral sites in patients to pass DNA purity standards?
    - Alternative (H_A) hypothesis: Yes, paper point sampling can adequately extract DNA from specific oral sites in patients to pass DNA purity standards
2. Can *Scardovia wiggsiae* be localized to hard tissue dental plaque in patients undergoing orthodontic treatment?
    - Alternative (H_A) hypothesis: Yes, *Scardovia wiggsiae* can be localized to hard tissue dental plaque in patients undergoing orthodontic treatment

Limitations and Recommendations:

A significant limitation in the two studies presented is the limited number of samples that could be analyzed and their limited profile. There were both financial and time constraints which are apparent in an orthodontic residency program. After data collection and during our sample processing phase of research, there was a power outage at the University of Nevada at Las Vegas

which affected the research laboratory. During this power outage many research samples were destroyed and unsalvageable being out of ideal storage conditions for an extended period of time. Originally samples form 48 patients were collected. This may be a common limitation to many clinical and epidemiologic studies, but it is hoped that the larger sample size in this study (n=105) may reduce any bias that could be evident in smaller samples

Not all samples were collected or processed on the same day, therefore it is always possible that other factors not directly associated with the parameters measured may have influenced outcomes. This is also an inherent risk in any type of biomedical study and every effort was made to ensure that samples were measured in duplicate or triplicate and all results were averaged to minimize any potential bias.

During data collection no temporal information (before and after orthodontic treatment), DMF indices, or oral hygiene scores were recorded which may have benefited a future study or help further delineate our results. A follow up study evaluating patient oral health status factors including gingival index, probing depth, plaque scores, DMF scores, and their correlation to the prevalence of *S. wiggsiae* may ultimately help to elucidate the nature of this bacterium.

Appendix A

UNLV

Biomedical IRB
Notice of Excluded Activity

DATE: February 6, 2015

TO: **Dr. Karl Kingsley,** School of Dental Medicine

FROM: Office of Research Integrity – Human Subjects

RE: Notification of IRB Action
Protocol Title: **The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population**
Protocol# 1502-5068M

This memorandum is notification that the project referenced above has been reviewed as indicated in Federal regulatory statutes 45CFR46.

The protocol has been reviewed and deemed excluded from IRB review. It is not in need of further review or approval by the IRB.

Any changes to the excluded activity may cause this project to require a different level of IRB review. Should any changes need to be made, please submit a Modification Form.

If you have questions or require any assistance, please contact the Office of Research Integrity – Human Subjects at IRB@unlv.edu or call 702-895-2794.

Office of Research Integrity – Human Subjects
4505 Maryland Parkway • Box 451047 • Las Vegas, Nevada 89154-1047
(702) 895-2794 • FAX: (702) 895-0805 • IRB@unlv.edu

Appendix B

Permission to Use Copyrighted Material

University of Nevada, Las Vegas

I, **Graydon Carr,** holder of copyrighted material entitled **Quantitative Comparison of Oral Site-Specific DNA Isolates Reveals Differential Outcomes,** authored by **Graydon Carr,** and **Karl Kingsley** originally published in **Journal of Advances in Biology and Biotechnology, October 2019**, hereby give permission for the author to use the above described material in total or in part for inclusion in a Master's thesis at the University of Nevada, Las Vegas.

I also agree that the author may execute the standard contract with ProQuest for storage and reproduction of the completed thesis, including the materials to which I hold copyright

February 24, 2020

Signature	Date
Graydon Carr	Resident
Name (typed)	Title

Appendix C

Permission to Use Copyrighted Material

University of Nevada, Las Vegas

I, **Graydon Carr,** holder of copyrighted material entitled **Oral Site Specific Sampling Reveals Differential Location for *Scardovia wiggsiae*,** authored by **Graydon Carr,** and **Karl Kingsley** originally published in **Microbiology Research Journal International, February 2020**, hereby give permission for the author to use the above described material in total or in part for inclusion in a Master's thesis at the University of Nevada, Las Vegas.

I also agree that the author may execute the standard contract with ProQuest for storage and reproduction of the completed thesis, including the materials to which I hold copyright

February 24, 2020

Signature	Date
Graydon Carr	Resident
Name (typed)	Title

Curriculum Vitae

Graduate College

University of Nevada, Las Vegas

Graydon Carr

Email: gcarrdds@gmail.com

Degrees:
Bachelor of Science – Cell and Developmental Biology, 2012
University of California at Santa Barbara, Santa Barbara

Doctor of Dental Surgery, 2017
The University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco

Thesis Title:
Oral Localization of Scardovia Wiggsiae

Thesis Examination Committee:
Chairperson, Karl Kingsley, Ph.D. M.P.H.
Committee Member, Katherine Howard, Ph.D.
Committee Member, Brian Chrzan, D.D.S., Ph.D.
Graduate Faculty Representative, Jennifer Pharr Ph.D.
Graduate Coordinator, Brian Chrzan, D.D.S., Ph.D.