EFFECT OF FIXED ORTHODONTIC APPLIANCES ON THE PRESENCE OF CARIOGENIC BACTERIA

By

Dr. Emma Paige Kozak

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Department of Preventive Dental Science
College of Dentistry
Rady Faculty of Health Sciences
University of Manitoba
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ABSTRACT

EFFECT OF FIXED ORTHODONTIC APPLIANCES ON THE PRESENCE OF CARIOGENIC BACTERIA

Paige Kozak[a]

[a]Graduate Resident, Division of Orthodontics, University of Manitoba

Introduction: The purpose of this study was to use a chair-side saliva immunoassay to determine the overall prevalence of high *Streptococcus mutans* levels in orthodontic patients and to determine the prevalence of high *S. mutans* levels as a function of dental crowding and bracket type at four time-points throughout orthodontic treatment.

Methods: 100 patients undergoing orthodontic treatment were selected, among which 35 used conventional brackets and 65 used self-ligated brackets. The sample population was comprised of 60 females and 40 males with overall mean age of 17.2 years. The chairside saliva assay Saliva-Check MUTANS™ was used to measure each subject at four time-points: immediately prior to bonding (T0), 3 months (T1), 6 months (T2) and 12 months (T3) into treatment. Bacteria levels as well as the amount of crowding were recorded at each time-point. Of the 400 anticipated data collection points, 8 were not recorded due to 6 patients being lost to follow-up. A repeated measures model was used to investigate the relationship between bracket type, crowding, and the risk of high bacteria levels. Specifically, a generalized linear mixed-effects model (GLMM) was used to account for the fact that the risk of high bacterial levels intrinsically varies between patients. A p-value ≤ 0.05 was significant.

Results: The overall prevalence of high *S. mutans* levels was found to be 81% at T0, 78% at T1, 68% at T2 and 47% at T3. Bracket and crowding effects on bacteria levels were found to be non-significant ($p>0.05$). Only the effect of time was found

to be significant, specifically, that T3 was different from T0, T1 and T2 ($p<0.0007$). An incidental finding in our study showed males had a greater prevalence of high S. *mutans* throughout orthodontic treatment, however this gender effect was not statistically significant ($p>0.05$).

Conclusions: The Saliva-Check MUTANS™ assays' high sensitivity and specificity make it an effective tool to measure *S. mutans* levels and its use may facilitate further research in the field of oral health in general and the etiology of WSLs specifically.

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1. INTRODUCTION

This study investigated whether there is any significant effect of fixed orthodontic appliances on the presence of cariogenic bacteria in the oral cavity using a chair-side saliva assay. As an extension of active orthodontic treatment with fixed appliances, variations in levels of crowding was also anticipated as treatment progressed and the effect of dental crowding on salivary bacterial levels was also investigated. In the event that a cause and effect relationship was established, variation among specific bracket systems would also be analyzed if the sample size caries enough power to draw significant conclusions. The information gleaned from this study is of importance since it will help orthodontists to identify patients at higher risk for enamel white spot lesions (WSL)/incipient caries before they are clinically visible. Knowing which patients are at highest risk for WSLs permits the orthodontist to take preventive measures and possibly choose a bracket system that is patient-tailored should bracket type be found to have a statistically significant effect on salivary *S. mutans* levels. Preventive measures may include supplemental oral hygiene instruction, oral and written, or the prescription of antibacterial mouth rinses, topical fluorides. If a correlation between reduction in bacterial levels and reduction in dental crowding was made, this would further validate orthodontic treatment beyond well-known orthodontic benefits of form, function and esthetics. This study may also help validate the use of Saliva-Check MUTANS™ chair side saliva assay if findings of high bacteria prevalence correlated with saliva assays, both lab-rendered and chair-side, reported in the literature.

2. LITERATURE REVIEW

Incipient caries or white-spot lesions (WSL) are one of the most common side-effects of orthodontic treatment. The etiology of these WSL is multifactorial and has been a challenge for orthodontists since the science of orthodontics was first developed. In 1982, Gorelick quantified the extent of the problem reporting that 49.6% of orthodontic patients had WSL on at least one tooth following orthodontic treatment.[1] More recent studies by Richter have found the incidence of WSL following orthodontic treatment on at least a single tooth to be an alarming 72.9% and that "the preventive therapy provided appeared to be ineffective".[2] Strong links between WSL and high levels of *S. mutans* have been established by multiple studies.[3] Currently, there appear to be no longitudinal studies that evaluate *S. mutans* levels over the entire course of orthodontic treatment using the chair-side saliva assay Saliva-Check MUTANS™. While there have been studies that found no statistically significant difference between bacteria levels in ligated versus self-ligating brackets[4–6], there have been very few that used the Saliva-Check MUTANS™ chair-side saliva immune assay. The relatively recent development of fast and user-friendly chair-side saliva immunoassay Saliva-Check MUTANS™ provides an additional tool in the orthodontists tool belt for identifying patients at especially high risk for WSL.[7] In addition to investigating a cause and effect relationship between high bacteria levels and bracket system, this study will attempt to establish links between high bacteria levels and severity of dental crowding over time.

3. PURPOSE

To determine the overall prevalence of high *S. mutans* levels in orthodontic patients.

To determine the prevalence of high *S. mutans* levels as a function of dental crowding at each time point.

To determine the prevalence of high *S. mutans* levels as a function of bracket type (ligated vs. self-ligated) at each time point.

4. NULL HYPOTHESES

4.1 Null Hypothesis #1(TIME vs Bacteria): There is no change in cariogenic bacteria levels throughout patients' fixed orthodontic treatment.

4.2 Null Hypothesis #2(BRACKET vs Bacteria): There is no difference in cariogenic bacteria levels in patients treated with self-ligated vs. non self-ligated fixed orthodontic appliances.

4.3 Null Hypothesis #3(CROWDING vs Bacteria): There is no difference in cariogenic bacteria levels in individual patients with varying levels of dental crowding.

