UNIVERSITI TEKNOLOGI MARA

RESEARCH PROPOSAL

Effectiveness of Thermoplastic Retainer Cleansing Agents and Patient Reported Outcome- A Randomised Controlled Trial

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1.0 INTRODUCTION

1.1 Background of study

After the completion of orthodontic treatment, patients will undergo a retention phase to prevent relapse. The use of orthodontic retainer during this phase can pose a lot of risks to the oral health especially if oral hygiene is not well maintained. Without adequate cleaning, retainers can attract plaque deposition and become a reservoir for oral microflora (Tiro, 2018). In turn, this can cause gingival inflammation and periodontal disease (Jaderberg, Feldmann, & Engstrom, 2012). Unfavourable oral microflora multiplication and opportunistic pathogens may affect the oral health of patients wearing retainers (Al Groosh et al., 2011). A study advocated that wearing thermoplastic retainer alters saliva buffer capacity, pH acidity and flow rate (Chang, Al-Awadi, Ready, & Noar, 2014). A variety of alternative cleaning agents have been discussed of which were mostly tested on acrylic orthodontic appliances and prostheses. To date however, few studies have been performed to investigate the effects of cleansing agents on thermoplastic retainers and changes in the oral environment.

1.2 Literature review

Orthodontic relapse or unfavourable changes in the alignment of teeth after the completion of orthodontic treatment are usually undesirable for the patients and clinicians (Alkadhimi & Sharif, 2019). Relapse was defined by the British Standards Institute (BSI) in 1983 as 'The return, following correction, of the original features of the malocclusion'.

Patients wearing retainers are at risk to develop caries (Al Groosh et al., 2011). Inadequate oral hygiene and frequent intake of sugary food and drinks will cause caries and periodontal disease (Tiro, 2018).

1.2.1 Types of retainer

Orthodontic retainers can be classified into removable and bonded fixed retainers. Choosing the type of retainers depends on a wide range of factors including the types of

orthodontic tooth movements, the outcome of orthodontic treatment, patients' age and compliance. Removable retainers require more patient compliance in comparison to bonded fixed retainers. The most common type of removable retainers are Hawley retainer and the vacuum-formed retainers (Mai et al., 2014). Others may include Begg 'wraparound' retainer and Hawlix 'aesthetic retainer'.

The removable vacuum-formed retainers were introduced by Ponitz in 1971 and further developed by Sheridan (Maurice J Meade & Millett, 2015). There are many terms referring to the invisible retainer such as vacuum-formed retainer, thermoplastic retainer, clear overlay retainer (Mai et al., 2014). Vacuum-formed retainers are made of polypropylene or polyvinylchloride material (Manzon, Fratto, Rossi, & Buccheri, 2018). In this research, the term thermoplastic retainer will be used. Thermoplastic retainers are widely sought after due to its aesthetics, ease of application and requires less lab work (Çifter, Gümrü Çelikel, & Çekici, 2017). A recent study concluded that thermoplastic retainers are more cost effective. As for the retention regime, wearing thermoplastic retainers part time is sufficient and does not cause any significant relapse (Gardner, Dunn, & Taloumis, 2003).

However, thermoplastic retainers are not the retainer of choice in cases that involved intrusion or extrusion movement and expansion. Patients with compromised periodontal health are also less suitable for thermoplastic retainers (Maurice J. Meade & Millett, 2013). Furthermore, among the major risks of wearing thermoplastic retainers is gingival recession. However in a study by (Çifter et al., 2017) gingival recession was not observed as the thermoplastic retainers were fabricated extending the gingival margin 2 mm buccally and 2-4mm lingually.

Patients wearing thermoplastic retainer with high sugar intake have increased risk of decalcification as the retainer may act as a reservoir (Alkadhimi & Sharif, 2019). This makes retainer maintenance even more important for patients wearing thermoplastic retainer. Previous published journal hypothesized that the irregular inner surface of the thermoplastic retainer makes cleaning more difficult and may favor more bacterial adherence as compared to Hawley retainer (Manzon et al., 2018).

1.2.2 Thermoplastic retainer maintenance

The most common and simple method of cleaning thermoplastic retainers involve brushing with a standard tooth brush and toothpaste under tap water (Chang et al., 2014). A study done in Germany on 450 orthodontic specialist practices found that 90% of these orthodontists recommend mechanical cleaning of removable orthodontic appliance with toothbrush and toothpaste (Eichenauer, Serbesis, & Ruf, 2011). During an in vitro study by Chang et al (2014), brushing with toothpaste was found to be 99% effective in reduction of *Streptococcus mutans.* Toothpaste composes of active and inactive ingredients. Among the inactive ingredients are abrasive agents which are effective in removing stains besides having anti-bacterial properties (Subramanian, Appukuttan, Tadepalli, Psg, & Victor, 2017). The frequently used abrasive agents are dicalcium phosphate dihydrate, calcium pyrophosphates, sodium carbonates, calcium carbonates and hydrated silicas. Previous papers that stated the risk of toothpaste causing abrasion were mostly on dentition and acrylic appliance. At present, there is no study that conclude using toothpaste can cause abrasion on thermoplastic retainers. This research will study on toothpaste as cleaning agent on thermoplastic retainers.

Besides toothpaste, chemical cleaning tablets or solutions has been advocated to increase effectiveness in cleaning retainers. Among the commercialized chemical cleaning agents available are Retainer Brite, Polident, Chlorhexidine and Listerine mouthwash. Commercial product such as Retainer Brite (Dentsply International Raintree Essix) as seen in Figure 1 requires soaking the retainers in a solution (Chang et al., 2014). Retainer Brite ingredients are as detailed (refer to 4.9.2 Materials). Based on the commercial website, Retainer Brite integrates both mechanical and chemical mode of cleaning. The bubbles from soaking the tablet in water will scrub the build-up of plaque and tartar from the appliance besides having anti-bacterial properties. The commercial products available might differ in the cleaning procedure. With improper cleaning, patients wearing thermoplastic retainer are at risk of developing caries, gingivitis or periodontal disease (Lara-Carrillo, Montiel-Bastida, Sanchez-Perez, & Alanis-Tavira, 2010). Little research has been done on the cleaning solutions and its effectiveness in controlling cariogenic bacteria on patients wearing thermoplastic retainer. Furthermore, there is no consistent recommendations on cleaning retainers (Eichenauer et al., 2011). Noteworthy, Levrini

et al, 2015 emphasized on cleaning the internal surface of appliance as it has been found to have more biofilm accumulation as compared to the external surface.

