Statistical Analysis Plan for Protocol

212224

Randomized Controlled Examiner-Blind Methodology Development Study to Investigate the Plaque Removal Efficacy of Manual Toothbrushes in Healthy Dentate Subjects

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STATISTICAL REPORTING AND ANALYSIS PLAN

RANDOMIZED CONTROLLED EXAMINER-BLIND METHODOLOGY DEVELOPMENT STUDY TO INVESTIGATE THE PLAQUE REMOVAL EFFICACY OF MANUAL TOOTHBRUSHES IN HEALTHY DENTATE SUBJECTS

Protocol Number: 212224

Phase: N/A

Document History

Document	Version Date	Summary of Changes (New analysis or Change in planned analysis)
Original Analysis Plan	01-Oct-2019	Not applicable (N/A)
Amendment 1	05-Nov-2019	Reference to amended protocol V3.0, dated 16-Oct-2019. Changes in definition of stratification groups in sections: 1.1 Study Design; 1.4 Sample Size Calculation; 3.2 Subgroups/Stratifications; 4.2.1 Demographic Characteristic; 4.4.1.2 Statistical Hypothesis, Model, and Method of Analysis.

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Abbreviation

Abbreviation	Term
AE	Adverse Event
ANCOVA	Analysis of Covariance
BDRM	Blinded Data Review Meeting
CH	Consumer Healthcare
CI	Confidence Interval
eCRF	Electronic Case Report Form
GSK	GlaxoSmithKline
ICF	Informed Consent Form
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-To-Treat
mL	Milliliter
N/A	Not Applicable
OHT	Oral Hard Tissue
OST	Oral Soft Tissue
PBT	Polybutylene Terephthalate
PT	Preferred Term
RPI	Rustogi Modified Navy Plaque Index
SAE	Serious Adverse Event
SD	Standard Deviation
SE	Standard Error
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
TPE	Thermoplastic Elastomer
TPI	6-site Turesky Modified Quigley and Hein Plaque Index

The purpose of this Statistical Reporting and Analysis Plan is to describe the planned analyses and outputs to be included in the Clinical Study Report for Protocol 212224 version 3.0 dated 16-Oct-2019.

1 Summary of Key Protocol Information

In vitro brushing models can be used to evaluate the cleaning capability of a toothbrush, typically by measuring the removal of artificial stain from the tooth surface. Such models are useful but limited in their ability to mimic the tenacity/adherent properties of dental plaque in vivo and variation in brushing technique within the general population. Clinical evaluation of self-performed plaque removal provides a more realistic assessment of the in-use performance of a toothbrush. This methodology development study will evaluate the plaque removal efficacy of a range of marketed manual toothbrushes, with differing bristle types and brush head designs, in healthy dentate subjects. Changes in supra-gingival plaque accumulation will be assessed after single brushing events and following an extended period of twice-daily use, using two different clinical measures of dental plaque. Data generated may inform the design of future clinical studies investigating the plaque removal efficacy of manual toothbrushes, with the aim of developing clinical models for the evaluation of GlaxoSmithKline Consumer Healthcare (GSK CH) toothbrushes.

1.1 Study Design

This will be a single center, 4-week, randomized, controlled, examiner-blind, four-treatment arm, parallel design, clinical methodology development study to investigate the plaque removal efficacy of marketed manual toothbrushes in healthy, dentate subjects. Changes in plaque level will be evaluated after first brushing, after two further single brushing events (Days 7 and 28) and following 7 and 28 days usage (twice daily brushing with assigned toothbrush product).

Supra-gingival plaque levels will be assessed using two established clinical measures:

- Rustogi-modification of the Navy Plaque Index (RPI), which focusses on plaque along the gingival margin and in the inter-proximal areas.
- 6-site Turesky modification of the Quigley and Hein Plaque Index (TPI), which focusses on plaque on the lower gingival third of the tooth surface.

Subjects will abstain from oral hygiene for a period of 12-18 hours prior to each assessment visit.

Clinical efficacy assessments will be performed at a single clinical site by a single dental examiner to eliminate inter-examiner variability. Intra-examiner variability will be monitored by performing repeat clinical assessments for both plaque indices, in randomly selected subjects, across the study period.

To ensure study subjects demonstrate the required propensity for plaque formation, only subjects with Day 0 pre-brushing mean RPI $_{gingival/interproximal} \geq 0.6$ will be randomized to study product groups (Visit 2). Subjects will be stratified by their Day 0 pre-brushing RPI $_{gingival/interproximal}$ score (lower: mean RPI $_{gingival/interproximal} \geq 0.6$ to ≤ 0.8; higher: mean RPI

$_{gingival/interproximal}$ > 0.8 to 1.0) to ensure study product groups are balanced for baseline supra-gingival plaque levels.

To standardize oral hygiene practice, eligible subjects will complete a lead-in period (minimum 5 days) prior to Visit 2, during which they will be provided with a standard flat trim toothbrush and regular fluoride toothpaste (both marketed products) to use in place of their own oral hygiene products. Use of these products will provide the study population with a standardized oral hygiene regimen prior to the Baseline visit and familiarize them with required usage regimen (1 minute timed brushing, twice daily) and completion of a diary after each brushing. The lead-in period will also serve to wash-out from any anti-bacterial ingredients contained in the subject's own oral care products, in use prior to screening.

The study toothbrushes differ in visual appearance. The level of blindness for this study is therefore described as 'examiner-blind'. Study toothbrushes will be dispensed by trained site personnel who are not involved in clinical efficacy assessment procedures and used by study subjects in a separate area from the clinical examination area. This will ensure the clinical examiner remains blinded to product group and so avoid bias in the clinical assessments. Clinical examination and product usage areas will be free from mirrors to minimize the visual impact of disclosed plaque on subject brushing behavior.