5. METHODS

5.1 ETHICS, PATIENT RECRUITMENT AND CONSENT

Ethics approval was granted by the University of Manitoba's Health Research Ethics Board (Appendix 12.4). New patients (or their legal guardian if under 18 yo) at the University of Manitoba orthodontic clinic, or either of the Children's Dental World locations in Winnipeg were verbally screened for interest in participating in the clinical trial described in this protocol. If interest was shown, the PI (or fellow G1,2 or 3 in the case of the PI's patients) would then present them with the information and consent disclosure form (Appendix 12.1). Once written consent was obtained, a sequential numerical code was assigned to the participant and a short survey and caries risk assessment (CRA)(Appendix 12.2) was administered verbally/conducted and saliva sample collected. Oral hygiene/diet instructions were also reviewed at this time. The baseline data were obtained prior to bonding fixed orthodontic appliances (T0).

5.2 SALIVA ASSAY

The immunoassay saliva test used in this trial was the Saliva-Check MUTANS ™ test. The participant began by chewing on a paraffin gum to stimulate salivary flow for one minute, followed by collection of the saliva into a mixing container. One drop of #1 reagent (alkaline solution) was added and mixed by tapping the container 15 times in a period of 10 seconds. Four drops of #2 reagent were then added and mixed the same way. Colour change to light green indicated a change in pH from alkaline to neutral and the solution was ready to be pipetted onto the

sample window of the test device. The test device was then left at room temperature for 15 minutes and a red line appeared in the Control window if the previous steps were done correctly. A second red line appeared in the Test window of the device if *Streptococcus mutans* concentration was high (> $5x10^5$ CFU/ml). A low *Streptococcus mutans* concentration (<$5x10^5$ CFU/ml) was indicated if after fifteen minutes there was no red line in the test window of the device.

5.3 DATA COLLECTION INTERVAL

In addition to the baseline data (T0), the same CRA, dental exam and saliva assay was performed throughout active orthodontic treatment at the following time points: 3months(T1), 6months(T2), and 12months(T3). The bracket system bonded and amount of crowding was also noted.

5.4 STATISTICAL METHOD

A repeated measures model was used to investigate the relationship between bracket type, crowding, time and the risk of High bacteria levels. The latter was the dependent variable in the regression model. Because the outcome is binary, a version of logistic regression was used which models the odds of the event for each predictor. To account for the repeated measures, which entail correlated data within subjects, the specific logistic regression model chosen was a generalized linear mixed-effects model (GLMM). The GLMM accounts for the fact that the risk of high bacterial levels intrinsically varies between subjects – some are at greater risk than others for various reasons outside our model/data. It can be thought of as an

extension to logistic regression, which assumes that all observations are independent. The GLMM regression model allowed us to evaluate the impact of time on our results. This term tells us whether the patients risk of high bacteria levels diverges over time, which would be indicative of a treatment effect. A preset threshold of significance of ($p < 0.05$) was assumed for this study. A test for crowding, which can vary over time as a within-subjects effect, was also evaluated.

Finally, the random subject effect was tested for using a logistic regression model with covariance parameters based on residual pseudo-likelihood. In this regression model, MI was derived from a *p*-value based on a mixture of chi-squares. Essentially, this tests whether subjects have different probabilities of high bacteria.

All analyses were carried out with PROC GLIMMIX of SAS version 9.3 (SAS Institute, Cary NC).

5.5 EXPECTED OUTCOME

Participants with a Low baseline bacteria level will have high bacteria levels after bonding of fixed orthodontic appliances, and those with high baseline levels will continue to be high. It is also predicted that participants with self-ligating bracket systems will have higher bacteria levels than those that are treated with ligated brackets. As orthodontic treatment progresses and crowding is resolved, it is reasonable to expect a drop in bacteria levels.

5.6 TIMELINE

Suspected timeline of study will be from October 2015 to June 2017.

5.7 ADVERSE/SERIOUS EVENTS

There were no anticipated adverse or serious events during this prospective study as it involved only a small saliva collection which was fast and painless as well as a short dental exam and survey that collected non-specific information anonymously.

5.8 POTENTIAL BENEFITS/HARMS

There were no anticipated potential harms to the participants of this trial as no identifying information was collected from the participants via a short survey/dental exam and the saliva samples collected were small, fast and painless. A potential benefit to participants was that they were told, if desired, if their cariogenic bacteria level was low or high chair-side. This, in and of itself, may have motivated patients to maintain/improve their oral hygiene. Participants were also entered into a draw for $500 once all four data collections were made. In general, a potential benefit from this study was the insight it provided into the effect of fixed orthodontic appliances on cariogenic bacteria levels allowing orthodontists to identify high-risk patients early using a simple chair-side saliva assay. This early assessment provided the orthodontist the opportunity to make patient-tailored choices for treatment including which bracket systems to use, and if the patient

would benefit from an oral antimicrobial rinse or topical fluorides to ultimately limit incipient caries during orthodontic treatment.

5.9 ALTERNATIVE TREATMENTS/PROCEDURES

There are no alternative pharmacological and non-pharmacological techniques applied to the orthodontic patients.

5.10 MINIMIZING POTENTIAL HARMS

All data collected were coded such that it could not be linked to individual participants. A single list of patients' chart numbers linked to the sequential code given for the trial were stored/locked in the Principal Investigators office, in a different drawer from the collected data. Only the PI and supervisor Dr. Robert Drummond had access to this list and it, along with the data collected. Data will be destroyed once the study is complete.

6. RESULTS

Overall, 100 patients undergoing orthodontic treatment were selected, among which 35 used conventional brackets and 65 used self-ligated brackets. The sample population was comprised of 60 females with mean age 18.2 ± 1.1 and 40 males with mean age 15.7 ± 0.7. The overall mean age was 17.2 ± 0.7. The chairside saliva assay Saliva-Check MUTANS™ was used to measure each subject at four time points: immediately prior to bonding - baseline (T0), at 3 months (T1), 6 months (T2), and 12 months (T3) into treatment. Bacteria levels and the amount of crowding were recorded at each time-point. Of the 400 anticipated data collection points, eight were not recorded as six patients were lost to follow-up (one of whom did not come for any appointments following bonding).