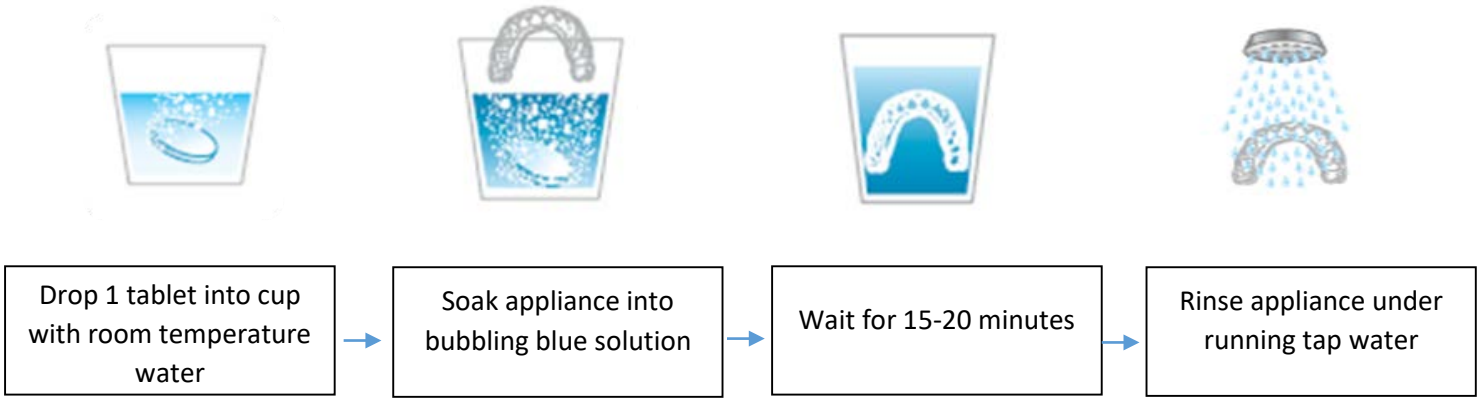


Figure 1: Retainer Brite Instructions from Dentsply International Raintree Essix

1.2.3 Risks and complications

Retainers in place intraorally reduces the physical cleaning effect of saliva and this predisposes patients to plaque accumulation, enamel demineralization and caries (Manzon et al., 2018). Enamel demineralization will progress to become white spot lesions (Ko-Adams et al., 2020). Recent studies have reported 70% of white spot prevalence and 5% cavity prevalence after completion of orthodontic fixed treatment (Tiro, 2017).

Intraorally, there is a balance of oral microbiota in a healthy individual (Al Groosh et al., 2011). Among the normal oral microflora present in patients with no caries are Mutans streptococci (Nishikawara et al., 2006). Mutans streptococci is divided into seven species, of these; the primary etiological species associated with caries are *Streptococcus mutans* and *Streptococcus sobrinus* (Yoo et al., 2007). Numerous published papers have associated *Streptococcus mutans* as early colonizers of plaque (Turkoz, Canigur Bavbek, Kale Varlik, & Akca, 2012) (Arab et al., 2016). This research will study on Mutans streptococci which was found to be a useful caries risk assessment (Batoni et al., 2001). The other group of bacteria that is associated with caries is Lactobacilli. This group of bacteria do not play a role in initiation of dental decay (Guo & Shi, 2013). Lactobacilli will be detected in abundance in deeper cavity on teeth (Batoni et

al., 2001). A recently published paper reiterated that *Streptococcus mutans* is found in the early stage of caries, while Lactobacilli is strongly related to cavities (Mummolo et al., 2020).

1.2.4 Saliva

Guo & Shi (2013) stated that whole saliva is a mixture of not only salivary gland secretions, it also contains bronchial and nasal secretions together with bacterial products, viruses, fungi and many others. Saliva aids in food digestion, lubrication, mucosal integrity, buffering capacity, and remineralization. Saliva anti-microbial properties are from amylase, complement, defensins, lysozyme, lactoferrin, lactoperioxidase, mucins, cystatins histatins and proline-rich gylcoproteins (Kaufman & Lamster, 2002). There are more than 700 oral microbial species found in the oral cavity that amounts to 10^8 to 10^9 CFU/mL (Guo & Shi, 2013). Extensive number of research has provided significant information on caries formation due to potentially cariogenic bacteria presence in saliva (Guo & Shi, 2013). The presence of cariogenic bacteria in saliva is influenced by multiple factors, such as frequent carbohydrate consumption and oral hygiene among others. Besides that, in a study by (Van Nieuw Amerongen, Bolscher, & Veerman, 2004) stated that saliva is also able to protect teeth against abrasion and attrition, retarding demineralization as well as promoting remineralization.

Unstimulated saliva are the saliva formed in the oral cavity without any exogenous stimuli (Ranganath, Shet, & Rajesh, 2012). It has been reported that unstimulated saliva flow rate is significantly different in females and males. This is due to different parotid and submandibular gland size (Inoue et al., 2006). Stimulated saliva is a means to increase saliva secretion after applying stimulants such as chewing paraffin gum (Mahvash Navazesh, 2008). This research will study on unstimulated saliva as it represent the 24 hours oral cavity. Previous studies has correlated between orthodontic appliance and changes in saliva flow rate, pH acidity and buffer capacity (Lara-Carrillo et al., 2010). The buffering function in saliva is from the presence of bisphosphonate, phosphate ions and proteins (Kaufman & Lamster, 2002). Low unstimulated saliva flow rate has been related to increased risk of developing oral disease. High flow rate indicates good physical cleaning action of saliva on substances in the oral cavity (Yamuna &

Muthu, 2017). As for saliva viscosity, the greater viscosity of saliva indicates lesser cleaning action (Ranganath et al., 2012). A recent published paper have found a significant decrease of pH and no significant changes in saliva flow following the placement of fixed appliances (Arab et al., 2016). To date, there is no data on saliva parameters during retention phase. Therefore, this research will also assess on saliva hydration, viscosity, quantity and pH.

1.3 Problem statement

It is always difficult to explain the importance of keeping the retainers clean during the discussion at post-debond of appliances. Moreover, orthodontists are often left to guess the best retainer cleansing agent and often suggest different cleaning methods based on their own experience. Most studies done on cleansing solutions, both commercial and home product has been in vitro. To date, there has been no randomised controlled trial (RCT) that states the best cleansing agent for retainers taking into consideration the bacterial colony forming unit (CFU) of cariogenic bacteria on thermoplastic retainer, cost and the ease of carrying out the procedure daily by the patient involved.