The safety and oral tolerability of each toothbrush will be monitored after first use and over the 28-day usage period by review of reported treatment emergent adverse events (TEAEs).

The schedule of activities table (Table 1-1) provides an overview of the subject visits and study procedures. The investigator may schedule visits (unplanned visits) in addition to those listed on the schedule of activities, to conduct evaluations or assessments required to protect the well-being of the subject.

Table 1-1 Schedule of Activities

Procedure/ Assessment	Visit 1 Screening		Visit 2 Day 0 Baseline	Visit 3 Day 7 (±1 day)	Visit 4 Day 28 (±2 days)
Informed Consent	X	Lead-In (Minimum 5 Days)[1]			
Demographics	X				
Medical History	X				
Prior/Current Medications/Treatments	X				
Subject Returns with Lead-In Toothbrush (in Toothbrush Holder), Regular Fluoride Toothpaste and Completed Diary			X		
Subject Returns with Study Toothbrush (in Toothbrush Holder), Regular Fluoride Toothpaste and Completed Diary				X	X[3]
Compliance Checks [2]			X	X	X
Concomitant Medications/Treatments			X	X	X
Subject Continuance				X	X
Oral Soft Tissue (OST) Examination	X		X	X	X
Oral Hard Tissue (OHT) Examination	X				X[7]
Oral Examinations to Determine Eligibility Against Inclusion/Exclusion Criteria and to Identify Scorable Teeth for RPI	X				
Pre-Brushing RPI/TPI Assessments			X	X	X
Confirm Pre-Brushing Mean RPI $_{gingival/interproximal}$ ≥ 0.6			X		
Inclusion/Exclusion Criteria	X		X		
Subject Eligibility	X		X		
Stratification and Randomization			X		
Dispense [Lead-In Toothbrush, Toothbrush Holder, Regular Fluoride Toothpaste, Diary and Timer]	X				
On-Site Supervised Brushing with Lead-In Toothbrush and Regular Fluoride Toothpaste; Complete Diary	X				
Dispense Study Toothbrush, Toothbrush Holder, Regular Fluoride Toothpaste and New Diary			X		
On-Site Brushing with Assigned Study Toothbrush and Fluoride Toothpaste; Complete Diary [4]			X	X	X
Post-Brushing OST Examination			X	X	X
Post- Brushing RPI/TPI Assessments			X	X	X
Repeat RPI/TPI Assessments [5]			X	X	X
Adverse Events (AEs) [6]	X		X	X	X
Study Conclusion					X

Footnotes:

1. No maximum duration is specified for the Lead-In period, however Visit 2 (baseline) appointments should be scheduled to provide a similar duration of lead-in period for most subjects.

2. Subjects will be required to bring their study supplies (minus timer) to every visit.
Staff will perform a visual check of the returned study supplies/review the completed diary.
- Visits 2: compliance with lead-in toothbrush/regular fluoride toothpaste usage.
- Visits 3-4: compliance with assigned study toothbrush/regular fluoride toothpaste usage.
Check compliance with Lifestyle Guidelines/Medication Requirements.
3. At the end of the study, subjects should be asked to return the labelled carton their study toothbrush was supplied in, together with the study toothbrush in its' toothbrush holder.
4. On-site brushings to be carried out after pre-brushing plaque assessments.
5. Two repeatability assessments should be completed - one for each clinical index - on each assessment day (one in the morning; one in the afternoon), alternating pre- and post-assessments for each index.
6. Adverse Events (AEs), and therefore all Serious Adverse Events (SAEs), will be collected from immediately after each subject consents to participate in the study (by the completion of the Informed Consent Form [ICF]) until 5 days after last use of study product.
7. At Visit 4, the OHT examination should follow the post-brushing OST examination.

1.2 Study Objectives

Study objectives and endpoints are defined in Table 1-2:

Table 1-2 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the within-treatment supra-gingival plaque removal efficacy of four manual toothbrushes after a single brushing event (first use).	Change from pre- to post-brushing in mean RPI - overall* (Day 0). Change from pre- to post-brushing in mean TPI - overall (Day 0). ** all sites assessed*
Secondary	
To evaluate the within-treatment supra-gingival plaque removal efficacy of four manual toothbrushes after single brushing events, following 7 and 28 days familiarization with the brush.	Change from baseline (Day 0 pre-brushing) to post-brushing in mean RPI and mean TPI - overall (Day 7, Day 28). Pre- and post-brushing mean RPI and mean TPI- overall (Day 7, Day 28).
To evaluate the within-treatment supra-gingival plaque removal efficacy of four manual toothbrushes at the gingival margin and from inter-proximal sites, after single brushing events, following 0, 7 and 28 days familiarization with the brush.	Change from baseline (Day 0 pre-brushing) to post-brushing in mean RPI and mean TPI - gingival margin sites (RPI only); inter-proximal sites (Day 0, Day 7, Day 28). Pre- and post-brushing mean RPI and mean TPI - gingival margin sites (RPI only); inter-proximal sites (Day 0, Day 7, Day 28).
To evaluate the within-treatment supra-gingival plaque removal efficacy of four manual toothbrushes after 7 and 28 days twice-daily brushing.	Change from baseline (Day 0 pre-brushing) in mean RPI and mean TPI - overall; gingival margin sites (RPI only); inter-proximal sites (Day 7 pre-brushing, Day 28 pre-brushing).
Safety	
To assess the safety and oral tolerability of the four manual toothbrushes after first use (Day 0) and over 28 days' use.	TEAEs

This clinical methodology development study will be considered successful if a numerical reduction in supra-gingival plaque levels is observed for each toothbrush after first use (Day 0, pre- to post-brushing), for both clinical measures.

