Clinical 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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CLINICAL PROTOCOL

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

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Sponsor Information

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Document History

Document	Version	Summary of Changes
Original protocol	1.0	Not applicable (N/A)
Amended protocol	2.0	1) Exclusion criteria number 33 "A subject who, in the opinion of the investigator or medically qualified designee, should not participate in the study" was deleted 2) Table 6-2 has been amended: "Lead-in toothbrush: disposed at site" "Toothbrush holder: ….; subject may keep it"
Amended protocol	3.0	The following change has been made: Amendment of inclusion criteria for visit 2 (baseline) so that interproximal and gingival areas only are considered • Mean RPI $_{gingival/interproximal}$ ≥6 Minor administrative changes have been made: - Clarification for OHT examination, section 9.2.3: 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 - Correcting typographical error for kappa score values, section 12.3.8: Changed to κ ≥ 0.6 - Product dispensing, section 6.1.2: Clarification on access to clinical areas during subject brushing by clinical examiner.

Amendments incorporate all revisions to date, including amendments made at the request of country health authorities, ethics committees (ECs) etc.

Principal Investigator Protocol Agreement Page

- I confirm agreement to conduct the study in compliance with the protocol and any amendments according to the current International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines.
- I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.
- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure site staff receives all appropriate information throughout the study.
- I agree to conduct this study in full conformance with the laws and regulations of the country in which the research is conducted and the Declaration of Helsinki.

Investigator Name:	
Investigator Qualifications:	
Investigator Signature:	PPD
Date of Signature/Agreement:	PPD DD-Mmm-YYYY

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1 PROTOCOL SUMMARY

Background and Rationale

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.

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 Rustogi Modified Navy Plaque Index (RPI) - overall* (Day 0). Change from pre- to post-brushing in mean Turesky Modified Quigley & Hein Plaque Index (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).

Objectives	Endpoints
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.	Treatment emergent adverse events (TEAEs)

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 treatment).

Supra-gingival plaque levels will be assessed using two established clinical measures: the Rustogi Modified Navy Plaque Index (RPI) and the 6-site Turesky Modified Quigley and Hein Plaque Index (TPI). Subjects will abstain from oral hygiene for a period of 12-18 hours prior to each assessment visit. Subjects with Day 0 pre-brushing mean RPI $_{\text{gingival/interproximal}} \geq$ 0.6 will be stratified by their Day 0 pre-brushing RPI score (*lower:* mean RPI $_{\text{gingival/interproximal}}$ $\geq$ 0.6 to $\leq$ 0.8; *higher:* mean RPI $_{\text{gingival/interproximal}}$ > 0.8 to 1.0) and randomized to study treatment (Visit 2).

To standardize oral hygiene practice, eligible subjects will complete a lead-in period (minimum 5 days) prior to Visit 2 during which they will brush with the toothbrush and regular fluoride toothpaste provided.

The safety and oral tolerability of each study toothbrush will be monitored after first use and over the 28-day usage period by review of reported TEAEs.

Study Products

Treatment Description	Lead-In Toothbrush	Study Toothbrushes			
		Commercially available manual toothbrushes			
Product Name	Oral B Sensi-Soft (ultra soft)	Oral B Indicator 123 (medium)	Dr Best Original (medium)	Dr Best Multi Expert (medium)	parodontax Interdental (soft)
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 milliliter (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.

Type and Planned Number of Subjects

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 ≥ 2/3^{rds} of the tooth surface assessable for RPI) at Screening (Visit 1), and a pre-brushing mean RPI $_{gingival/interproximal}$ ≥ 0.6 at Visit 2. 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 treatment.

Statistical Analyses

A modified Intent-To-Treat (mITT) population will be used for efficacy analyses.

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). Change from baseline in mean RPI $_{overall}$ and mean TPI $_{overall}$ will be analyzed using analysis of covariance (ANCOVA). For RPI, the model will include treatment as a fixed effect and the baseline RPI $_{overall}$ value as a covariate. For TPI, RPI stratification level (lower/higher) will be included as an extra factor. Change from baseline will be summarized for each primary outcome variable by treatment group (adjusted mean change, 95% confidence intervals [CIs] and p-value for change from zero). No formal sample size has been generated for this methodology development study, thus the p-values will be interpreted with caution. Size of effect will be a point of focus.