In spite of improvements in dental materials and techniques, caries and white spot on tooth surfaces are common. During retainer application, there is reduced or less saliva to flush the teeth area from acid accumulation produced by cariogenic bacteria and alters the pH saliva. Imbalance of saliva properties might have a direct influence on the bacterial count of plaque samples taken from patients wearing thermoplastic retainers. Hence, there is a need to investigate on saliva parameters during retention phase as the attachment and colonization of bacteria are influenced by multiple factors.

There is also an increasing drive to incorporate patient related outcome measures such as patient acceptance and experience in healthcare provision. Previous reports were on participants' level of knowledge, expectation in orthodontic retention and patient acceptance following different treatment protocols but no consideration on the taste, smell, cleaning time, time allocation and cost with regards to the retainer maintenance from the patients' point of view.

1.4 Study significance

Retainer hygiene advice needs to be effective and reliable. This study will provide evidence to guide Orthodontists in making recommendations on the most effective cleansing agent for thermoplastic retainers. The result of this study will illustrate the amount of cariogenic bacteria on thermoplastic retainers and any alteration on saliva parameters upon using three different cleansing agents. This study will also provide an insight on patient reported outcome.

2.0 OBJECTIVES

2.1 General Objective

To assess the effectiveness of three cleansing agents; tap water, toothpaste and Retainer Brite against cariogenic bacteria.

2.2 Specific Objectives

- To quantify and compare the presence of Mutans Streptococci (MS) between the three cleansing agents using colony counting method
- To investigate salivary parameters of unstimulated saliva during retention period
- To evaluate patient reported outcome on the different cleansing agents using Likert scale

3.0 NULL HYPOTHESES

- There is no significant difference in the presence of Mutans Streptococci (MS*)* between three cleansing agents.
- There is no significant difference in salivary parameters in unstimulated saliva.
- There is no significant difference in patient reported outcome on different cleansing agents.

4.0 METHODOLOGY

4.1 Ethical approval

Ethical approval will be obtained from the UiTM Research Ethics Committee.

4.2 Study design

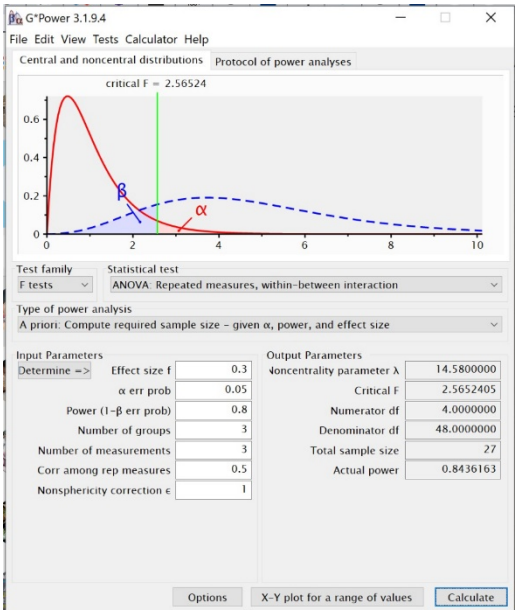
This study is designed as a three arm parallel-group randomisation controlled trial. Patient flow diagram as in (Figure 2).

4.3 Study setting

This study will take place at UiTM Orthodontic Dental Clinic (Faculty of Dentistry UiTM Sungai Buloh)

4.4 Sample size calculation

Sample size calculation will be carried out using the G* Power for Analysis of Variance in which the significance level will be set at 0.05 and statistical power at 0.8 with effect size of 0.3. A sample size of 9 patients will be required for each intervention group. However, in anticipation of 10% drop out, this study will recruit 10 patients per group. Hence, a total sample size of 30 patients. Below is picture of the sample size calculator.



4.5 Sample selection

Patients who are ready for debond at the Department of Orthodontics UiTM Sungai Buloh will be briefed on the trial. Written informed consent will be obtained from patient and will be screened based on inclusion and exclusion criteria as in Table 1.

Inclusion criteria	Exclusion criteria
Patients fitted with thermoplastic retainer Above 18 years old Caries free patients Healthy periodontal status	Pregnant Patients on regular medication Smokers Patients with systemic disease Prosthetic tooth in retainer

Table 1: Patient inclusion and exclusion criteria

4.6 Randomisation:

Patients will be randomly allocated to 3 groups;

- **Group A** (tapwater as control), **Group B** (toothpaste) or **Group C** (Retainer Brite)
- The list of randomisation will be generated by using an online randomisation system based on block sizes of 6 with stratification according to gender.

Allocation concealment will be done through sequentially numbered, opaque, sealed envelope. The envelope will contain the number that will indicate the allocated group intervention for the patient. The main supervisor will be responsible for the envelope selection process by the patients.

4.7 Blinding

It is not possible for the researcher and patients to be blinded to the treatment intervention. Samples will be labelled with anonymous ID number to ensure blinding during microbiological analysis process and data analysis.

4.8 Outcome measures

The study objectives and type of variables are as in Table 2

Study objectives	Type of variables	
	Independent	Dependent
To quantify and compare the presence of Mutans Streptococci between three cleansing agents using colony counting method	Cleansing agent Plaque sample Saliva sample	Colony forming unit (CFU)
To investigate the salivary parameters of unstimulated saliva during retention period	Visit/ Time	Salivary hydration, viscosity, pH, quantity
To evaluate patient reported outcome on the different cleansing agents	Cleansing agent	Patient response/ experience

Table 2: Study objectives and type of variables

4.9 Materials

4.9.1 Retainer

Thermoplastic retainers will be fabricated with 0.04-inch plastic material (Essix ACE; Dentsply International, York, Pa), covering all tooth surfaces up to half of occlusal surface of the most distal tooth in the arch and will be trimmed 2 mm occlusally away from the gingival margin.