The results begin with simple data summaries (Tables 6.1 and 6.2) outlining bracket type and crowding levels at time points T1, T2 and T3. Within the tables, data are reported as both a raw number and a percentage of the total sample. At the bottom of each table, the frequency of missing data points were reported. At time point T1, there was only one missing data point out of 100 (Table 6.1). At time point T2, there was also only one missing data point out of 100 (Table 6.1). At time point T3, there were six missing data points out of 100 (Table 6.1).

Table 6.1: Crowding levels at T0, T1, T2 and T3, stratified by treatment.

Crowding Levels vs Bracket Type @ T0, T1, T2, T3				
	Crowding at T0 (baseline)			
Bracket Type	None	Mild/Moderate	Severe	Total
Non Self-ligated	3 (8.6%)	25 (71.4%)	7 (20.0%)	35
Self-ligated	6 (9.2%)	44 (67.7%)	15 (23.1%)	65
Total	9	69	22	100
Frequency Missing	0			
	Crowding at T1 (3 months)			
Bracket Type	None	Mild/Moderate	Severe	Total
Non Self-ligated	18 (51.4%)	15 (42.9%)	2 (5.7%)	35
Self-ligated	52 (81.3%)	10 (15.6%)	2 (3.1%)	64
Total	70	25	4	99
Frequency Missing	1			
	Crowding at T2 (6 months)			
Bracket Type	None	Mild/Moderate	Severe	Total
Non Self-ligated	30 (85.7%)	5 (14.3%)	0 (0%)	35
Self-ligated	60 (93.8%)	3 (4.7%)	1 (1.6%)	64
Total	90	8	1	99
Frequency Missing	1			
	Crowding at T3 (12 months)			
Bracket Type	None	Mild/Moderate	Severe	Total
Non Self-ligated	33 (97.1%)	1 (2.9%)	0 (0%)	34
Self-ligated	59 (98.3%)	1 (1.7%)	0 (0%)	60
Total	92	2	0	94
Frequency Missing	6			

The prevalence of high and low bacterial levels at each time point is shown in Table 6.2. The overall prevalence of high *S. mutans* levels was found to be 81% at baseline (T0), 78% at 3 months (T1), 68% at 6 months (T2) and 47% at 12 months (T3). Male prevalence values were higher than female values at each time point. Odds ratios(OR) and corresponding confidence intervals(CI) with *p*-values were calculated to quantify this gender difference. At T0 the OR was 2.13x with CI(0.70-

6.48), p=0.18. At T1 the OR was 1.36x with CI(0.52-3.60), p=0.53. At T2 the OR was 1.78x with CI(0.73-4.34), p=0.20. At T3 the OR was 1.36x with CI(0.60-3.10), p=0.46. Based on our accepted *p*-value of ≤ 0.05, gender difference was not statistically significant (Table 6.2 and Figure 6.1).

Table 6.2: Bacteria levels over time

Bacteria Levels vs Time				
		# Patients with Each Bacteria Level		
Time	Gender	High	Low	Total
T0 (0 months)	Male	35 (87.5%)	5 (12.5%)	40
	Female	46 (76.7%)	14 (23.3%)	60
	Overall	81 (81.0%)	19 (19.0%)	100
T1 (3 months)	Male	32 (80.0%)	8 (20.0%)	40
	Female	44 (74.6%)	15 (25.4%)	59
	Overall	77 (77.8%)	22 (22.2%)	99
T2 (6 months)	Male	30 (75.0%)	10 (25.0%)	40
	Female	37(62.7%)	22 (37.3%)	59
	Overall	67 (67.7%)	32 (32.3%)	99
T3 (12 months)	Male	20 (51.3%)	19 (48.7%)	39
	Female	24 (43.6%)	31 (56.4%)	55
	Overall	44 (46.8%)	50 (53.2%)	94
Frequency Missing	8 out of 400 samples total			

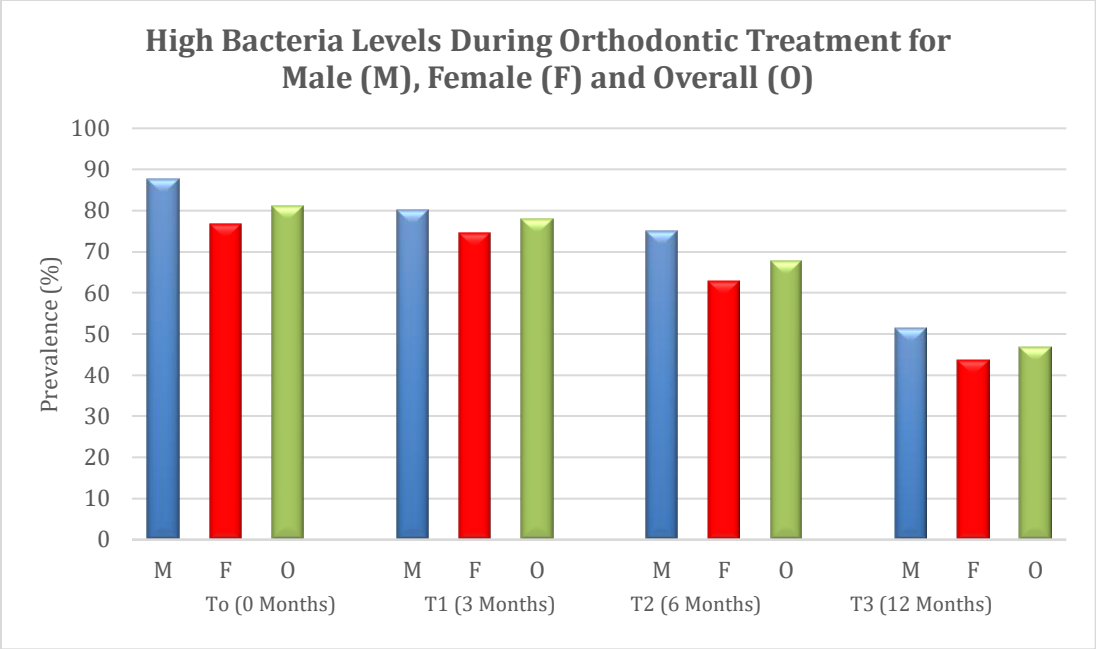

Figure 6.1: Bacteria levels over time

A generalized linear mixed-effects model (GLMM) was used to compare bracket, crowding and time effects on bacteria levels (Tables 6.3 & 6.4). Table 6.3 shows that GLMM results were non-significant ($p>0.05$) for bracket and crowding effects and only the effect of time was found to be significant. Since time was significant, pairwise contrasts were calculated using least squares means(Table 6.4). These contrasts tested each time point against the others and shows specifically, that the rate of high bacteria levels at 12 months (T3)($p<0.0001$) was different from other intervals of T0, T1 and T2 but the rest were not different from each other.

