Version 061020

Protocol

CLINICAL RESEARCH STUDY PROTOCOL

Evaluation of Toothbrush Bristles in Plaque Reduction

Study Number: CLP-2020-06-01-1

Investigator: Dr. Franklin Garcia-Godoy

Sponsor: Sunstar Americas, Inc.

I. PROTOCOL APPROVAL SIGNATURES

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Dr. *Franklin Garcia-Godoy,* UTHC **DATE**

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II. INTRODUCTION

Toothbrushes with tapered bristles were designed to clean plaque accumulated interdental area effectively. *In vitro* studies showed that the effectiveness in artificial plaque reduction is greater than that of the conventional toothbrushes with end rounded bristles. However, clinical studies in dental plaque and gingivitis have not showed consistent result in the superiority comparing to conventional toothbrushes[1]. The purpose of this cross-over clinical research is to evaluate the cleaning efficacy of toothbrushes tufted with two types of tapered bristles and end rounded bristles in removal of interdental plaque with clinical measurement (Plaque Index) and objective measurement (digitally measured plaque area). This Proof of Concept study will
demonstrate the influence by a type of processing bristles on removal of interdental plaque and determine an agreement between the different evaluation methods.

III. STUDY OBJECTIVES

The primary objective of this study is to evaluate and compare toothbrushes with three different bristle types in the reduction of plaque index **(PI)** for interdental area and digitally measured interdental plaque area after one-time brushing.

Secondary objectives are to evaluate toothbrushes with three different bristle types in the changes of PI, digitally measured interdental plaque area and gingival abrasion one week after using the toothbrushes, and to assess an agreement between the different evaluation methods **(PI** and a plaque area by image analysis)

IV. STUDY GROUPS

Three different bristles will be tufted into the same tuft pattern. All toothbrush head and handle are identical (Sonic TB without turning on the sonic vibration). The study samples will be provided by sponsor. The bristle dimensions (height, base diameter of filament) will be same. The difference of three toothbrushes is a type of processing bristles.

A: Tapered bristles (double tapered J-hook)
B: Tapered bristles (single tapered J-hook)
C: End rounded bristles

Table 1. Physical characteristics of the bristles in all test groups

	Product A	Product B	Product C
Name	Double tapered J-hook (OTJ)	Single tapered J-hook (STJ)	End rounded (Control)

Individual filament heights in all tufts	10and13mm	10 and 13 mm	13 and 13 mm
Tall filament end	Chemically tapered	Chemically tapered	Rounded
Short filament end	Chemically tapered	Rounded	Rounded
Filament's base diameter, milli inch (mm)	7 (0.18)	7 (0.18)	7 (0.18)
Filament material	PBT**	PBT**	Nylon

*Pending, to check with supplier, ** Polybutylene terephthalate

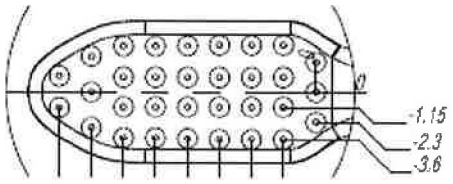


Figure 1. Toothbrush head schematic showing tuft patterns (circles) used in all test groups

V. SUBJECT INFORMATION AND CONSENT

The clinical investigation, including the consent form, will be reviewed by an IRB in accordance with Title 21 of the Code of Federal Regulations, Parts 50 and 56. Subject consent will be obtained prior to participation in any study procedures as required by the Food and Drug Administration (FDA) GCP guidelines. Subjects will be given ample opportunity to read the consent form and to have all questions regarding study conduct answered prior to signing and dating the consent form. Each subject will be provided with an exact copy of the informed consent form to retain for his or her records.

Informed consent means the knowing consent of an individual, so situated so as to exercise free power of choice without undue inducement or constraint or coercion. The elements of information necessary for such consent include: a statement that the study involves research; an explanation of the procedures to be followed and their purpose; identification of any procedures that are experimental; any expected discomforts, risks or benefits; approximate number of subjects involved in the study; any appropriate alternative procedures or treatments; description of the confidentiality of subject records; name and phone number of the individual to contact with inquiries concerning the research; explanation of compensation or free medical treatment available for research-related injury; statement that the subject is free to withdraw consent and to discontinue participation at any time; statement that participation is voluntary; indication of any additional costs to the subject; assurance that the subject will be notified of new findings relevant to the subject's participation; statement that it is within the Principal Investigator's discretion to drop the subject from participation at any time.