4.9.2 Cleansing materials

Cleansing materials that will be used in this study are as follows;

- Colgate Cushion Clean toothbrush with soft bristle
- Toothpaste (Colgate Total Range) 150g with active ingredients of sodium fluoride 0.24%, triclosan 0.3% and inactive ingredients such as hydrated silica, glycerin, sorbitol, PVM/MA copolymer, sodium lauryl sulfate, cellulose gum, flavor, sodium hydroxide, propylene glycol, carrageenan, sodium saccharin and titanium.
- Retainer Brite tablets for Oral Care, Clear Aligners and Mouthguards is manufactured from Dentsply International Raintree Essix. Ingredients are: sodium bicarbonate, citric acid, sodium carbonate, potassium persulfate compound, corn syrup solids, sodium

percarbonate, sodium sulfate, sorbitol, tetraacetylethylenediamine, PEG-180, sodium lauryl sulfoacetate, magnesium stearate.

All patients will be provided with a dental kit according to group;

- **Group A**; Toothbrush and toothpaste
- **Group B**; Toothbrush and toothpaste
- **Group C**; Toothbrush, toothpaste and Retainer Brite cleaning tablets

4.9.3 Clinical items

No.	Item
1.	Personal protective equipment (Nitrile examination gloves, face mask and plastic gown)
2.	Examination set (mouth mirror with handle, WHO Periodontal probe, Dental Probe, Dental Tweezer)
3.	Sterile Cotton Swab (Individually packed)
4.	15mL centrifuge tube
5.	1.5 mL microcentrifuge tubes with 0.5 mL Phosphate Buffered Saline
6.	pH indicators strips pH 0-14 (universal indicator)
7.	Icebox (4.5L)

Table 3: Clinical items

4.9.4 Laboratory items

No.	Items
1.	1.5 mL Microcentrifuge tubes
2.	1.5 mL Microcentrifuge rack
3.	Phosphate Buffered Saline Solution
4.	15 mL Centrifuge Tube Rack
5.	Micropipette (Sartorius)
6.	200 µl Universal Pipet Tips, Non-sterile
7.	1000 µl Universal Pipet Tips
8.	Cryogenic Storage Boxes, Polypropylene, 100-wells
9.	Autoclave tape
10.	Masking tape
11.	Heavy duty Aluminium Foil
12.	Forcep
13.	Ethanol 70% (denaturated)
14.	Sterile spreader
15.	Parafilm

16.	Disposable Sterile Petri Dish 90x15mm
17.	Mitis Salivarius Agar
18.	Bacitracin
19.	Potassium Tellurite 3.5%
20.	Sucrose
21.	Anaerogen 2.5 L
22.	BHI Broth
23.	Inoculating Loops
24.	API kit
25.	Existing facilities such as autoclave, biological safety cabinet, incubator, chiller 4°C

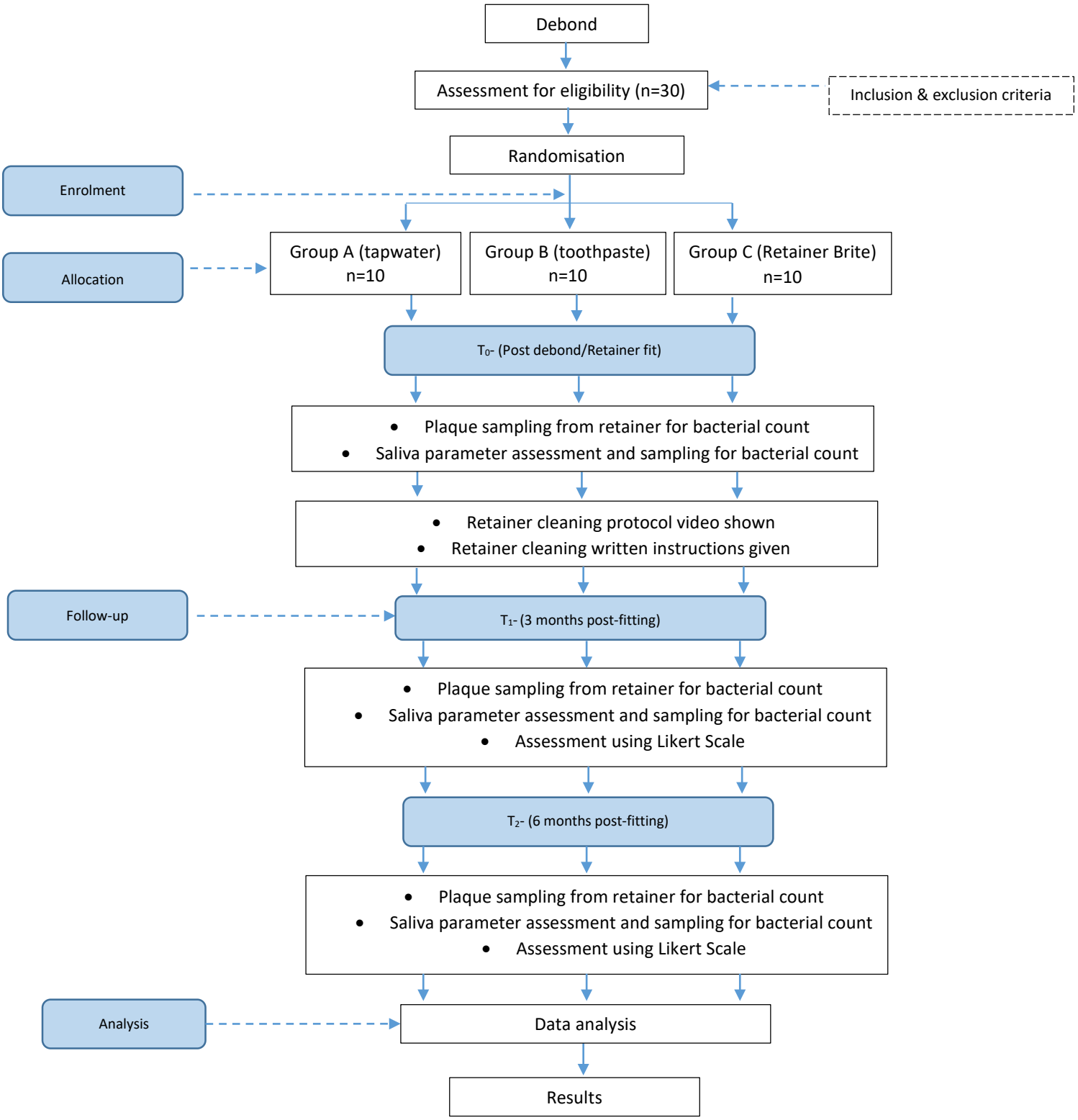
Table 4: Laboratory items

4.9.5 Miscellaneous

- Paper (Double A A4 80gsm) and print ink (Canon PG 47 Black Catridge)

Refer to Appendix a for budget estimation and details

Figure 2. CONSORT participant flow diagram



4.10 General procedure

4.10.1 On the day of debond:

- Fixed appliances will be removed followed by scaling, polishing and alginate impression for a standard retainer fabrication will be carried out.
- Retainer will be fitted on the same day of debonding.
- Patients will be assessed for eligibility based on inclusion and exclusion criteria.
- Basic periodontal examination (BPE) will be carried out to screen patient periodontal status. The dentition will be divided into 6 sextant. A World Health Organisation probe (WHO BPE probe) with a "ball end" and a black band from 3.5 mm to 5.5 mm will be "walked around" the sulcus in each sextants using light probing force (20-25 grams) as outlined by The British Society of Periodontology 2011 Guidelines. The scoring codes are as below in Table 5.