Table 6.3: Repeated-measures logistic regression model Type III tests of fixed effects

Type III Tests of Fixed Effects	
Effect	p'value
Bracket/Type	0.3818
Time	0.0007
Time*Bracket/Type	0.9802
Crowding	0.6796

Table 6.4: Repeated-measures logistic regression model, differences of time least squares means

Differences of Time Least Squares Means			
Time A (months)	Time B (months)	Estimate	p-value
0	3	-0.07741	0.8752
0	6	0.4769	0.3628
0	12	1.4461	0.0086
3	6	0.5543	0.1475
3	12	1.5235	<0.0001
6	12	0.9692	0.0046

7. DISCUSSION

In this study the prevalence of high S. mutans levels at baseline (prior to bonding, T0) was 81%. This appears to mirror three previous studies[8–10], but differs from two[7,11] similar studies in this relatively new field of research. A table summarizing current research evaluating the prevalence of S. *mutans* in saliva can be found in Appendix 5.4.

Karaoğlanoğlu et al (2010) study reported a S. *mutans* prevalence of greater than 10^5 CFU/mL in 69% of females and 65% of males.[8] The result obtained in our study of 81% was slightly higher than would be expected looking at the Karaoğlanoğlu study alone, however another study by Lombardo (2013) study found that all 20 orthodontic patients (100%) had a S. *mutans* prevalence of greater than 10^5 CFU/mL.[9] The result obtained in our study of 81% falls neatly in between the Karaoğlanoğlu and Lombardo studies. A study by Jung (2014) analyzed patients at the end of their orthodontic treatment and results were presented as an average of all 58 patients at each time point.[10] At the time of debond, and one week post-debond, the average bacteria prevalence was low (< 10^5 CFU/mL), while at five and 13 weeks post-debond, the average bacterial prevalence was high (>10^5 CFU/mL). Attempting to compare these results to the prevalence of 81% found in our study was difficult because the distribution of the mean in the Jung study is unknown. At best, we could only infer that at five weeks and 13 weeks post-debond, the prevalence of high bacteria levels was greater than 50%. The prevalence results of our study at baseline (T0) of 81% correlates modestly with a prevalence of

greater than 50% at 5 and 13 weeks post-debond in the Jung study. The prevalence results of our study at 12 months into orthodontic treatment (T3) show 53.2% of participants have low bacteria levels. This correlates modestly with a prevalence of greater than 50% of subjects in the Jung study having low bacteria levels at the time of debond and 1 week post-debond. These correlations, although vague, may imply that as orthodontic treatment progressed over time, including a period beyond debond of up to five weeks, high bacteria levels diminish. This theory would be supported by a similar decreasing trend in our study's high bacteria level prevalence (Table 6.2). Futhermore, once an extended period of time had passed post-debond (greater than five weeks post debond), it could be hypothesized that a person's natural bacterial level increased back to a level closer to that of their original level prior to bonding in the first place.

In contrast to our findings, a study by Gao (2012) evaluated 190 pediatric patients and found only a 39.0% prevalence of test subjects with high S. *mutans* levels using a culture-based assay and only a 28.4% prevalence using the Saliva-Check MUTANS™ assay.[7] PCR estimates were not given in the article, instead the PCR results were used to validate the accuracy of the other two methods. The result obtained in our study of 81% was much higher and correlates poorly with the values reported in the Gao study. It was possible that patient age played a significant role in levels of salivary S. *mutans*. Also, it has been shown that there is variation of oral microbiome among different ethnicities and geographic regions[12], which could explain why the Gao study results, based on a Hong Kong population

sample, varied from this Canadian study. Another study by Edith (2010) investigated the prevalence of salivary S. mutans levels in 30 orthodontic patients using a conventional cuture-based assay (Dentocult SM) at 4 stages of treatment.[11] The four stages of treatment were: initial (prior to bonding), one month after bonding, canine retraction, and anterior segment retraction. Results showed that at the initial time point, 45.5% of males and 42.1% of females had high bacteria levels. One month into treatment 45.5% of males and 52.6% of females had high bacteria levels. During canine retraction 81.8% of males and 89.5% of females had high bacteria levels. During anterior segment retraction 90.9% of males and 84.2% of females had high bacterial levels. The baseline prevalence results obtained in our study of 81% correlate best with mid-orthodontic treatment points of canine and anterior segment retraction in the Edith study, however the timing appears to be contradictory. The Edith study showed a steady increase in high bacteria prevalence as treatment progressed in contrast to our study (Table 6.2) and the Jung (2014) study which implied a steady decrease. Differences in prevalence trends during orthodontic treatment could be attributed to limited sample size in the Edith study. Our study showed a statistically significant ($p=0.0007$) interaction of time with high bacteria levels. Since time was significant, pairwise contrasts were calculated (Table 6.4). These contrasts tested each time point against the others. This logistic regression model showed that the rate of high bacteria levels at 12 months (T3) was different from the rest (T0, T1 & T2), but the rest were not different from each other.

The bracket, bracket*time and crowding effects were found to be non-significant. Only the effect of time was significant. *P*-values can be found in the table of "Tests of Fixed Effects" (Table 6.3).

An incidental finding in our study was that males had a greater prevalence of high S. *mutans* throughout orthodontic treatment, however this difference was not statistically significant. This finding has been mirrored in three other studies that reported no significant differences between gender[8,10,11].