1.3 Treatments

There are 5 products used in this study – 1 lead-in toothbrush and 4 investigational/study toothbrushes.

The study products are manual toothbrushes.

- **Oral B Indicator 123 (medium)** and **Dr Best Original (medium)**: these are standard, flat trim toothbrushes, comprising only end-rounded polybutylene terephthalate (PBT) bristles, designed to clean the surfaces of the teeth gently and effectively.

- **Dr Best Multi Expert (medium)**: the toothbrush head contains co-extruded and endrounded bristles, with 'silky' and 'tapered' filaments, and has been designed to clean the surfaces of teeth, the inter-proximal areas and along the gingival margin.
  - End-rounded bristles are standard bristles, combining PBT and titanium dioxide pigment.
    Filament tips are ground ('polished') to a smooth lozenge-shaped end designed to clean the surface of the teeth gently and effectively.
  - Co-extruded bristles combine PBT with a thermoplastic elastomer (TPE) core and are reported to have a whitening/polishing effect on the tooth surface.
  - Silky and tapered filaments are made of PBT. Silky filaments are longer and thinner than standard bristles; tapered filaments are end-rounded, with the ends chemically eroded into a fine tip.
    These bristles are designed for effective cleaning inter-proximally and along the gingival margin ('hard to reach areas').
- **Parodontax Interdental (soft)**: the toothbrush head contains 'tapered' PBT filaments and has been designed to clean the inter-proximal areas.

The plaque removal efficacy of each brush will be evaluated using regular fluoride toothpaste and a 1 minute brushing time.

The following products (presented in Table 1-3) will be supplied by the Global Clinical Supplies group, GSK CH:

Table 1-3 Study Products

Treatment Description	**Study Treatments** Commercially available manual toothbrushes			
Product Name	Oral B Indicator 123 (medium; compact head) (UK market)	Dr Best Original (medium; regular head) (German market)	Dr Best Multi Expert (medium; compact head) (German market)	Parodontax Interdental (soft; compact head) (German market)
Pack Design	Individual toothbrush in its' commercial pack			
Dispensing Details	One brush at Visit 2 (after randomization)			
Product Master Formulation Code	N/A	MFC CCI [redacted]	MFC CCI [redacted]	MFC CCI [redacted]
Dose/Application	N/A			
Route of Administration	Oral topical use			
Usage Instructions	Subjects will dose the toothbrush with a strip of regular fluoride toothpaste (full brush head) then brush their entire dentition for 1 timed minute, twice daily (morning and evening). **On-site**: Subjects will rinse with 10 mL tap water post-brushing for 5 seconds.			

	Off-site: Subjects will be permitted to rinse with tap water post-brushing, according to their normal habit.
Return Requirements	**Used Samples**: Destroy at site using site disposal procedures. **Unused Samples**: Return to 3rd party vendor

Lead-in toothbrush **is not** considered as investigational/study product. All analysis described in this document will be based on 4 study toothbrushes.

1.4 Sample Size Calculation

The study will be conducted in male and female subjects in good general and oral health, aged 18-65 years, with ≥ 20 natural, permanent teeth and ≥ 40 gradable tooth surfaces (defined as ≥ 2/3rds of the tooth surface assessable for RPI) at Screening (Visit 1), and a pre-brushing mean RPI $_{gingival/interproximal}$ ≥ 0.6 at Visit 2.

No formal sample size has been produced. However, based on similar studies (He et al. 2009, Nathoo et al. 2004), evaluating the plaque removal efficacy of manual toothbrushes, approximately 20 evaluable subjects per product group is considered sufficient to provide reliable estimates of treatment effect for the purposes of this methodology development study and to aid in the design of future clinical studies. Sufficient subjects will be screened (approximately 200 subjects) to enter approximately 100 subjects into the lead-in period and so ensure approximately 80 subjects are randomized to study products. No allowance will be made for dropouts; none is expected for the primary objective given the clinical assessments will be made shortly after randomization.

2 Planned Analyses

2.1 Interim Analysis

No interim analysis is planned for this study.

2.2 Final Analyses

The final planned analyses will be performed after the completion of the following sequential steps:

1. All subjects have completed the study as defined in the protocol.
2. All required database cleaning activities have been completed and database has been locked.
3. All criteria for unblinding the randomization codes have been met and the randomization codes have been distributed.

3 Considerations for data analyses and Data Handling Conventions

3.1 Baseline Definition

In this study, baseline values are defined as the Day 0 pre-brushing measurements.

3.2 Subgroups/Stratifications

Subgroups are not defined for this trial.

Subjects will be stratified according to their Day 0 pre-brushing mean $RPI_{gingival/interproximal}$ score. The stratification factor will give rise to two strata.

- **Stratum 1 (lower):** mean $RPI_{gingival/interproximal} \geq 0.6$ to ≤ 0.8
- **Stratum 2 (higher):** mean $RPI_{gingival/interproximal} > 0.8$ to 1.0

3.3 Centers Pools

Since this is a single center study, pooling of centres is not applicable.

3.4 Timepoints and Visit Windows

The timepoints and visits for this study are defined in the Section 1-1 “Schedule of Activities” of the protocol and in Table 1-1 of this document. Any deviation from the study schedule may be reviewed on case-by-case basis at the Blinded Data Review Meeting (BDRM) to determine whether the data should be excluded from the analysis populations. A time window non-compliance listing will be produced for the BDRM.