0	No pockets >3.5 mm, no calculus/overhangs, no bleeding after probing *(black band completely visible)*
1	No pockets >3.5 mm, no calculus/overhangs, but bleeding after probing *(black band completely visible)*
2	No pockets >3.5 mm, but supra or subgingival calculus/ overhangs *(black band completely visible)*
3	Probing depth 3.5-5.5 mm *(black band partially visible, indicating pocket of 4-5mm)*
4	Probing depth >5.5 mm (black band entirely within the pocket, indicating pocket of 6 mm or more)
*	Furcation involvement

Table 5. Scoring codes

- Informed consent will be obtained.
- Patients will be allocated to the treatment intervention following the randomisation procedure (refer to 4.6 Randomisation).

4.10.2 Retainer fit

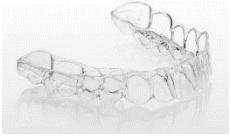
- During the retainer fit, patients will be provided with dental kit (refer to 4.9.2 Cleansing materials)
- A video will be shown and given to each subject explaining on respective cleaning protocol;
    - **Group A** as control will clean their thermoplastic retainer by ONLY brushing under running tap water in the morning and at night.
    - **Group B** will clean their thermoplastic retainer by ONLY brushing with toothpaste and rinse under running tap water in the morning and at night.
    - **Group C** will clean their thermoplastic retainer by ONLY brushing under running tap water and soaking the thermoplastic retainer into the bubbling blue solution, produced when 1 Retainer Brite tablet is dropped into a cup of warm (not hot) water. After 15 minutes, rinse retainer under running tap water. This will be done only at night and to brush under running tap water in the morning.

- The general retainer wear instructions that will be explained to patients are;
    - Retainers are to be worn full time except during meals, sports and brushing in the morning and at night. When not worn, kindly ensure the retainer is kept safe in a retainer case.
    - Apart from the morning and night cleaning, at other times throughout the day the retainer can be rinsed under running tap water.
    - After eating, floss teeth and rinse mouth with water before inserting retainers into mouth.
    - Do not use any other toothpaste, cleaning tablets or mouthwash that are not provided during the experiment.
    - Do not hesitate to call the clinic if retainers are unfit, sharp, broken or lost.
- Written instructions will be provided accordingly in English and Bahasa Malaysia.

4.10.3 Plaque sampling

- Plaque sample will be collected with sterile applicator on the labial and palatal/lingual inner surface on the thermoplastic retainer covering 10 teeth as in Figure 2.
- The swab will be placed in a 1.5 mL microcentrifuge tube with Phosphate Buffered Saline (PBS) and placed in an ice box as in Figure 2.

Sterile applicator



Thermoplastic retainer

Microcentrifuge tube

Ice box

Figure 2: Plaque sampling

4.10.4 Saliva parameter assessment and sampling

- Saliva parameter assessments will be on hydration, viscosity, quantity and pH.
- This procedure will take 30 minutes for each patient.
- For hydration, patients will be required to sit and relax, instructions will be explained to the patient.
    - The lower lip will be pulled and dabbed gently with gauze. The time taken for new droplets of saliva to form on the lower lip (orifice of the minor salivary glands) will be recorded.
- For saliva viscosity, quantity and pH, patients will rinse their mouth with sterile water several times and then to relax for five minutes.
    - Patients will be required to sit in an upright position at rest with the head bent down to allow the saliva to pool in the floor of mouth. Every 1 minute, the saliva pooled at the floor of mouth will be spitted into a sterilized gradient tube and this will be repeated for a total of 5 minutes.

- Visual assessment will be done on the saliva to differentiate the rate of production.
- The quantity of the saliva collected will be noted.
- A pH test strip will be placed in the collected resting saliva sample for 10 seconds and colour of the strip will be assessed.

- The findings and interpretation for each saliva parameter are as in Table 6:

Saliva Parameter	Findings	Interpretation
Hydration	Greater than 60 seconds	Low
	30-60 seconds	Normal
	Less than 60 seconds	High
Viscosity	Clear and watery	Sufficient production
	Bubbly and sticky	Low production
Quantity	Less than 0.1 mL/min	Very Low
	0.1-0.3 mL/min	Low
	More than 0.3 mL/min	Normal
Saliva pH	Red	Highly acidic (pH 5.0-5.8)
	Yellow	Moderately acidic (pH 6.0-6.8)
	Green	Healthy (pH 6.8-7.8)

Table 6: Saliva findings and interpretation

- The saliva sample will be placed in an icebox together with the plaque sample.
- The ice box will then be transferred to the laboratory for microbiological analysis (refer to 4.11).
- Written retainer cleaning instructions will be provided to take home as attached in Appendix B.
- The following appointments will be given for plaque sampling and saliva assessment and sampling at 3 months post-fitting (T_1) and 6 months post-fitting (T_2).

4.11 Microbiological analysis

- Following the plaque and saliva sampling, bacterial count process will be carried out.
- A micropipette will be used to transfer 100 µL of sample from original sample to the 1st tube.
- Micropipette will be used to transfer 100 µL from the 1st tube to the 2nd tube. This process will be repeated until all tube diluted as in Figure 3.