In addition, the agreement entered into by the subject should include no exculpatory

language by which the subject is made to waive, or appear to waive, any of his/her legal rights or to release the institution from liability or negligence.

VI. SUBJECT POPULATION

Number of Subjects

A total number of 10 subjects will be enrolled. Volunteers must read and sign the Informed Consent Form after the nature of the study has been fully explained.

Inclusion Criteria

Individuals may be included in the study provided they meet all of the following inclusion criteria:

- Must have read, understood and signed an informed consent prior to being entered into the study.
- Must be 18 to 70 years of age, male or female.
- Have at least 20 natural or restored teeth.
- Must have average Plaque Index of Ramfjord teeth at baseline greater than 2 (Quigley and Hein)[4]I at screening.
- Agree not to have a dental prophylaxis or any other elective, non-emergency dental procedures (other than those provided during the study) any time during the study.
- Agree to refrain from regular oral hygiene regimen for 24 hours and eating for 4 hours before the appointment in the study.
- Agree to abstain from the use of any dental products other than those provided in the study.
- Agree to comply with the conditions and schedule of the study.

Exclusion Criteria

Individuals are not eligible for participation in this study if any of the following are noted:

- Physical limitations or restrictions that might preclude normal tooth brushing.
- Evidence of gross oral pathology
- Presence of severe gingivitis with 30 or more sites showing bleeding on probing.
- Evidence of major soft tissue lesions or trauma at the baseline visit as determined by the Investigator/Examiner.
- Chronic disease with concomitant oral manifestations
- Subjects who are currently undergoing, or require, extensive dental work, orthodontic treatment or periodontal surgery or orthodontic treatment in the preceding 3 months
- Currently using bleaching trays
- Eating disorders
- Recent history of substance abuse
- Smoking >10 cigarettes/day

- Participation in other clinical studies within 14 days of screening

VII. STUDY DESIGN OVERVIEW

This cross-over group, examiner blinded, randomized, single center study will enroll subjects to target 10 subjects completing the study. Subjects will visit the clinical site 7 times including screening/enrollment visit. Subjects will be instructed to refrain from regular oral hygiene regimen for 24 hours and from eating for four hours before each appointment. The study examiner and staff will screen for subjects that meet the enrollment criteria. Subjects will be randomly assigned to one of three treatment groups. The study staff responsible for random assignments will be blinded to the product treatments.

- Group 1: A -> B -> C
- Group 2: B -> C -> A
- Group 3: C ->A-> B

After distribution of an assigned test toothbrush, subjects will receive a basic brushing instruction for Bass method by a study staff but without actual brushing. Subjects will perform toothbrushing using the assigned product for 2 minutes under supervision. Plaque Index (Navy PI), Gingival Abrasion Score and standardized digital dental photos will be taken before/after the one-time brushing.

Subjects will use the assigned toothbrush for a week until the next visit for evaluation. At the visit, the used toothbrush shall be returned to the study staff to avoid mixing up with next test product. PI and Gingival Abrasion Score will be recorded, and the standardized digital dental photos will be taken. After one week of wash-out period, subjects will return to the clinical site to start the next test product. The study schedule is showed in Table 2. Subjects will be instructed to keep a diary given by the study staff at Visit 1 throughout the study. Adverse experiences will be noted and recorded during the study.

Table 2. Study Schedule

Events	Visit 1	Visit2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
		First product		Second product		Third product	
Informed consent, demographics, Inclusion/Exclusion criteria, medical history, concurrent meds	X						
Update of Inclusion/Exclusion criteria	X*	X	X	X	X	X	X
Clinical endpoints (PI and Gingival Abrasion)		X	X	X	X	X	X
Digital dental photos		X	X	X	X	X	X
Test product distribution		X		X		X	
Instruction of brushing		X		X		X	
Clinical endpoints (PI and Gingival Abrasion) immediate post-brushing		X		X		X	
Digital dental photos immediate post-brushing		X		X		X	
Wash-out period products distribution (TP and TB)	X						
Used test product collection			X		X		X
Diary disbursement and collection		X					X
Compliance check			X		X		X
Adverse events		X	X	X	X	X	X

Note: Visit 1, 3 and 5 will be followed by a 1 week "washout" period.
*Evaluation of PI (Quigley and Hein) is included.