Secondary outcome variables will be analyzed as the primary outcome variables and include change from baseline in:

- Mean RPI $_{overall}$ (Day 7, Day 28);
- Mean TPI $_{overall}$ (Day 7, Day 28);
- Mean RPI $_{gingival\ margin}$ (Day 0, Day 7, Day 28);
- Mean RPI $_{inter\text{-}proximal}$ (Day 0, Day 7, Day 28);
- Mean TPI $_{inter\text{-}proximal}$ (Day 0, Day 7, Day 28).

For change from baseline calculations, the baseline value will be the mean Day 0, pre-brushing plaque score.

Summary statistics (mean, median, standard error (SE), standard deviation (SD), minimum, maximum) will be presented for each outcome variable at each assessment time point by treatment group. Percent change from baseline will also be presented. Plots of raw means (± SE) for each outcome variable at each timepoint and box plots will be presented by treatment group.

1.1 Schedule of Activities

The schedule of activities table 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 $_{gingival/interproximal} \geq 0.6$			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. 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.

2 INTRODUCTION

2.1 Study Rationale

Dental plaque is a diverse and organized community of microorganisms on the tooth surface and is recognized as the causative agent of gingivitis (inflammation of the gingivae surrounding teeth in response to the presence of dental plaque) re. Plaque-induced gingivitis is a reversible condition which, if left untreated, can progress to periodontitis. Maintenance of good gingival health is therefore important in the prevention of periodontal disease. Gingivitis can be treated and prevented with regular effective oral hygiene (Brook 2003, Ower 2003), principally via mechanical cleaning (toothbrushing) (Chapple et al. 2015).

Since the introduction of simple toothbrushes with nylon bristles and straight plastic handles in the 1930s, the modern toothbrush has evolved to include new materials, different bristle shapes and stiffness, complex bristle tuft arrangements and novel handle designs, all with a view to improving plaque removal, particularly from 'hard to reach' areas (inter-proximal regions, third molars) of the mouth. A systematic review of manual toothbrush studies (59 publications with a total of 212 brushing exercises) reported 30-53% plaque removal after a single brushing occasion (depending on the clinical index used) and concluded that bristle tuft arrangement and duration of use contributed to the plaque removal efficacy of a brush. The review also noted that modifications to brush design can lead to better cleaning (for example, a change from vertical to angled bristle tuft arrangements can improve inter-proximal plaque removal) (Slot et al. 2012).

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 (Stookey et al. 1982). Such models are useful but limited in their ability to mimic the tenacity/adherent properties of dental plaque *in vivo* and the 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 clinical 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 use (with twice-daily brushing) 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 GSK CH toothbrushes.

Complete information for the four study toothbrushes and the lead-in toothbrush may be found in the single reference safety document (SRSD), which for this study is the Safety Statement.

2.2 Background

Regular mechanical plaque removal by toothbrushing is key to maintaining good gingival health and the prevention of periodontal disease (Brook 2003, Chapple et al. 2015, Kinane and Attström 2005, Ower 2003, Tai et al. 2006). Single-use, short-term (1-4 weeks) and longer-term (>4 weeks) clinical studies evaluating the plaque removal efficacy of manual and powered toothbrushes are reported in the scientific literature (Cronin et al. 2001a, Cronin et al. 2001b, Cronin et al. 2000, Dorfer et al. 2001, Jan Haun et al. 2002, Nathoo et al. 2004,

Nathoo et al. 2000, Robinson et al. 2006, Sharma et al. 2012, Sharma et al. 2005, Sharma et al. 2000a, Sharma et al. 2000b, Sharma et al. 2011, Singh et al. 2001, Slot et al. 2012, Williams et al. 2003). Studies involving a period of 'home use' allow study subjects time to familiarize themselves with a new toothbrush and may provide a more representative picture of product performance. However, a systematic review of 59 manual toothbrush studies noted a toothbrush which removes more plaque after a single use can also perform better over time (Slot et al. 2012). Trials of longer duration (>30 days treatment) are required to determine the impact of a toothbrush on gingival health (American Dental Association 2016, Robinson et al. 2006).

Many clinical indices have been developed to assess supra-gingival plaque accumulation; the two most commonly used in toothbrush evaluation studies (Robinson et al. 2006, Slot et al. 2012) are the 6-site Turesky modification of the Quigley and Hein Plaque Index (TPI) (Lobene et al. 1982, Turesky et al. 1970), which focusses on plaque accumulation on the lower gingival third of the tooth surface, and the Rustogi-modification of the Navy Plaque Index (RPI) (Rustogi et al. 1992), which focusses on plaque accumulation along the gingival margin and in the inter-proximal areas. The two measures are strongly correlated. Neither scale is linear, however, increasing scores indicate increasing plaque coverage of the tooth surface.