All data included will be by nominal visits and visit windows will not be considered.

4 Data Analysis

Data analysis will be performed by Syneos Health with oversight from GSK CH. The statistical analysis software used will be SAS version 9.4 or higher.

Prior to database closure a BDRM will be conducted in which various aspects of the trial will be discussed and agreed.

Unless otherwise described, all listings will be produced for all randomized subjects.

4.1 Populations for Analysis

4.1.1 Subject Disposition

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomized. An enrolled subject is a subject who has signed informed consent and is eligible to proceed beyond the screening visit. The number of subjects screened, subjects not randomized, and subjects enrolled will be presented for all screened subjects in Table 14.1.1.

Table 14.1.1 will also display the number and percentage of screen failure subjects (subjects not randomized) with reasons why subjects are not randomized. Percentages for screen failure subjects will be based on the total number of subjects screened.

Subject disposition will also be summarized as the number and percentage of subjects in each of the defined analysis populations, who complete the study and who discontinue the study broken down by reason for discontinuation. The summary will be presented by study product group and overall. The percentages are based on the total number of subjects randomized.

Subject disposition including demographic data (age, sex and race), screening date, study product start date and time, study product end date and time, duration of study product in days (defined as [(last date of study product administration - start date of study product) + 1] for all subjects completing the study as per protocol, and [(date of withdrawal - start date of study product) + 1] for all subjects dropping out of the study), subject status (completer, Yes/No), study completion/withdrawal date, duration in the study in days (defined as [(date of completion or withdrawal - start date of study product) + 1], and the primary reason for withdrawal will be listed (Listing 16.2.1.1) by study product group for all randomized subjects.

Subject disposition information will be listed for non-randomized subjects (Listing 16.2.1.2), displaying subject number, demographic information (age, sex and race), screening date, reason for screen failure and any further details of reason for screen failure.

4.1.2 Protocol Deviations

Protocol deviations will be tracked by the study team throughout the conduct of the study. Data will be reviewed prior to unblinding and closure of the database to ensure all important deviations are captured and categorized.

Important deviations of the protocol procedures may include, but will not be limited to the following:

- Consent procedures
- Inclusion/Exclusion criteria
- Study procedures

The specific details of important protocol deviations will be listed in Protocol Deviation Management Plan and assessment process will be specified in the Blind Data Review Plan. Subjects with important protocol deviations will be identified at the BDRM.

The number and percentage of subjects with at least one important protocol deviation with reasons for deviations will be summarized in Table 14.1.2 by study product group for all randomized subjects and listed in Listing 16.2.2.1 for all randomized subjects.

All protocol deviations collected on the protocol deviation electronic Case Report Form (eCRF) will be listed in Listing 16.2.2.2 by study product group for all randomized subjects. The listing will present date of deviation, type of deviation, and deviation description.

4.1.3 Analysis Populations

The analysis populations defined for this study are presented in Table 4-1.

Table 4-1 Analysis Populations

Population	Definition / Criteria	Analyses Evaluated
Safety	Comprise all randomized subjects who complete at least one use of study product. This population will be based on the product the subject actually received.	Safety Analysis
Modified Intent-To-Treat (mITT)	Comprise all randomized subjects who have a post-baseline plaque assessment. This population will be based on the product to which the subject was randomized. Any subject who receives a randomization number will be considered to have been randomized.	Demographic and Baseline Characteristics, Efficacy Analysis
RPI Repeatability	Comprise all subjects who have a repeat clinical efficacy assessment for RPI index at any visit.	Exploratory Repeatability Analyses
TPI Repeatability	Comprise all subjects who have a repeat clinical efficacy assessment for TPI index at any visit.	Exploratory Repeatability Analyses

NOTE:
Please refer to Attachment 1: List of Data Displays which details the population to be used for each displays being generated.

Due to the exploratory nature of the study, the Per Protocol population will not be defined. The primary population for assessment of efficacy will be the mITT population. Repeatability Population will be used only to support repeatability analyses.

The numbers of subjects included in each of the analysis populations will be presented in Table 14.1.1 by study product group and overall. Subjects excluded from any of the analysis populations will be listed in Listing 16.2.3.1 by study product group.

4.2 Subject Demographics and Other Baseline Characteristics

Demographic and baseline characteristics summaries will be produced for the mITT.

4.2.1 Demographic Characteristics

Descriptive statistics (number of subjects [n], mean, standard deviation [SD], median, minimum and maximum for continuous variables and frequency count [n] and percentage [%] of subjects for categorical variables) will be presented for demographic variables by study product group and overall. These variables include age, gender and race and will be presented for mITT population (Table 14.1.3). In addition, stratification groups defined as Day 0 pre-brushing mean RPI $_{gingival/interproximal}$ score (categorized as: lower - mean RPI $_{gingival/interproximal} \geq 0.6$ to ≤ 0.8; and higher - mean RPI $_{gingival/interproximal} > 0.8$ to 1.0) will also be summarized for mITT population (Table 14.1.3).

Demographic information will be listed (Listing 16.2.4.1) for all randomized subjects by study product group.

4.2.2 General Medical History

Medical history data will be listed (Listing 16.2.4.2) for all randomized subjects with start date and end date or ongoing at the start of the study.

4.3 Treatments (Study Product, Rescue Medication, other Concomitant Therapies, Compliance)

The study product kit allocations will be listed (Listing 16.1.6.1), including kit number and study product information.