VIII. CLINICAL SUPPLIES

Test Materials

The sponsor will provide the following items to the study site:

- 3 test toothbrushes with different types of bristles (A, **B,** C)
- Toothbrush for wash-out period
- Toothpaste throughout the study period

Packaging and Labeling

The toothbrushes will be identically labeled by the sponsor.

Delivery and Inventory:

Immediately upon receipt of study supplies at the clinical site, study personnel will account for all products. The study staffs are responsible to maintain the accountability log.

Storage:

Study supplies will be maintained under secure conditions, until assignment to subjects.

Return of Study Supplies:

At the conclusion of the study, all unused product will be returned to the Sponsor. Used products will also be returned to the Sponsor.

IX. TREATMENT REGIMEN

Qualified subjects meeting all inclusion and exclusion criteria will be provided their assigned toothbrush (A, **B** or C) at Visit 2, 4 and 6 for use for immediate post-brushing evaluation and during the one-week treatment phase of the study. Subjects will be instructed to brush their teeth as instructed with their assigned toothbrush 2 times daily (after breakfast, before going to bed) for 2 minutes, using only the provided toothpaste for one-week study period.

Following their one week of use, they will then go through a one-week "washout" period and will follow their normal oral hygiene using study provided toothbrush and fluoride toothpaste. The same fluoride toothpaste will be used throughout the whole study period. Subjects will refrain from using other oral hygiene products throughout the study period.

X. PRODUCTUSECOMPLIANCE

To demonstrate compliance with product use, each subject will complete a daily diary indicating the time of each brushing for the one-week duration of the study. The subjects will be requested to return their diary and assigned toothbrush at the end of the study period.

XI. RESTRICTIONS

Subjects will be advised of the following restrictions during the study period:

- To avoid using other oral hygiene products throughout the study period.
- To avoid starting or changing treatments, medications including OTC drugs and supplements that potentially modify plaque accumulation for the duration of the study.
- To refrain from starting or changing habits (e.g. eating chewing gum) to potentially modify subject's plaque accumulation condition for the duration of the study.

XII. STUDY PROCEDURE

Screening and Enrollment Visit (Visit 1)

Potential subjects will be instructed to refrain from regular oral hygiene regimen for 24 hours and eating for 4 hours before the appointment in the study.

Male and female subjects will read and sign the informed consent prior to enrollment. Subjects will be asked about demographics, medical history, general health status and current medication usage, and the information will be recorded.

Plaque Index (Quigley and Hein) of a whole mouth will be evaluated to verify that the subjects meet the inclusion and exclusion criteria (average PI>2).

If the participants meet the study criteria, they will be assigned to one of three treatment groups randomly based on computer generated randomized numbers.

Subjects will be instructed to use a toothpaste and a toothbrush (for wash-out period) provided by the study staff until the next visit. The appointment of next visit will be scheduled within 7 days ± one day.

Visit 2

The following will happen at this visit:

Potential subjects will be instructed to refrain from regular oral hygiene regimen for 24 hours and eating for 4 hours before the appointment in the study. The study staff will

update of Inclusion/Exclusion Criteria, medical history, and concurrent medications.

Whole-mouth Plaque Index (Navy PI) and Gingival Abrasion Score will be evaluated by a qualified examiner following visualize dental plaque with a disclosing agent (Mira-2-Ton, Miradent) and record the scores on the Case Report Form for the baseline information. Successively, standardized dental digital photographs of three close-up views of the retracted views for plaque area measurement: front, left and right lateral sides.

Assigned toothbrush and a fluoride toothpaste will be given to each subject. the study staff will instruct Bass method to each subject (without using a toothbrush) jTAJ1and provide the assigned toothbrush with a half inch length of the toothpaste. Subjects will brush their teeth for 2 minutes with Bass method under a supervision of the study staff. Whole-mouth Navy PI and Gingival Abrasion Score will be recorded, and standardized dental digital photographs will be taken again for immediate post-brushing evaluation.

Subjects will then be instructed to use assigned product to use 2 times daily, after breakfast and before bedtime, for one week for 2 minutes with instructed brushing method. They will maintain a daily diary of their product usage. Compliance will be assessed by subject diary.

Next appointment will be scheduled within 7 days ± one day. Subjects will be instructed to refrain from regular oral hygiene regimen for 24 hours and eating for 4 hours before the appointment in the study. They will be advised to follow the instructions carefully and return to the study center with their assigned toothbrush and diary for their next examination.

Visit 3

After one week, subjects will return to the office to return the used toothbrush and their dairy. The following will happen at this visit:

The study staff will update of inclusion/exclusion criteria, medical history and concurrent medications. Adverse experiences will be noted and recorded during the study.