There was good confidence in the Saliva-Check MUTANS ™ assay because it has a very high sensitivity of 97.6% and a specificity of 90.6% when compared to the gold standard Taqman real-time PCR.[7] A chair-side saliva assay was chosen over a lab-rendered assay because its efficiency, availability, relatively low cost and ease-of use permitted the collection of a larger sample size and therefore more powerful clinical study. The chairside assay used in this study also had a higher sensitivity and specificity than a conventional culture-based assay.[7]

The first limitation in comparing this study's data to the literature was finding studies that categorize their results into groups that had a natural division at the 10^5 CFU/mL bacteria count. This was relatively straight forward in studies that used the Saliva-Check MUTANS™ testing kits but required more in-depth analysis of the data in studies that used other testing products like Dentocult SM strips or samples that were analyzed in the lab.

Another challenge in data comparison of bacteria prevalence was that other studies evaluated different time points than ours, or had extremely small sample sizes or had targeted different population ages. In our study, the retention rate of study participants was excellent. This may be attributed in part to a generous participation draw of $500. The winner was determined by electronic random number generator.

The sample size of 100 participants tested at four time points was limited by the availability of patients at the very start of their orthodontic treatment, the fixed timeline afforded by the master's program of which I was enrolled, the extensive requirements of the research and ethics board required prior to commencing data collection for a clinical study, and the significant expense of the testing kits.

The etiology of decreasing bacteria prevalence throughout orthodontic treatment found in our study was likely multifactorial since time was the only variable tested that was statistically significant. A second hypothesis for the decreasing trend could be that S. *mutans* was being competitively inhibited by other bacteria as the oral flora adapted to changes introduced by orthodontic appliances. A third hypothesis for the decreasing trend could be that as orthodontic treatment progresses, S. *mutans* found in the saliva precipitates out of solution and is deposited onto brackets and their surrounding enamel.

Clinically, the use of chairside saliva assays is in its infancy and further research is required to assess the specific impact of high S. *mutans* levels on the prevention of WSLs. Future research may make it possible for an individual's entire microbiome

to be sequenced so that a personalized medicine approach can be applied by the dentist to maximize caries prevention of the individual.

Revisiting the null hypotheses, the findings of this research and their comparison with existing literature provides sufficient evidence to reject null hypothesis #1 and to accept null hypotheses #2 and #3.

Regarding the Null Hypotheses, the following conclusions can be drawn.

Null Hypothesis #1(TIME vs Bacteria): There was no change in cariogenic bacteria levels throughout a patients fixed orthodontic treatment. **Rejected**.

Null Hypothesis #2(BRACKET vs Bacteria): There was no difference in cariogenic bacteria levels in patients being treated with self-ligated vs. non self-ligated fixed orthodontic appliances. **Accepted**.

Null Hypothesis #3(CROWDING vs Bacteria): There was no difference in cariogenic bacteria levels in individual patients with varying levels of dental crowding. **Accepted**.

8. CONCLUSIONS

The overall prevalence of high *S. mutans* levels immediately prior to bonding orthodontic brackets was 81% using the Saliva-Check MUTANS™ chairside saliva assay. This prevalence decreased as orthodontic treatment progressed. The effects of bracket type and crowding on high bacteria levels were found to be non-significant. The effect of gender on high bacterial levels was observed to be higher in males at each time point, however this difference was not significant. The Saliva-Check MUTANS™ assays' high sensitivity and specificity make it an effective tool to measure *S. mutans* levels and its use may facilitate further research in the field of oral health in general and the etiology of WSLs specifically.

9. RAW DATA

TABLE 9.1: Type of bracket system and crowding and bacterial levels of 100 patients at 4 time points throughout orthodontic treatment. 8 samples lost to follow-up highlighted in blue.

Patient	Bracket Type	Crowding 0 months	Bacteria 0 months	Crowding 3 months	Bacteria 3 months	Crowding 6 months	Bacteria 6 months	Crowding 12 months	Bacteria 12 months
1	self litigated	mild / mod	High	mild / mod	High	none	High	none	High
2	non self litigated	mild / mod	Low	mild / mod	High	mild / mod	Low	none	High
3	self litigated	mild / mod	High	none	High	none	High	none	High
4	non self litigated	mild / mod	High	none	High	none	Low	none	Low
5	non self litigated	mild / mod	Low	none	High	none	Low	none	Low
6	self litigated	severe	High	none	High	none	Low	none	High
7	self litigated	severe	Low	none	High	none	Low	none	Low
8	non self litigated	mild / mod	High	mild / mod	High	none	High	none	High
9	self litigated	none	Low	none	Low	none	High	none	Low
10	non self litigated	severe	High	mild / mod	High	none	High	none	High
11	non self litigated	mild / mod	High	none	Low	none	Low	none	Low
12	non self litigated	none	Low	none	High	none	High	none	High
13	self litigated	severe	High	none	High	none	High	none	High
14	non self litigated	severe	High	mild / mod	High	none	High	none	Low
15	self litigated	mild / mod	High	none	High	none	High	none	High
16	non self litigated	severe	High	mild / mod	High	none	High	none	Low
17	self litigated	mild / mod	High	none	High	none	High	none	High
18	self litigated	severe	High	mild / mod	Low	mild / mod	High	mild / mod	High
19	self litigated	severe	High	severe	High	mild / mod	High	none	Low
20	non self litigated	mild / mod	High	mild / mod	High	mild / mod	High	none	Low
21	self litigated	severe	High	none	High	none	High	none	High
22	self litigated	mild / mod	High	mild / mod	High	mild / mod	Low	none	High
23	non self litigated	none	High	none	High	none	High	none	Low
24	non self litigated	mild / mod	Low	mild / mod	High	none	Low	none	Low
25	self litigated	mild / mod	High	mild / mod	High	none	High	none	Low
26	self litigated	mild / mod	High	none	Low	none	High	none	Low
27	self litigated	mild / mod	Low	none	High	none	High	none	High
28	self litigated	mild / mod	High	none	Low	none	Low	none	Low
29	non self litigated	mild / mod	High	none	Low	none	High	none	High