Randomization details will be listed, including the randomization number, stratification group, planned randomized study product, actual study product and the randomization date (Listing 16.1.7.1).

4.3.1 Study Product Compliance and Exposure

To promote compliance throughout the study, subjects will be provided with a diary at Screening (Visit 1) and at Baseline (Visit 2) to record each brushing with their lead-in toothbrush and study toothbrush respectively. They will also use the diary to note any missed/additional brushings, the reasons for any missed/additional brushings, any issues with the toothbrush or toothpaste used, oral problems, illnesses and any new medications/treatments. Subjects will attend each study visit with the lead-in/study products provided (used and unused) for a visual check of product usage, and with their completed diary for review by study staff.

Subjects will be instructed to self-perform oral hygiene with the lead-in toothbrush and their assigned study toothbrush (using the regular fluoride toothpaste provided) according to the usage instructions provided at the study site and detailed in the diary.

A supervised brushing will be carried out at the study site at the end of Visit 1 to facilitate subject compliance with brushing instructions and diary completion. Other on-site brushings (Visits 2-4) will be carried out in the presence of study staff but will not be supervised.

The number of missed or additional brushings will be captured as protocol deviations and transcribed from the diary into the eCRF. Subjects will be re-instructed in the correct usage requirements and diary completion as needed.

No summaries of lead-in product compliance will be provided.

Study product compliance data will be summarized for the mITT population in Table 14.2.1.1. Total number of brushings, compliance (%), number of missed brushings and number of additional brushings will be summarized using descriptive statistics as separate categories by study product group. Total number of brushings is defined as: [(date of last brushing – date of first brushing + 1) multiplied by 2 – number of missing brushings + number of additional brushings]. Compliance (%) is defined as: [100 x (Total number of brushings / Expected number of brushings)], where expected number of brushings is defined as: [(date of last brushing - date of first brushing + 1) multiplied by 2].

Study product brushing compliance (total number of brushings / compliance (%) / missed brushings / additional brushings) will be listed in Listing 16.2.5.1 for all randomized subjects by study product groups.

On-site study product brushings will also be summarized for the mITT population in Table 14.2.1.2 by visit. In addition, details (including subject number, date and start time of brushing) for on-site study product brushings will be listed (Listing 16.2.5.2) for all randomized subjects by study product group.

4.3.2 Prior and Concomitant Medication

Any medications, treatments or vaccine (including over-the-counter or prescription medicines, vitamins and/or herbal supplements) taken during the study, 30 days prior signing the informed consent, must be recorded in the eCRF with indication, reason for use, unit dose, daily dose and start/stop dates of administration. All subjects will be questioned about concomitant medication/treatments at each site visit.

Prior medication/treatments are defined as medication/treatments taken in the 30 days prior signing the ICF and before the first use of study product (i.e., assigned study toothbrush).

Prior medications will be listed by subject, with drug name, GSK drug synonym, reason, dose, frequency, route, start and end dates, study day relative to study product start and end date (Listing 16.2.4.3) by study product group for all randomized subjects.

Concomitant medications and concomitant non-drug treatments are defined as medications or non-drug treatment/procedures taken after first use of study product. Any medication, which are ongoing at the time of first use of study treatment will also be considered as concomitant.

Unknown dates will not be imputed. If the stop date is unknown, or incomplete, and the medication cannot be considered as stopped prior to the date of signing the ICF then the

medication will be considered as a concomitant medication (unless partial start or stop dates indicate differently).

Concomitant medications and concomitant non-drug treatments will be listed (Listing 16.2.4.4) similarly to prior medication with either ongoing or end date displayed by study product group for all randomized subjects.

4.4 Analysis of Efficacy

The mITT population will be considered as primary population for primary analyses.

4.4.1 Primary Efficacy Endpoint

4.4.1.1 Primary Efficacy Endpoint Definition

The main focus of the analysis will be the ability of each study toothbrush to reduce supra-gingival plaque after first use (Day 0, pre- to post-brushing), as measured by mean RPI $_{overall}$ and mean TPI $_{overall}$.

For RPI, each tooth surface is divided into nine zones (A to I); A-C along the gingival margin; D-F directly above A-C (D and F being the inter-proximal zones); G-H across the middle of the tooth and I covers the incisal area. The plaque will be disclosed and the presence or absence of plaque in each zone recorded, generating a total of 18 scores per tooth. RPI scale is described in Table 4-2 below.

Table 4-2 RPI Scoring

Score	Description
0	Plaque absent
1	Plaque present

For TPI, each tooth surface is divided into 3 areas; three scores are recorded facially (mesiofacial, facial, distofacial) and three scores lingually (mesiolingual, lingual and distolingual) generating a total of six scores per tooth.

The plaque will be disclosed and scored for each site as described in Table 4-3.

Table 4-3 TPI Scoring

Score	Description
0	No plaque
1	Slight flecks of plaque at the cervical margin of the tooth
2	Thin, continuous band of plaque (1millimetre (mm) or smaller) at the cervical margin of the tooth
3	Band of plaque wider than 1mm but covering less than 1/3 of the area
4	Plaque covering at least 1/3 but less than 2/3 of the area
5	Plaque covering 2/3 or more of the crown of the tooth

Mean RPI $_{overall}$ and mean TPI $_{overall}$ will be calculated for each subject as the total score for all tooth sites assessed divided by the total number of tooth sites assessed.

Change from baseline will be derived for each individual tooth site first before calculating the mean overall change for all sites assessed. Changes from baseline will be derived, summarized and analyzed.