Toothbrush studies evaluating self-performed plaque removal efficacy differ widely in design, plaque index, plaque accumulation period, duration and brushing regimen (Robinson et al. 2006, Slot et al. 2012). A working group convened 'to review the evidence for primary prevention of periodontitis by preventing gingivitis' conducted meta-reviews to determine the plaque control efficacy of different self-performed toothbrushing regimens and highlighted the need for a systematic review of the effect of toothbrush design (filament characteristics and bristle configuration) on plaque removal (Chapple et al. 2015). In the absence of professional or industry-accepted guidelines to establish equivalence or superiority, GSK CH wishes to develop a clinical model to evaluate the plaque removal efficacy of manual toothbrushes.

The performance of four manual toothbrushes with differing filament types/bristle configurations will be assessed after single brushing events (with and without a period of familiarization) and after 7 and 28 days twice-daily brushing, using two established plaque indices (RPI and TPI). Data generated may inform the design of future clinical studies investigating the plaque removal efficacy of manual toothbrushes.

2.3 Mechanism of Action/Indication

Twice daily mechanical plaque removal by toothbrushing with fluoride toothpaste continues to be recommended as the primary means of controlling dental plaque and maintaining gingival health (Chapple et al. 2015, Laudenbach and Simon 2014, Sälzer et al. 2015).

The plaque removal efficacy of a manual toothbrush is determined by three main factors: the design of the toothbrush; the frequency and duration of use; the skill of the individual using the toothbrush (Cugini and Warren 2006, Frandsen 1986). A range of different bristle types (end-rounded, tapered, co-extruded, rectangular, silky), bristle diameters, that is, 'stiffness' (extra soft 0.127-0.152 millimeters [mm]; soft 0.152-0.178 mm; medium 0.178-0.203 mm; hard 0.203-0.23 mm) and tuft configurations are used in manual toothbrushes. Brush head design is determined by the specific cleaning requirements and target user (for example, dentin hypersensitivity, whitening, gum health) of the finished toothbrush.

The study treatments are manual toothbrushes.

- **Oral B Indicator 123 (medium)** and **Dr Best Original (medium):** these are standard, flat trim toothbrushes, comprising only end-rounded 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 end-rounded 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 polybutylene terephthalate (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.

3 STUDY OBJECTIVES AND ENDPOINTS

Table 3-1 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 Rustogi Modified Navy Plaque Index (RPI) - overall* (Day 0). Change from pre- to post-brushing in mean Turesky Modified Quigley & Hein Plaque Index (TPI) - overall (Day 0). * all sites assessed
Secondary	

Objectives	Endpoints
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.

4 STUDY DESIGN

4.1 Overall 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 treatment).

Supra-gingival plaque levels will be assessed using two established clinical measures: the RPI and the TPI. Subjects will abstain from oral hygiene for a period of 12-18 hours prior to each assessment visit. Subjects with Day 0 pre-brushing mean $RPI_{gingival/interproximal} \geq 0.6$ 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) and randomized to study treatment (Visit 2).

The mean RPI $_{gingival/interproximal} \geq 0.6$ is calculated based on the 10 areas for inter-proximal & gingival margins (A, B, C, D, F – facial and lingual sites) as follow: RPI $_{gingival/interproximal}$ sum score/number of gingival and interproximal sites.

To standardize oral hygiene practice, eligible subjects will complete a lead-in period (minimum 5 days) prior to Visit 2 during which they will brush with the toothbrush and regular fluoride toothpaste provided.

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 TEAEs.

4.2 Rationale for Study Design

Published studies evaluating the plaque removal efficacy of toothbrushes differ widely in design, plaque index, plaque accumulation period, duration and brushing regimen (single use, short and longer-term use) (Robinson et al. 2006, Slot et al. 2012). The results of this study may inform the design of future plaque removal efficacy studies.

Supra-gingival plaque accumulation will be assessed using two established clinical measures.

- Rustogi-modification of the Navy Plaque Index (RPI) (Rustogi et al. 1992) 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) (Lobene et al. 1982, Turesky et al. 1970) which focusses on plaque on the lower gingival third of the tooth surface.