Whole-mouth Navy Plaque Index and Gingival Abrasion Score will be evaluated by a qualified examiner following visualize dental plaque and record. Then, standardized dental digital photographs of three close-up views of the retracted views: front, left and right lateral sides.

Subjects will follow their normal oral hygiene using a toothbrush and a toothpaste for "wash-out" period given at Visit 1.

Next appointment will be scheduled to return within 7 days ± one day, and subjects will be instructed to refrain from regular oral hygiene regimen for 24 hours and eating for 4 hours before the appointment in the study.

Visit4

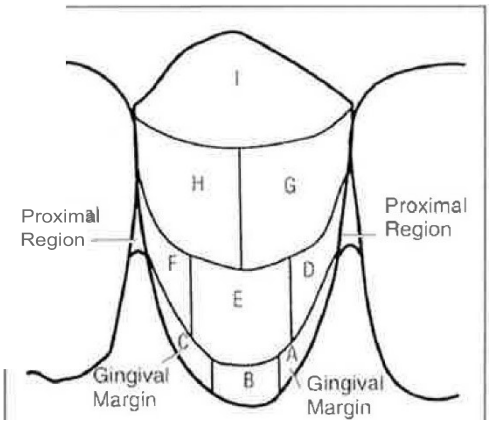
After a 1-week washout, subjects will return to the office to begin the second period of the

study, using the same study procedures and be assigned the alternate toothbrush as in Visit 2.

At the end of Visit 5 (examination for the second product) they will go through a 1 week wash out period and then return for Visit 6 (baseline for the third product).

Rustogi Modified Navy Plaque Index (Rustogi et al. J Clin Dent. 1992)[5]

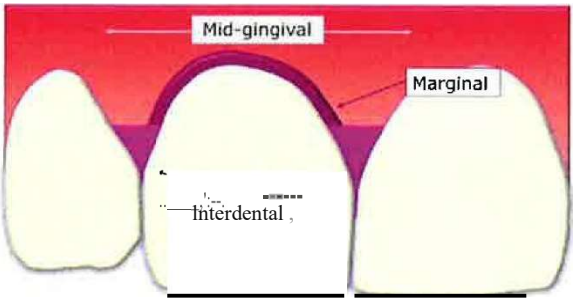
After disclosing dental plaque on teeth, the Rustogi Modified Navy Plaque Index (Navy PI) is evaluated as either present or absent (1 or 0) on each of the nine areas of the buccal and lingual tooth surfaces except for third molars. Whole tooth, marginal, and approximal areas are defined as shown below. Score of each area (from A to I) will be recorded for further assessments. In this study, the PI should be evaluated by only trained and calibrated examiners.



Gingival Abrasion Score (Van der Weijden et al. J Clin Periodontol. 2004)[6]

After the gums were dried with compressed air, Mira-2-Ton® disclosing solution was applied for better visualization of areas where the surface of the oral epithelium had been abraded. The gingival tissues were divided into three areas: marginal (cervical free gingiva), interdental (papillary free gingiva) and mid-gingival (attached gingiva).

The lesions were assessed as small (;5;2 mm), medium (3-5 mm) and large (>5 mm) using a periodontal probe. Those lesions measuring between 2 and 3 mm were assigned a score of small or medium according to nearest mm mark on the probe.



Standardized Digital Dental Photographs A4J

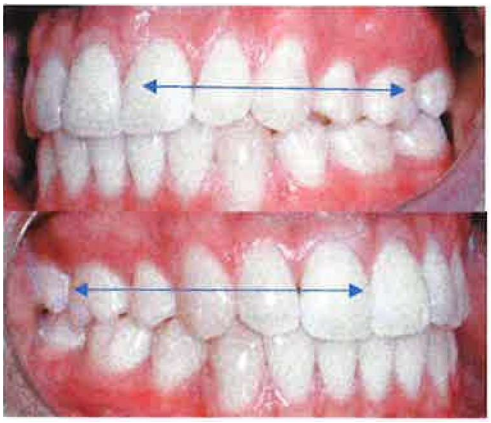
Dental photographs will be taken by a digital SLR camera (model, maker) with a ring flash to distribute the light evenly with intraoral exposure. A macro lens (85 mm or more) will be used to take a close-up photo.