Descriptive statistics (n, missing, mean, SD, standard error [SE], median, minimum, and maximum values) of the pre-brushing, post-brushing and change from Day 0 pre-brushing in mean RPI $_{overall}$ and mean TPI $_{overall}$ scores will be presented in Table 14.2.2.1.1 for mean RPI $_{overall}$ and in Table 14.2.2.2.1 for mean TPI $_{overall}$ for all subjects in mITT population by study product group.

The listings of RPI and TPI derived scores by subject will be presented for all randomized subjects (Listing 16.2.6.1.1 for RPI derived scores and Listing 16.2.6.2.1 for TPI derived scores). These listings will include subject number, visit, pre-brushing mean scores, post-brushing mean scores, change from pre-brushing to post-brushing and change from Day 0 pre-brushing.

All RPI index data collected in the eCRF will be listed in Listing 16.2.6.1.2 for all randomized subjects while TPI index data collected in the eCRF will be listed in Listing 16.2.6.2.2 for all randomized subjects.

4.4.1.2 Statistical Hypothesis, Model, and Method of Analysis

In this study, baseline is defined as the Day 0 pre-brushing mean plaque score for each index (RPI/TPI). Primary analysis will focus on the ability of each study toothbrush to reduce supra-gingival plaque after first use (Day 0, pre- to post-brushing).

Mean RPI $_{overall}$ and mean TPI $_{overall}$ will be calculated for each subject as the total score for all tooth sites assessed divided by the total number of tooth sites assessed. Change from baseline will be derived for each individual tooth site first before calculating the mean overall change for all sites assessed.

Plaque removal efficacy after first use (change from baseline) will be tested under the null hypothesis:

- H_0: there is no change from the baseline plaque level;
- H_1: there is a change from the baseline plaque level.

Change from baseline in mean RPI $_{overall}$ and mean TPI $_{overall}$ will be analyzed using analysis of covariance (ANCOVA).

Change from baseline will be summarized for each primary outcome variable by study product group (adjusted mean change, percent change from mean baseline, 95% confidence intervals [CIs] and p-value for change from zero). Percent change from mean baseline will be derived as: 100 x (Adjusted Mean Change / Mean Baseline Value). Adjusted Mean Change will be derived from ANCOVA model.

No formal sample size has been generated for this methodology development study, thus the p-values will be interpreted with caution.

For analysis of mean RPI $_{overall}$, the model will include product group as a fixed effect and the baseline mean RPI $_{overall}$ value as a covariate (Table 14.2.2.1.2). RPI stratification level will not be used in the analysis as the actual value will be used as a covariate.

For analysis of mean TPI $_{overall}$, the model will include product group as a fixed effect, the baseline mean TPI $_{overall}$ value as a covariate and RPI stratification level [lower (defined as Day 0 pre-brushing mean RPI $_{gingival/interproximal} \geq 0.6$ to ≤ 0.8) or higher (defined as Day 0 pre-brushing mean RPI $_{gingival/interproximal} > 0.8$ to 1.0] as an extra factor (Table 14.2.2.2.2).

Size of effect will be a point of focus. As this is a methodology development study, no adjustments will be made for multiple comparisons.

The assumption of normality and homogeneity of variance in the ANCOVA models will be investigated. Violation of this assumption will be overcome using a suitable data transformation or a non-parametric technique (e.g. Wilcoxon Sign Rank test).

4.4.1.3 Supportive Analyses

N/A

4.4.2 Secondary Efficacy Variables

See Section 4.5.

4.4.3 Handling of Missing Values/Censoring/Discontinuations

Missing data will not be replaced or imputed. Dropouts will be included in analyses up to the point of discontinuation.

4.5 Analysis of Secondary Objectives

The mITT population will be considered as primary population for secondary analyses.

4.5.1 Efficacy (Secondary)

The secondary efficacy endpoints are:

- Mean RPI $_{overall}$ (Day 7 pre- and post-brushing, Day 28 pre- and post-brushing)
- Mean TPI $_{overall}$ (Day 7 pre- and post-brushing, Day 28 pre- and post-brushing)
- Mean RPI $_{gingival\ margin}$ (Day 0 pre- and post-brushing, Day 7 pre- and post-brushing, Day 28 pre- and post-brushing), defined as mean RPI score for sites A, B and C
- Mean RPI $_{inter\text{-}proximal}$ (Day 0 pre- and post-brushing, Day 7 pre- and post-brushing, Day 28 pre- and post-brushing), defined as mean RPI score for sites D and F
- Mean TPI $_{inter\text{-}proximal}$ (Day 0 pre- and post-brushing, Day 7 pre- and post-brushing, Day 28 pre- and post-brushing), defined as mean TPI score for mesial and distal sites

Secondary outcome variables will be derived, based on relevant tooth sites, as the primary outcome variables.