30	non self litigated	mild / mod	High	mild / mod	High	none	High	none	High
31	non self litigated	mild / mod	High	mild / mod	High	none	High	none	High
32	non self litigated	mild / mod	High	none	High	none	High	none	High
33	self litigated	mild / mod	Low	mild / mod	Low	none	High	none	Low
34	self litigated	mild / mod	High	none	High	none	High	none	Low
35	self litigated	mild / mod	High	none	High	none	High	none	Low
36	self litigated	mild / mod	High	none	High	none	High	none	High
37	self litigated	mild / mod	High	none	High	none	High	none	High
38	non self litigated	mild / mod	High	none	High	none	High	none	High
39	self litigated	severe	High	none	High	none	High	none	Low
40	self litigated	mild / mod	High	none	High	none	Low	none	Low
41	self litigated	mild / mod	High	none	High	none	High	none	High
42	self litigated	severe	High	none	High	none	High	none	High
43	non self litigated	mild / mod	High	none	High	none	High	none	High
44	self litigated	severe	High	mild / mod	High	none	High	none	High
45	self litigated	mild / mod	High	none	High	none	High	none	High
46	non self litigated	mild / mod	High	mild / mod	High	none	High	none	High
47	non self litigated	mild / mod	High	mild / mod	High	none	Low		
48	non self litigated	mild / mod	High	none	High	none	High	none	Low
49	non self litigated	severe	High	none	Low	none	High	none	Low
50	self litigated	mild / mod	High	none	High	none	Low		
51	self litigated	mild / mod	High	none	High	none	High	none	High
52	non self litigated	mild / mod	High	mild / mod	High	none	High	none	High
53	self litigated	severe	High	none	High	none	High	none	Low
54	self litigated	mild / mod	High	none	High	none	High	none	Low
55	non self litigated	mild / mod	High	none	High	none	High	none	High
56	self litigated	mild / mod	High	none	High	none	High	none	High
57	self litigated	mild / mod	High	mild / mod	High	none	High	none	Low
58	non self litigated	mild / mod	High	none	High	none	High	none	High
59	non self litigated	severe	High	severe	High	mild / mod	High	none	Low
60	non self litigated	mild / mod	High	mild / mod	High	none	High	none	High
61	self litigated	severe	High	mild / mod	Low	none	High	none	Low
62	self litigated	mild / mod	High	none	Low	none	Low	none	Low
63	non self litigated	mild / mod	High	mild / mod	High	mild / mod	High	mild / mod	Low
64	self litigated	mild / mod	High	none	High	none	High	none	Low
65	non self litigated	mild / mod	Low	none	High	none	Low	none	Low
66	self litigated	severe	Low	none	High	none	High	none	Low
67	non self litigated	severe	Low	severe	Low	mild / mod	High	none	High
68	self litigated	none	Low	none	High	none	Low	none	Low

69	self litigated	severe	High	none	High	none	High	none	High
70	self litigated	mild / mod	High	none	High	none	Low	none	Low
71	self litigated	mild / mod	High	none	High	none	High	none	High
72	self litigated	mild / mod	High	none	High	none	High	none	High
73	self litigated	mild / mod	High	none	High	none	High	none	Low
74	self litigated	mild / mod	High	none	High	none	Low	none	Low
75	non self litigated	mild / mod	High	mild / mod	High	none	High	none	High
76	non self litigated	mild / mod	High	none	Low	none	Low	none	Low
77	non self litigated	mild / mod	High	none	High	none	Low	none	Low
78	non self litigated	mild / mod	High	none	High	none	High	none	High
79	self litigated	mild / mod	High	none	Low	none	Low	none	Low
80	self litigated	mild / mod	Low	none	Low	none	Low	none	High
81	self litigated	severe	High	severe	High	severe	High		
82	self litigated	mild / mod	High	none	High	none	High	none	Low
83	self litigated	mild / mod	High	none	High	none	High	none	High
84	self litigated	mild / mod	Low	none	Low	none	Low	none	High
85	self litigated	mild / mod	Low	none	High	none	Low		
86	self litigated	mild / mod	Low	none	Low	none	Low	none	Low
87	non self litigated	none	High	none	Low	none	Low	none	Low
88	self litigated	mild / mod	Low	none	Low	none	Low	none	Low
89	self litigated	severe	High	mild / mod	High	none	High	none	High
90	self litigated	mild / mod	Low	none	High	none	Low	none	Low
91	self litigated	mild / mod	High	none	Low	none	Low	none	Low
92	self litigated	mild / mod	High	none	High	none	High	none	High
93	self litigated	mild / mod	Low	none	Low	none	High	none	Low
94	self litigated	none	High	none	High	none	Low	none	Low
95	self litigated	none	High	none	High	none	High	none	Low
96	self litigated	none	High	none	Low	none	Low	none	Low
97	self litigated	mild / mod	High	mild / mod	High	none	Low	none	Low
98	self litigated	none	High	none	Low	none	Low		
99	self litigated	mild / mod	High	none	High	none	High	none	Low
100	self litigated	mild / mod	High						

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12. LIST OF APPENDICES:

APPENDIX 12.1: Consent form for participation in study
APPENDIX 12.2: Modified caries risk assessment form
APPENDIX 12.3: University of Manitoba Health Research Ethics Board (HERB) Approval; Delegated Review and Annual Approval
APPENDIX 12.4: Summary of current research evaluating S. *mutans* prevalence in saliva.

APPENDIX 12.1: Consent form for participation in study

Clinical Study: Effect of Fixed Orthodontic Appliances on the Presence of Cariogenic Bacteria

Thank you for your interest in participating in this clinical study. Dr. Paige Kozak is a Masters of Dentistry Orthodontic resident and in her role as the Principal Investigator for this trial she will be collecting data in the form of a short survey, dental exam and saliva sample.

This study is being conducted to establish whether or not there is a link between having braces and having high levels of bacteria that cause cavities. The study will also look at whether or not the type of braces affects the same bacteria levels.

Your data will be collected using a chair-side survey followed by a dental exam and the collection of a small saliva sample. Your participation will take less than 5 minutes to complete and can be done during your regular orthodontic appointment, ideally while waiting for an instructor to verify your treatment of the day. Extra appointments outside of your regular treatment times are not required. Data will be collected before your braces have been bonded as well as after they have been bonded at an interval of 3months, 6months, 12 months, 18months etc until your braces come off. A final sample will be collected 3months after your braces have been removed. Every time data is collected your name will be entered in a draw for $500.