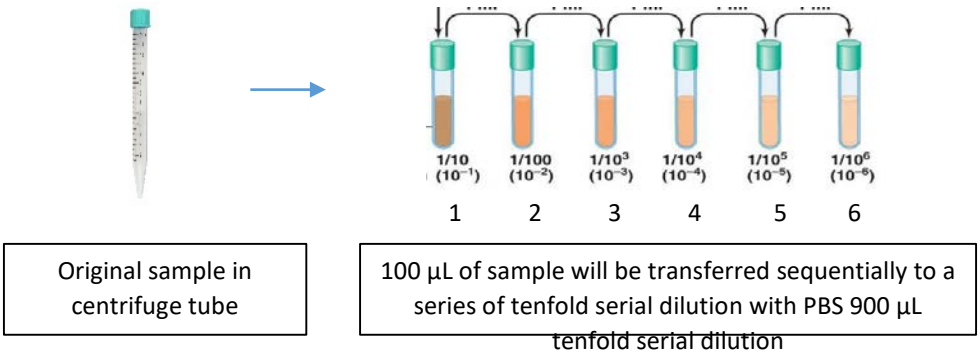


Figure 3: Sampling serial dilution

- Following the tenfold serial dilution, 100 µL of sample from each diluted tube will be evenly distributed on mitis salivarius agar with bacitracin and 20% sucrose as seen in the figure 4 below to observe Mutans streptococci (MS) (Ko-Adams et al., 2020).
- The plate will be placed in an incubator at 37°C for 24-48 hours to allow the colony to grow (Aydogan Akgun, Senisik, & Sesli Cetin, 2019).

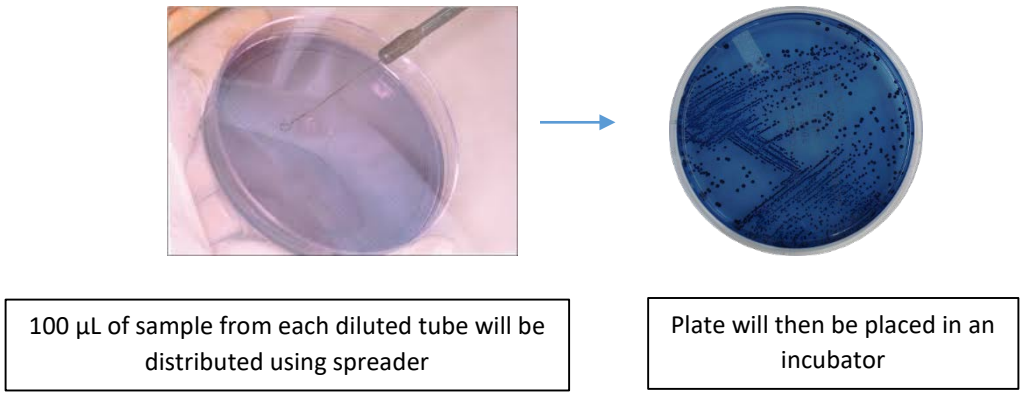
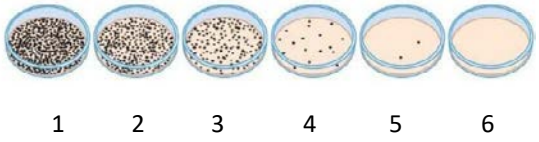


Figure 4: Sampling plating

- Then, colony grown on the plates will be placed on a grid background on the colony counter. The number of viable microbial organism will be counted using the magnifying glass on the colony counter as seen in Figure 5.



Each plate will be labelled according to the tube dilution factor

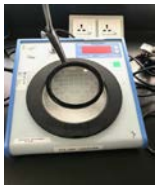


Plate will be placed on the colony counter

Figure 5: Bacterial count

- To determine the MS colony numbers in a given 100 µL sample, the plate with the most optimal colony count which is (30 to 300 counts) will be chosen. The colony count will be multiplied by the tube dilution factor and then divided by the volume transferred from the dilution tube to the culture plate.
- The microbial count will be expressed as CFU in 1 mL (CFU/mL) using Miles and Misra Method (1938) as stated (Lombardo et al., 2013):
    - Low if $<10^5$ CFU/mL
    - Moderate if between 10^5 and 10^6 CFU/mL
    - High if $>10^6$ CFU/mL

4.12 At 3 months post-fitting (T_1)

- Plaque sample will be collected with sterile applicator on the labial and palatal/lingual inner surface on the thermoplastic retainer covering 10 teeth.
- The swab will be placed in a microcentrifuge tube with PBS and placed in an ice box.
- Next, saliva parameter assessment and sampling as previously done in 4.10.5.
- The saliva sample will be placed in an icebox together with the plaque sample.
- The ice box will then be transferred to the laboratory for microbiological analysis as previously done in 4.11.

- The assessment for patient reported outcome will be using a Likert Scale as attached in Appendix C.
- Contents of the assessment will be taste, smell, cleaning time, convenience, cost and time allocation.

4.13 At 6 months post-fitting (T_2)

- Plaque sampling and saliva parameter assessment and sampling will be carried out as previously done at day of debond (T_0) and 3 months post-fitting (T_1).
- The same set of assessment questions will be used as at 3 months post-fitting (T_1).

4.14 Pilot study

- A pilot study will be conducted earlier to identify potential drawback regarding the randomisation process, sample collection procedures, microbiological analysis and assessment form. (Zailinawati Abu Hassan FRACGP, 2006).
- The pilot study will involve 5 patients from the targeted group who will not be recruited in this study.

4.15 Limitation of study

- Patients' compliance to wearing and cleaning thermoplastic retainers as per-instructions.
- Breakage or losing of retainer.

5.0 EXPECTED RESULTS (Dummy table)

Objective 1: To quantify and compare the presence of Mutans Streptococci (MS) between three cleansing agents at retainer fit (T_0), 3 months post-fitting (T_1) and 6 months post-fitting (T_2) using colony counting method.

- Statistical test: Repeated measures Analysis of Variance (ANOVA)

Time	Cleaning group	Sample	Mean (CFU/mL)	95% CI
T_0	Tap water	Plaque sample		
		Saliva sample		
	Toothpaste	Plaque sample		
		Saliva sample		
	Retainer Brite	Plaque sample		
		Saliva sample		
T_1	Tap water	Plaque sample		
		Saliva sample		
	Toothpaste	Plaque sample		
		Saliva sample		
	Retainer Brite	Plaque sample		
		Saliva sample		
T_2	Tap water	Plaque sample		
		Saliva sample		
	Toothpaste	Plaque sample		
		Saliva sample		
	Retainer Brite	Plaque sample		
		Saliva sample		

<u>Comparison of mean CFU/mL among three different cleaning groups based on time</u>

T_0 – Retainer fit

T_1 – 3 months post-fitting

T_2 – 6 months post-fitting

Statistical test: Repeated measures ANOVA within group analysis

Comparison		Tap water		Toothpaste		Retainer Brite	
		MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value
Plaque sample	T_0						
	T_1						
	T_2						
Saliva sample	T_0						
	T_1						
	T_2						