Summary statistics (n, missing, mean, SD, SE, median, minimum, maximum) of pre-brushing, post-brushing and change from Day 0 pre-brushing will be presented for each secondary outcome variable at each assessment time point by study product group in the following tables:

- Table 14.2.2.1.1 for mean RPI $_{\text{overall}}$
- Table 14.2.2.2.1 for mean TPI $_{\text{overall}}$
- Table 14.2.2.3.1 for mean RPI $_{\text{gingival margin}}$
- Table 14.2.2.4.1 for mean RPI $_{\text{inter-proximal}}$
- Table 14.2.2.5.1 for mean TPI $_{\text{inter-proximal}}$

Change from baseline of secondary efficacy variables will be analyzed using ANCOVA model with product group as a fixed effect and the appropriate baseline value as a covariate, as defined below:

- For mean RPI $_{\text{overall}}$: baseline mean RPI $_{\text{overall}}$
- For mean TPI $_{\text{overall}}$: baseline mean TPI $_{\text{overall}}$ and RPI stratification level (lower/higher)
- For mean RPI $_{\text{gingival margin}}$: baseline mean RPI $_{\text{gingival margin}}$
- For mean RPI $_{\text{inter-proximal}}$: baseline mean RPI $_{\text{inter-proximal}}$
- For mean TPI $_{\text{inter-proximal}}$: baseline mean TPI $_{\text{inter-proximal}}$ and RPI stratification level (lower/higher)

Change from Day 0 pre-brushing to each analysis time points (Visit 2 Day 0 post-brushing, Visit 3 Day 7 pre- and post-brushing and Visit 4 Day 28 pre- and post-brushing) will be summarized for each secondary outcome variable by product group (adjusted mean change, percent change from mean baseline, 95% CIs and p-value for change from zero) in the following analysis tables:

- Table 14.2.2.1.2 for mean RPI $_{\text{overall}}$
- Table 14.2.2.2.2 for mean TPI $_{\text{overall}}$
- Table 14.2.2.3.2 for mean RPI $_{\text{gingival margin}}$
- Table 14.2.2.4.2 for mean RPI $_{\text{inter-proximal}}$
- Table 14.2.2.5.2 for mean TPI $_{\text{inter-proximal}}$

Percent change from mean baseline will be derived as: 100 x (Adjusted Mean Change / Mean Baseline Value). Adjusted Mean Change will be derived from ANCOVA model.

The assumption of normality and homogeneity of variance in the ANCOVA models will be investigated. Violation of this assumption will be overcome using a suitable data transformation or a non-parametric technique (e.g. Wilcoxon Sign Rank test).

Actual mean plaque scores (± SE) for all secondary efficacy endpoints will be plotted over time by study product group for all subjects in mITT population in the following figures:

- Figure 14.2.2.1.1 for mean RPI $_{\text{overall}}$
- Figure 14.2.2.2.1 for mean TPI $_{\text{overall}}$

- Figure 14.2.2.3.1 for mean RPI $_{\text{gingival margin}}$
- Figure 14.2.2.4.1 for mean RPI $_{\text{inter-proximal}}$
- Figure 14.2.2.5.1 for mean TPI $_{\text{inter-proximal}}$

Pre-brushing values will be set at the nominal time recorded (Days 0, 7 and 28), while post-brushing values will be set at a small nominal time after (Days 0.5, 7.5 and 28.5).

All RPI index data collected in the eCRF will be listed in Listing 16.2.6.1.2 for all randomized subjects while TPI index data collected in the eCRF will be listed in Listing 16.2.6.2.2 for all randomized subjects.

The listings of derived scores of secondary efficacy variables by subject will be presented for all randomized subjects as follows:

- Listing 16.2.6.1.1 for mean RPI $_{\text{overall}}$
- Listing 16.2.6.2.1 for mean TPI $_{\text{overall}}$
- Listing 16.2.6.1.3 for mean RPI $_{\text{gingival margin}}$
- Listing 16.2.6.1.4 for mean RPI $_{\text{inter-proximal}}$
- Listing 16.2.6.2.3 for mean TPI $_{\text{inter-proximal}}$

These listings will include subject number, visit, pre-brushing mean scores, post-brushing mean scores, change from pre-brushing to post-brushing, and change from Day 0 pre-brushing.

4.5.2 Pharmacokinetic (Secondary)

N/A

4.6 Analysis of Safety

All safety data will be reported for the Safety Population as per actual study product received. The safety profile of the study products will be assessed with respect to AEs, OST, OHT and incidents.

4.6.1 Adverse Events and Serious Adverse Events

All AEs will be reviewed by the Clinical Research Scientist or Designee prior to database lock and will be coded to a system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA).

AEs will be classified as oral and non-oral on the AE page of eCRF.

TEAEs are defined as an AEs that occur on or after the start date of first study product usage (if this date is missing a suitable alternative will be used e.g., date of randomization). AEs with an onset date/time prior to the first study product usage will be considered as non-treatment emergent.

The following summary tables and listings will be presented by study product group and overall:

- Table of TEAEs by SOC and PT (Table 14.3.1.1.1).
- Table of TEAEs by Oral/Non-Oral and PT (Table 14.3.1.1.2).

- Table of treatment-related TEAEs by SOC and PT (Table 14.3.1.2.1).
- Table of treatment-related TEAEs by Oral/Non-Oral and PT (Table 14.3.1.2.2).
- Table of treatment-emergent SAEs by SOC and PT (Table 14.3.1.1.3) [only produced if there are more than 5 SAEs].
- Listing of all AEs (Listing 16.2.7.1 for all randomized subjects; Listing 16.2.7.2 for non-randomized subjects).
- Listing of deaths (Listing 14.3.2.1).
- Listing of non-fatal SAEs (Listing 14.3.2.2).
- Listing of TEAEs leading to study or product discontinuation (Listing 14.3.2.3).
- Listing of TEAEs classified as Oral (Listing 14.3.2.4).

In the event that there is nothing to report, a null table or listing will be produced.