Your participation in this clinical study is completely voluntary. You are not required to provide any personal information such as your name, address or telephone number, and you don't have to answer any questions you don't want to. Also, if informed consent is obtained from parents/legal guardians but the minor patient does not want to participate, they don't have to. The only personal information that is recorded is your chart number so that the follow-up appointment can be booked. Only the Principal Investigator and her Supervisor Dr. Robert Drummond will have access to this list and it will be destroyed once the statistical analysis is complete.

The risks of participating are low. If there is a survey question that you find sensitive, there is no obligation to answer it. The dental exam and collection of the saliva sample is quick and painless. Sometimes, the research Supervisor, Dr. Robert Drummond may help collect data to ensure that the results we are getting are reliable.

Your participation is important to us and will help us to determine if there is a direct link between braces and high levels of cavity causing bacteria. If a link is found, this may lead to a more assertive approach to reducing these bacterial levels in patients with braces, with an ultimate goal of preventing white spots/incipient caries from forming while undergoing orthodontic treatment. As an added bonus, the saliva test is fast enough for us to let you know what your bacteria level is immediately. If you have any questions about this study, please do not hesitate to contact Dr. Paige Kozak at 204-789-3545.

This study has been approved by the University of Manitoba Health Research Ethics Board.

I consent to participate in this survey ○ Yes ○ No

_____	_____	_____
Patient/Legal Guardian (Print)	Patient/Legal Guardian (Signature)	Date
_____	_____	_____
Witness (Print)	Witness (Signature)	Date

APPENDIX 12.2: Modified caries risk assessment form

Caries Risk Assessment Form — Children Age 6 and Over/Adults

Patient #:_____ Date:_____ Age:_____ Gender: M / F

Time Point (months):	Baseline	3m	6m	12m	18m	____m	3m Post Tx

Disease Indicators (Any one "YES" signifies likely "High Risk" and to do a bacteria test **)	YES = CIRCLE	YES = CIRCLE	YES = CIRCLE
1.Visible cavities or radiographic penetration of the dentin	YES		
2.White spots on smooth surfaces	YES		
3.Restorations last 3 years	YES		

Risk Factors (Biological predisposing factors)

4.S. Mutans HIGH by Saliva Check Mutans chair side saliva assay		YES	
5.Visible heavy plaque on teeth		YES	
6.Frequent snack (> 3x daily between meals)		YES	
7.Deep pits and fissures		YES	
8.Recreational drug use		YES	
9.Inadequate saliva flow by observation		YES	
10.Saliva reducing factors (medications/radiation/systemic)		YES	
11.Exposed roots		YES	
12.Orthodontic appliances		YES	

Protective Factors

13.Lives/work/school fluoridated community			YES
14.Fluoride toothpaste at least once daily			YES
15.Fluoride toothpaste at least 2x daily			YES
16.Fluoride mouthrinse (0.05% NaF) daily			YES
17.5,000 ppm F fluoride toothpaste daily			YES
18.Fluoride varnish in last 6 months			YES
19.Office F topical in last 6 months			YES
20.CHX prescribed/used one week each of last 6months			YES
21.Xylitol gum/lozenges 4x daily last 6 months			YES
22.Calcium and phosphate paste during last 6 months			YES
23.Adequate saliva flow (> 1 ml/min stimulated)			YES

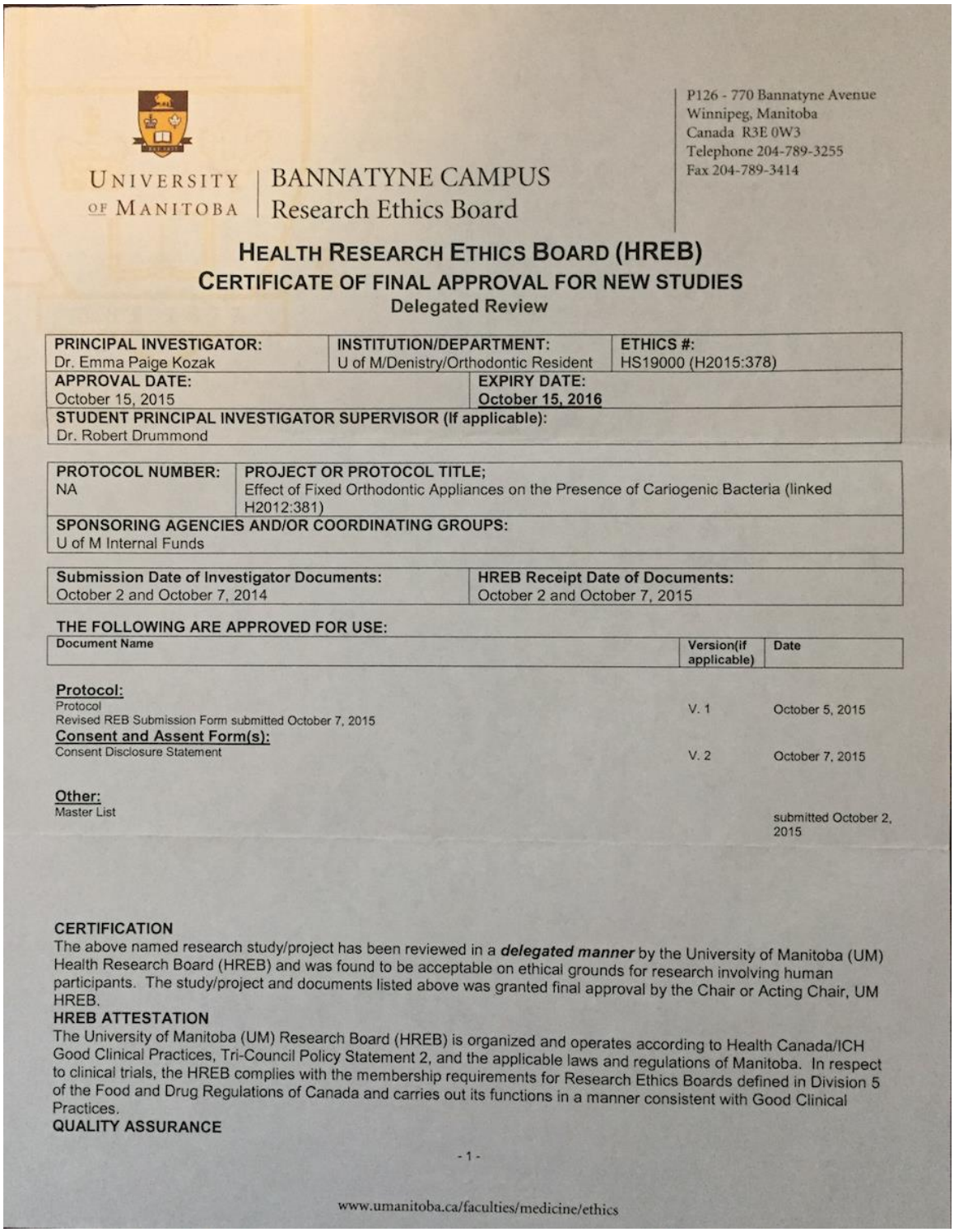
24.CARIES RISK ASSESSMENT (CIRCLE):	HIGH	MODERATE	LOW