Comparison of bacterial count within each group based on time

Objective 2. To investigate salivary parameters of unstimulated saliva during retention period

- Statistical test: repeated measures ANOVA

Time	Cleaning group	Mean	p value
T_o	Tap water		
	Toothpaste		
	Retainer Brite		
T_1	Tap water		
	Toothpaste		
	Retainer Brite		
T_2	Tap water		
	Toothpaste		
	Retainer Brite		

Comparison of mean among three different cleaning groups based on saliva parameter (hydration/ viscosity/ quantity/ pH)

Objective 3: To evaluate patient reported outcome on different cleansing agents using Likert Scale

- Statistical analysis: Repeated measure ANOVA

Variables	Group	mean	SD	p- value
Fresh	Tap Water			
	Toothpaste			
	Retainer Brite			
Clean	Tap Water			
	Toothpaste			
	Retainer Brite			
Taste	Tap Water			
	Toothpaste			
	Retainer Brite			
Smell	Tap Water			
	Toothpaste			
	Retainer Brite			
Time	Tap Water			
	Toothpaste			
	Retainer Brite			
Ability to clean	Tap Water			
	Toothpaste			
	Retainer Brite			

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Appendix A. Budget estimation.

NO	BRAND	DESCRIPTION	SIZE	QTY	PRICE (RM)	TOTAL (RM)
1.	ISRCTN	RCT Registration	-	1	-	**1209.41**
2.	Double A	Double A A4 80gsm Paper	1 ream	2	15.00	**30.00**
3.	Canon	Canon PG47 Black Cartridge	1 unit	2	34.00	**68.00**
4.	Colgate	Colgate Twinpack (TP)	12pcs free 2	4	14.99	**539.64**
5.	Colgate	Colgate Total Toothpaste 150g	12pcs free 4	4	91.20	**364.80**
6.	Others	Dentsply Retainer Brite 96 Tablets Teeth Oral care Clear Aligners Mouthguards	288 tablets / 3 packs	8	299.00	**2,429.00**
7.	Local	Disposable Facemask 3 ply	50pcs/box	2	75.00	**150.00**
8.	Local	Examination Glove, Size S	100pcs/box, 10box/case	1	200.00	**200.00**
9.	Biologix	15ml Centrifuge Tube,Flat-top Cap, PP Tube, Sterile, DNase & RNase Free, Conical Bottom, Bulk Pack,	25tubes/bag	6	32.00	**192.00**
10.	Biologix	1.5ml Microcentrifuge Tubes, Clear, DNase & RNase Free, Non-Sterile 500 Pieces/Bag/Pack, 10 Packs/Case	500 Pieces/Bag/Pack 10 Packs/Case	1	543.00	**543.00**
11.	Merck	OmniPur 10X PBS Liquid Concentrate [Phosphate Buffered Saline] Sterile	1 Litre	1	430.00	**430.00**
12.	China	Sterile Cotton Swab with wooden stick 6" Individually packed in peel packed	1000 pcs / box	1	221.00	**221.00**
13.	Merck	pH-indicator strips pH 0 - 14 Universal indicator Mquant	100 strips	1	51.00	**51.00**
14.	Biologix	Centrifuge Tube Rack for 15ml Centrifuge Tubes, PP, 50-well	20 Bags / Case	1	214.00	**214.00**
15.	Sigma, USA	LABPL tapes, white polyester W x L 12.7 mmx 6.4 m	1	1	334.00	**334.00**
16.	Biologix	Cryogenic Storage Boxes, Polypropylene, 100-wells, -90ºC to 121ºC, 5 Assorted Colors	5 Boxes	1	85.00	**85.00**
17.	Sartorius	mLINE Manual Pipettor, 1-ch 10-100µ	1	1	940.00	**940.00**
18.	Sartorius	mLINE Manual Pipettor, 1-ch 100-1000µ	1	1	940.00	**940.00**
19.	Biologix	200µl Universal Pipet Tips, PP, Bulk, Non-sterile, DNase & RNase Free, Yellow 1000 tips/bag	10 bags / case	1	368.00	**368.00**
20.	Biologix	1000µl Universal Pipet Tips, PP, Bulk, Non-sterile, DNase & RNase Free, Yellow 1000 tips/bag	10 bags / case	1	368.00	**368.00**
21.	Brandon	Disposable Sterile Petri Dish 90x15mm	500 pcs / box	2	171.00	**342.00**
22.	Sigma	Mitis Salivarius Agar	500 g	1	730.00	**730.00**
23.	Sigma	Bacitracin	1g	1	442.00	**442.00**

24.	Sigma, USA	Sucrose for molecular biology	500g	1	224.00	**224.00**
25.	Sigma	Potassium Tellurite 3.5%, 10x2mL Storage 2-8°C	1	1	217.00	**217.00**
26.	Wheaton, USA	Spreader L-Shape Sterile Inner Pack	Pack 5/ bag		334.00	**334.00**
27.	Biologix, USA	Inoculating Loops, Sterile	25 pieces/pack, 40bag/case	1	236.00	**236.00**
28.	Biomerieux	API kit 20 Strep, 25 strips + 25 media	1	2	889.00	**1778.00**
29.	Oxoid	BHI Broth	500g	1	410.00	**410.00**
30.	Oxoid	BHI Agar	500g	1	415.00	**415.00**
31.	Oxoid	Anaerogen 2.5 L (for use in 2.5L jar)	10 pack	1	114.00	**114.00**
32.	Essix ACE	Thermoplastic Retainer		30	120.00	**3600.00**
33.		Alsoft Pure Hand Sanitizer	500 mL	3	50.00	**150.00**
Total						**18, 668.85**

Appendix B.1. Retainer instructions in English

Patients will be given the written instructions following cleaning intervention group.

Group A (tap water)

- Retainers are to be worn full time except during meals, sports and brushing in the morning and at night. When not worn, kindly ensure the retainer is kept safe in a retainer case.
- After eating, floss teeth and rinse mouth with water before inserting retainers into mouth.
- Retainers are to be cleaned by ONLY brushing under running tap water (for 20 seconds) in the morning and at night.
- Apart from the morning and night cleaning, at other times throughout the day the retainer can be rinsed under running tap water.
- Do not use any other toothpaste, cleaning tablets or mouthwash that are not provided during the experiment.
- Do not hesitate to call the clinic if retainers are unfit, sharp, broken or lost.