4.6.2 Other Safety Variables

Other Safety Variables are listed below:

- OST examination
- OHT examination
- Incidents

OST Examination

The OST examination will be accomplished by direct observation and palpation, using retraction aids as appropriate, and will include examination of the oral labial mucosa (including lips), buccal mucosa, and mucogingival folds, gingival mucosa, hard palate, soft palate, tonsillar area, pharyngeal area, tongue, sublingual area, submandibular area and salivary glands. The results of the examination will be recorded in the eCRF as either normal or abnormal with details of any abnormalities. Any abnormality or worsening of a pre-existing condition observed by the clinical examiner or reported by the subject from the OST examination carried out at Screening will be recorded as an AE.

OST examinations will be performed at Screening and before and after each on-site tooth brushing at Day 0, Day 7 and Day 28.

OST will be summarized (number of subjects and percentages with abnormalities, without abnormalities or OST not examined) by study product groups in Table 14.3.4.1 for all subjects in Safety Population. In addition, OST will be summarized by shift table (Table 14.3.4.2) comparing normal/abnormal results of pre-brushing to post-brushing at each visit. OST examinations will be listed (Listing 16.2.9.1) for all randomized subjects.

OHT Examination

Where possible, the OHT examination should be conducted by a single dental examiner or clinically qualified designee for all subjects. Examination of the oral hard tissues (all facial, lingual/ palatal, mesial/distal and occlusal surfaces) will be accomplished by direct/indirect observation, using retraction aids as appropriate. Enamel irregularities, tooth fracture,

defective/faulty restorations (all direct & indirect restorations including fixed/removal prostheses), carious lesions, non-carious hard tissue loss (abrasion, attrition, abfraction and erosion), tooth staining and any other hard tissue irregularity (e.g. hypo/hypermineralisation, decalcification) will be recorded. Observations will be recorded as either absent or present, and conditions noted as present will be described in the eCRF. Any abnormality or worsening of a pre-existing condition observed by the clinical examiner or reported by the subject from the OHT examination carried out at Screening will be recorded as an AE. Findings from this Screening examination will be used to determine subject eligibility.

OHT examinations will be performed at Screening and at Visit 4 Day 28, following the post-brushing OST examination.

OHT will be summarized (number of subjects and percentages with abnormalities, without abnormalities or OHT not examined) by study product group in Table 14.3.4.3 for all subjects in Safety Population. OHT examinations will be listed (Listing 16.2.9.2) for all randomized subjects.

Incidents

All study products supplied are for use only in this clinical study.

A study product incident is any malfunction or deterioration in the characteristics and/or performance of a device as well as any inadequacy in the labeling or the instructions for use which, directly or indirectly, might lead to or might have led to the death of a subject/user/other person or to a serious deterioration in his/her state of health.

All Incidents will be listed in Listing 16.2.9.3. In the event that there is nothing to report, a null listing will be produced.

4.7 Analysis of Other Variables

Additional/Exploratory endpoint for this study is to analyze the repeatability of dental plaque scoring.

The clinical examiner selected for this study will have demonstrated their ability to replicate their own scores (intra-examiner repeatability) on a tooth site-by-tooth site basis. Given both the RPI and the TPI allow accurate repeat assessment, repeat assessments will be performed for both clinical measures throughout the treatment period (Visits 2 to 4) to monitor consistency of scoring.

One repeat assessment will be completed for each clinical index on each clinical assessment day (one in the morning, one in the afternoon), alternating between pre- and post-brushing assessments for each index. Repeatability assessments should be separated by a minimum of 10 minutes and, where possible, the clinical examiner should assess a different subject in the intervening period. The examiner should not have sight of their previous scores when carrying out a repeat assessment.

‘Repeat’ subjects will be selected from those in attendance on a particular study day.

The repeat dental plaque assessments (for both RPI and TPI indices) will be compared to the original assessments. Repeat assessments will not be used in any efficacy analyses.

The first and repeat plaque assessments on each tooth site will be cross tabulated. A weighted kappa coefficient (κ), along with the 95% CI will be calculated to assess the intra-examiner reliability.

Fleiss-Cohen weighted kappa will be calculated for the repeatability analysis. Reliability will be deemed

- excellent if $\kappa > 0.75$;
- fair to good if $0.4 \leq \kappa \leq 0.75$;
- poor if $\kappa < 0.4$.

A $\kappa \geq 0.6$ will be considered indicative of good repeatability across the study period.

Repeatability analysis will be conducted on RPI Repeatability and TPI Repeatability Populations.

Results for RPI scoring repeatability will be presented in Table 14.2.3.1. Results for TPI scoring repeatability will be presented in Table 14.2.3.2.

5 Changes to the Protocol Defined Statistical Analysis Plan

Any changes from the originally planned statistical analysis specified in the protocol are outlined in Table 5-1.

Table 5-1 Changes to Protocol Defined Analysis Plan

Protocol	**Reporting & Analysis Plan**	
Statistical Analysis section	**Statistical Analysis Plan**	**Rationale for Changes**
12.3 Definition of Analysis Populations The Repeatability population will comprise all subjects who have a repeat clinical efficacy assessment at any visit.	4.7 Analysis of Other Variables Repeatability analysis will be conducted on RPI Repeatability and TPI Repeatability Populations.	Repeatability Population will be separated for each index, in order to minimize the number of missing records caused by the fact that the subjects have repeated assessments for only one of the indices.
12.3.5 Secondary Analyses In this section it is noted that Plot of change from baseline for Days 7 and 28 will be created.	N/A	It was decided that the Plots of change from baseline for Days 7 and 28 will not be produced.
12.3.5 Secondary Analyses In this section it is noted that Box Plots of Actual Values for Days 0, 7 and 28 will be created.	N/A	It was decided that the Box Plots of actual values for Days 0, 7 and 28 will not be produced.

6 Attachment 1: List of Data Displays

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