25.TYPE OF FIXED ORTHODONTIC BRACKET SYSTEM	Self-Ligated? YES / NO TYPE:_____

26.CROWDING: ASSESS ARCH WITH MOST CROWDING	NONE(or spacing)	MILD-MOD	SEVERE

Doctor signature/#: _____ Date:_____

APPENDIX 12.3: University of Manitoba Health Research Ethics Board (HERB) Approval; Delegated Review and Annual Approval



UNIVERSITY OF MANITOBA | BANNATYNE CAMPUS Research Ethics Board

P126 - 770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Telephone 204-789-3255
Fax 204-789-3414

HEALTH RESEARCH ETHICS BOARD (HREB)
CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES
Delegated Review

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Emma Paige Kozak	U of M/Denistry/Orthodontic Resident	HS19000 (H2015:378)
APPROVAL DATE: October 15, 2015	**EXPIRY DATE:** October 15, 2016	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable): Dr. Robert Drummond		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE;
NA	Effect of Fixed Orthodontic Appliances on the Presence of Cariogenic Bacteria (linked H2012:381)
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: U of M Internal Funds	

Submission Date of Investigator Documents:	HREB Receipt Date of Documents:
October 2 and October 7, 2014	October 2 and October 7, 2015

THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol: Protocol Revised REB Submission Form submitted October 7, 2015	V. 1	October 5, 2015
Consent and Assent Form(s): Consent Disclosure Statement	V. 2	October 7, 2015
Other: Master List		submitted October 2, 2015

CERTIFICATION

The above named research study/project has been reviewed in a ***delegated manner*** by the University of Manitoba (UM) Health Research Board (HREB) and was found to be acceptable on ethical grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM HREB.

HREB ATTESTATION

The University of Manitoba (UM) Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada, R3E 0W3
Telephone : 204-789-3255
Fax: 204-789-3414

UNIVERSITY OF MANITOBA | Research Ethics - Bannatyne
Office of the Vice-President (Research and International)

HEALTH RESEARCH ETHICS BOARD (HREB)

CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR: Dr. Emma Paige Kozak	**INSTITUTION/DEPARTMENT:** U of M/Dentistry/Orthodontic Resident	**ETHICS #:** HS19000 (H2015:378)
HREB MEETING DATE (If applicable):	**APPROVAL DATE:** October 3, 2016	**EXPIRY DATE:** October 15, 2017
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable): Dr. Robert Drummond		

PROTOCOL NUMBER: NA	**PROJECT OR PROTOCOL TITLE:** Effect of Fixed Orthodontic Appliances on the Presence of Cariogenic Bacteria (linked H2012:381)
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: U of M Internal Funds	

Submission Date of Investigator Documents: September 17, 2016	**HREB Receipt Date of Documents:** September 19, 2016

REVIEW CATEGORY OF ANNUAL REVIEW: **Full Board Review** ☐ **Delegated Review** ☒

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name(if applicable)	Version(if applicable)	Date

Annual approval

*Annual approval implies that the most recent **HREB approved** versions of the protocol, Investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.*

Consent and Assent Form(s):

CERTIFICATION

The University of Manitoba (UM) Health Research Board (HREB) has reviewed the annual study status report for the research study/project named on this ***Certificate of Annual Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM HREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

HREB ATTESTATION

The University of Manitoba (UM) Health Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

APPENDIX 12.4: Summary of current research evaluating S. *mutans* prevalence in saliva.

Study	Orthodontic Patients?	Sample Size	Male	Female	Age	Test	Timepoints	S. mutans prevalence greater than 10^5 CFU/mL	Gender difference?
Karaoğlanoğlu (2010)	No	135	71	62	20 – 30 years	Ivoclar Vivadent AG (48 hrs incubation)	Single timepoint	69% of females and 65% of males	Females higher but not statistically significant
Lombardo (2013)	Yes (during treatment)	20	5	15	19 – 23 years	Mitis salivarius agar (48 hrs incubation)	T0 (prior to bonding, T1 4 weeks; T2 8 weeks	100%	N/A
Jung (2014)	Yes (post treatment)	58	20	38	23.4 years	Real Time PCR	T1 (debond; T2 1 week post; T3 5 weeks post; T4 13 weeks post	<50% at T1+T2; >50% at T3+T4 (only the following averages were given in Log_{10} /mL: T1 4.73, T2 4.79, T3 5.02, T4 5.22)	No
Gao (2012)	No	190	N/A	N/A	3 – 4 years	1) Dentocult SM; 2) Saliva Check MUTANS; 3) Real Time PCR	Single timepoint	39% (Dentocult SM; 28.4% (Saliva Check MUTANS; (Results not given for PCR)	N/A
Edith (2010)	Yes (during treatment)	30	11	19	16.5 years	Dentocult SM	T0 (prior to bonding, T1 4 weeks; T2 Canine retraction; T3 Incisor retraction	T0 45.5% males, 42.1% females; T1 45.5% males, 52.6% females; T2 81.8% males, 89.5% females; T3 90.9% males, 84.2% females	No statistically significant difference