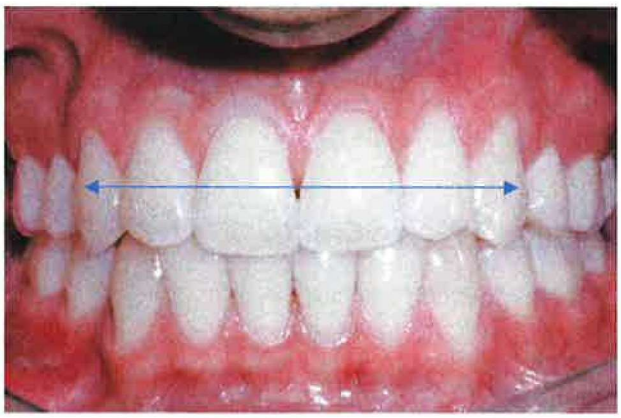
To standardize the exposing views, the same magnification (1:1 or 1:2 views) and same views at the same distance from the subject shall be used for all subjects and all visits.

Study staffs will expose the cheek retracted views. Retracted views from front, left and right lateral will be taken at the same setting. When an image of the front of the mouth is taken, the subject's bicuspids may be focused on to obtain the overall sharpness. Images from left and right lateral may be centered between the cuspid and the first premolar.

A length of tooth height (mm) of one of maxillary central incisors will be recorded using a periodontal prove for future analyses.

After image acquisition with same spatial resolution and contrast resolution for an existing image analysis software, the image of each non-molar tooth will be assessed. The entire visible tooth area and the area of plaque of each tooth will be calculated into either mm^2 or number of pixels .





XIII. ADVERSE EXPERIENCES

Adverse Event Definition and Handling

An adverse event is any unexpected or serious medical occurrence in a clinical investigation subject during the study, whether or not related to the study product. All adverse events observed by the investigator and/or reported by the subject will be recorded throughout the entire study and documented. The Investigator will be asked to make a judgement on all adverse events as to their severity and possible relation to the study treatments.

More specifically, adverse events would include:

- Any unexpected event not seen before study initiation.
- Any pre-existing event that recurs with increased intensity or increased frequency subsequent to initial product treatment.

Adverse events will be reported to the investigator and the sponsor will be notified within 24 hours of any serious adverse events. All adverse events should be recorded on the appropriate case report form, including date of onset, severity, duration, treatment and follow-up observation.

The following definitions will be used for grading severity of adverse events:

- Mild - Either asymptomatic, or subject is aware of the sign, symptom or event, but it is easily tolerated.
- Moderate - Discomfort enough to cause interference with usual activity and may warrant intervention.
- Severe - Incapacitating with inability to do usual activities.

The investigator will make an assessment of the likelihood that there is a reasonable possibility of a causal relationship between a study product and the adverse event. This will be captured using the following criteria: product related, non-product related, non- product related but protocol related. The causal relationship to the product will be assessed as: definitely related, probably related, possibly related, probably not related or definitely not related.

Serious Adverse Event

A serious *adverse event* is *any adverse event that:*

- Results in death
- Is life threatening (i.e., immediate risk of death as the event occurred). A life-threatening event does not include an event that, had it occurred in a more severe form, might have caused death, but as it occurred did not create an immediate risk

of death. For example, hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though hepatitis of a more severe nature can be fatal. Similarly, an allergic reaction resulting in angioedema of the face would not be life threatening, even though angioedema of the larynx, allergic bronchospasm, or anaphylaxis can be fatal.

- Results in persistent or significant disability or incapacity (i.e., a substantial, persistent disruption in a subject's ability to conduct normal life functions).
- Requires inpatient hospitalization or prolongation of an existing hospitalization. Hospitalization or prolongation of a hospitalization constitutes criteria for an adverse event to be serious; however, it is not in itself considered a serious adverse event. In the absence of an adverse event, a hospitalization or prolongation of a hospitalization should not be reported as a serious adverse event by the participating Investigator. This is the case in the following situations:
  - The hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol.
  - The hospitalization or prolongation of hospitalization is part of a routine procedure followed by the center. This should be recorded in the study file.

    In addition, a hospitalization for a pre-existing condition that has not worsened does not constitute a serious adverse event.
  - Results in cancer
  - Results in a congenital anomaly or birth defect.

Additionally, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event(s) may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse. If there is any doubt whether the adverse event constitutes a serious adverse event, the information will be treated as a serious adverse event.