Group B (toothpaste)

- Retainers are to be worn full time except during meals, sports and brushing in the morning and at night. When not worn, kindly ensure the retainer is kept safe in a retainer case.
- After eating, floss teeth and rinse mouth with water before inserting retainers into mouth.
- Retainers are to be cleaned by ONLY brushing with toothpaste (for 20 seconds) and rinse under running tap water in the morning and at night.
- Apart from the morning and night cleaning, at other times throughout the day the retainer can be rinsed under running tap water.
- Do not use any other toothpaste, cleaning tablets or mouthwash that are not provided during the experiment.
- Do not hesitate to call the clinic if retainers are unfit, sharp, broken or lost.

Group C (Retainer Brite)

- Retainers are to be worn full time except during meals, sports and brushing in the morning and at night. When not worn, kindly ensure the retainer is kept safe in a retainer case.
- After eating, floss teeth and rinse mouth with water before inserting retainers into mouth.
- Retainers are to be cleaned by ONLY brushing under running tap water (for 20 seconds) and soaking the thermoplastic retainer into the bubbling blue solution, produced when 1 Retainer Brite tablet is dropped into a cup of warm (not hot) water. After 15 minutes, rinse retainer under running tap water. This will be done only at night and to brush under running tap water in the morning.
- Apart from the morning and night cleaning, at other times throughout the day the retainer can be rinsed under running tap water.
- Do not use any other toothpaste, cleaning tablets or mouthwash that are not provided during the experiment.
- Do not hesitate to call the clinic if retainers are unfit, sharp, broken or lost.

Appendix B.2. Retainer instructions in Bahasa Malaysia

<u>Kumpulan A</u>

- *Alat pemegang gigi hendaklah dipakai sepenuh masa kecuali semasa makan, bersukan dan menggosok gigi pada waktu pagi dan malam. Sekiranya tidak dipakai, pastikan alat pemegang gigi disimpan dengan selamat di casing.*
- *Selepas makan, gosok gigi dan kumur mulut dengan air sebelum alat pemegang gigi dipakai semula.*
- *Alat pemegang gigi hendaklah dibersihkan dengan HANYA memberus (selama 20 saat) bawah air paip yang mengalir pada waktu pagi dan waktu malam.*
- *Jangan gunakan ubat gigi, tablet pembersih atau ubat kumur yang tidak disediakan semasa tempoh uji kaji.*
- *Sila hubungi klinik sekiranya alat pemegang gigi kurang selesa, tajam, patah atau hilang.*

<u>Kumpulan B</u>

- *Alat pemegang gigi hendaklah dipakai sepenuh masa kecuali semasa makan, bersukan dan menggosok gigi pada waktu pagi dan malam. Sekiranya tidak dipakai, pastikan alat pemegang gigi disimpan dengan selamat di casing.*
- *Selepas makan, gosok gigi dan kumur mulut dengan air sebelum alat pemegang gigi dipakai semula.*
- *Alat pemegang gigi hendaklah dibersihkan dengan HANYA memberus (selama 20 saat) menggunakan ubat gigi yang disediakan dan bilas alat pemegang gigi di bawah air paip yang mengalir pada waktu pagi dan waktu malam.*
- *Jangan gunakan ubat gigi, tablet pembersih atau ubat kumur yang tidak disediakan semasa tempoh uji kaji.*
- *Sila hubungi klinik sekiranya alat pemegang gigi kurang selesa, tajam, patah atau hilang.*

<u>Kumpulan C</u>

- *Alat pemegang gigi hendaklah dipakai sepenuh masa kecuali semasa makan, bersukan dan menggosok gigi pada waktu pagi dan malam. Sekiranya tidak dipakai, pastikan alat pemegang gigi disimpan dengan selamat di casing.*
- *Selepas makan, gosok gigi dan kumur mulut dengan air sebelum alat pemegang gigi dipakai semula.*
- *Pada waktu malam, alat pemegang gigi hendaklah dibersihkan dengan HANYA menggosok di bawah air paip yang mengalir (selama 20 saat) dan merendam alat pemegang didalam larutan yang berwarna biru yang dihasilkan apabila 1 tablet Retainer Brite diletakkan ke dalam secawan air suam (tidak panas). Selepas 15 minit, bilas alas alat pemegang gigi di bawah air paip yang mengalir. Pada waktu pagi, alat pemegang gigi hendaklah dibersihkan dengan hanya memberus dibawah air paip yang mengalir (selama 20 saat).*
- *Jangan gunakan ubat gigi, tablet pembersih atau ubat kumur yang tidak disediakan semasa tempoh uji kaji.*
- *Sila hubungi klinik sekiranya alat pemegang gigi kurang selesa, tajam, patah atau hilang.*

Appendix C. Assessment form to evaluate patient reported outcome on the different cleansing agent

ID: ……

Date: ……

Instructions: Please circle the level of your satisfaction on the given cleansing agent ……based on the questions below:

1. How **FRESH** do you feel after cleaning your retainer with the cleansing agent?

1	2	3	4	5
Not at all	Slightly	Moderately	Very	Extremely

2. How **CLEAN** do you feel after cleaning your retainer with the cleansing agent?

1	2	3	4	5
Not at all	Slightly	Moderately	Very	Extremely

3. How much do you like or dislike the **TASTE** of the retainer after using the cleansing agent?

1	2	3	4	5
Hate	Don't like	Don't mind	Like	Love

4. How much do you like or dislike the **SMELL** of the retainer after using the cleansing agent?

1	2	3	4	5
Hate	Don't like	Don't mind	Like	Love

5. Can you allocate the recommended **TIME** of **…….** to clean your retainer?

1	2	3
Never	Sometimes	Always

6. Are you able to clean your retainer with the cleansing agent daily as recommended?

1	2
Yes	No

Appendix D. GANTT Chart

No.	Description	Year 1			Year 2			Year 3			Year 4		
		2019	2020			2021			2022			2023	
		Sept - Dec	Jan - Apr	May - Aug	Sept - Dec	Jan - Apr	May - Aug	Sept - Dec	Jan - Apr	May - Aug	Sept - Dec	Jan - Apr	May - Aug
1	Literature review												
2	DRP & Ethics presentation												
3	Subject recruitment												
4	Data collection												
5	Data analysis												
6	Thesis write -up												
7	Submission of research paper												
8	Thesis submission for viva												
9	Research send for publication												