XIV. SUBJECT COMPLETION AND WITHDRAWAL

Subject Completion

Only subjects who complete all procedures and comply with all areas of the protocol will be deemed to have completed the study. Subjects will be compensated for their time and travel and for successfully completing all provisions and procedures of the study. If a subject voluntarily withdraws from the study, or if withdrawn by the Investigator for any reason, he/she will be compensated on a pro rata basis. Subject compensation will be

documented in the Informed Consent and will be approved by the IRB.

Subject Withdrawal

Subjects are free to withdraw at any time during the clinical trial. Subjects may also be withdrawn from the study at any time at the discretion of the Investigator. All subject withdrawals will be documented in the study file. Where possible, the reason for the withdrawal will be documented. Any withdrawals as the result of an adverse event will be followed-up at the discretion of the Study Investigator. Withdrawn subjects will not be replaced.

Subject Discontinuation

A subject will be considered discontinued from the study at any time under the following circumstances:

- Any subject who violates any condition of the entrance criteria after having been entered into the study
- Any subject who develops a confounding concomitant illness (as determined by the subject, Research Coordinator, or Investigator) or a serious adverse event
- Any subject who becomes uncooperative, does not adhere to the requirements of the study protocol, or refuses to complete the study

XV. EFFICACY

Primary Outcome Variables

The primary outcome variables to be assessed are:

1) the reduction of plaque index (Navy PI) for interdental area after one-time brushing in a whole mouth.
2) the reduction of digitally measured interdental plaque area on non-molar teeth, facial/buccal aspect after one-time brushing by image analysis.

Secondary Outcome Variables

1) the change of plaque index for interdental area in a whole mouth one week after using the toothbrushes.
2) the change of digitally measured interdental plaque area on non-molar teeth one week after using the toothbrushes.
3) correlation between plaque index and plaque area by image analysis in non-molar teeth, facial/buccal aspect.
4) The change of gingival abrasion score in whole mouth at one week after using the toothbrushes.

As necessary, additional exploratory statistical data analysis may be performed to further advance evaluation of the results.

XVI. STATISTICAL ANALYSIS AND DATA MANAGEMENT

Since this study is a Proof of Concept study, the sample size calculation was not performed.

Descriptive statistics for all subjects will be performed for background and demographic variables. Continuous variables are expressed as frequencies (n), mean +/- SD and categorical variables are expressed as frequencies (n) and percentages (%).

Student *t* test or Wilcoxon *rank sum* test where appropriate, will be used to compare among different bristles. For intragroup changes of endpoints before/after one-time brushing or use for one-week, paired t test or Wilcoxon signed rank test will be used where appropriate. Spearman Rank Order Correlation was used to analyze correlations between the variables.

Safety parameters will include oral soft and hard tissues and subjective reports as well as any AEs. The analysis will include all observed effects which initially occurred or worsened following treatment. Any adverse effects will be summarized classified according to their intensity (mild, moderate, or severe) and relationship (definitely related, probably related, possibly related, probably not related or definitely not related) to study product.

All statistical tests will be conducted using Minitab 18.

XVII. RERFERENCES

1) Hoogteijling F, Hennequin-Hoenderdos NL, Van der Weijden GA, Slot DE. The effectof tapered toothbrush filaments compared to end-rounded filaments on dental plaque, gingivitis and gingival abrasion: a systematic review and meta-analysis. Int *J* Dent Hyg. 2018;16(1):3- 12. doi:10.1111/idh.12272
2) Ni L, Tang R, He T, et al. Clinical effect of a manual toothbrush with tapered filaments on dental plaque and gingivitis reduction . Am *J* Dent. 2017;30(5):272 - 278.
3) Xu Z, Cheng X, Conde **E,** Zou Y, Grender J, Ccahuana-Vasquez RA. Clinical assessment of a manual toothbrush with Crisscross and tapered bristle technology on gingivitis and plaque reduction. Am *J* Dent. 2019;32(3):107-112.
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5) Rustogi KN, Curtis JP, Volpe AR, Kemp JH, McCool JJ, Korn LR Refinement of the Modified Navy Plaque Index to increase plaque scoring efficiency in gumline and interproximal tooth areas. J Clin Dent. 1992;3(Suppl C):C9-C12.

6) Van der Weijden GA, Timmerman MF, Versteeg PA, Piscaer M, Van der Velden U. High and low brushing force in relation to efficacy and gingival abrasion. J Clin Periodontol. 2004;31(8):620-624. doi:10.1111/j.1600-051x.2004.00529.x