Both scales are descriptive, based on the examiner's subjective assessment of surface plaque coverage. The two measures are strongly correlated (Cugini and Warren 2006); increasing scores indicate increasing 'severity' (more of the tooth surface covered by plaque). RPI is the more demanding of the two to score, requiring the examiner to make 18 assessments per tooth, compared to 6 for TPI. American Dental Association (ADA) guidelines (American Dental Association 2016) and a systematic review of powered toothbrush studies (Robinson et al. 2006) recommend use of the TPI, however, RPI is reported to yield numerically greater pre- to post-brushing differences than the TPI (Slot et al. 2012). The ability of both measures to characterize the plaque removal capability of the study toothbrushes will be investigated here. Data generated may inform the choice of index in future brushing studies.

Single-use brushing studies are often used as screening tools to give an initial indication of cleaning performance. However, it is not unreasonable to expect toothbrushes that perform well after a single brushing to show greater plaque removal with continued use (Slot et al. 2012). A longer period of 'home use' allows study subjects to familiarize themselves with a new toothbrush and provides a more representative picture of product performance over time. Longer term toothbrushing studies would also be required to investigate gingival health benefits. In this methodology development study, plaque removal efficacy will be investigated after three separate (on-site) single brushing events (without and with a period of familiarization with the subject's assigned study toothbrush):

- Day 0: first use, no familiarization;
- Day 7: 1 week's familiarization;

- Day 28: 4 weeks' familiarization;

and after an extended period of twice daily brushing (7 and 28 days). The 28 day treatment period has been selected to help inform the design of future longer term studies, while facilitating subject visit scheduling for this study.

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.

Toothbrush efficacy studies typically include a period of abstinence from oral hygiene prior to clinical assessment visits (i) to standardize oral hygiene practice in the study population and (ii) ensure sufficient plaque growth on the tooth for efficacy evaluation. A systematic review of manual toothbrush studies (59 publications) reported 23–25 hours as the most common period of plaque accumulation prior to brushing efficacy evaluation, 12-18 hours was the next most common (Slot et al. 2012). In this study, subjects will abstain from toothbrushing for 12-18 hours prior to Visits 2-4 to align with other GSK CH brushing studies.

To ensure study subjects demonstrate the required propensity for plaque formation, only subjects with Day 0 pre-brushing mean RPI $_{\text{gingival/interproximal}} \geq 0.6$ will be randomized to study treatment (Visit 2). Interproximal and gingival margins were chosen due to the unlikelihood that plaque will be present on the smooth surfaces of the tooth due to contact/rubbing with cheeks and tongue. Subjects will be stratified by their Day 0 pre-brushing RPI score (*lower:* mean RPI $_{\text{gingival/interproximal}} \geq 0.6$ to ≤ 0.8; *higher:* mean RPI $_{\text{gingival/interproximal}} > 0.8$ to 1.0) to ensure treatment groups are balanced for baseline supra-gingival plaque levels.

A systematic review of manual toothbrush studies (59 publications with a total of 212 brushing exercises) reported 30-53% plaque removal after a single brushing occasion (depending on the clinical index used) and concluded that bristle tuft arrangement and duration of use contributed to the plaque removal efficacy of a brush. Four marketed manual toothbrushes with differing brush head designs, and therefore anticipated differences in cleaning performance, have been selected for evaluation. The dental profession typically recommend ≥ 2 minutes brushing at least twice daily for good oral hygiene, however, studies reported in the scientific literature indicate brushing duration to be much shorter (working estimate 45 seconds) (Gallagher et al. 2009). A 1 minute brushing time has been selected for this study as representative of consumer behavior.

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 the clinical examination area and the examiner won't be allowed in the room during this period. This will ensure the clinical examiner remains blinded to treatment 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.

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.

4.3 Justification for Dose

The study products are manual toothbrushes, intended for topical oral use. Subjects will be provided with a regular fluoride toothpaste to use with the lead-in toothbrush and with their assigned study toothbrush for the duration of the study.

The usage regimen of twice daily brushing (morning and evening) will be the same for all subjects and is based on widely recommended oral hygiene practice/typical consumer habit. Study subjects will be required to brush for 1 timed minute with their assigned study toothbrush and the regular fluoride toothpaste provided (according to their normal habit) on each brushing occasion, for 28 days (± 2 days). Study toothbrushes will be evaluated under normal use conditions; no professional oral hygiene instruction will be given.

Eligible subjects will complete a supervised brushing with the lead-in toothbrush and the regular fluoride toothpaste provided at the end of the Screening visit (while still at the study site) to enable staff to confirm correct usage and encourage compliance with the required brushing regimen throughout the study.

4.4 End of Study Definition

A subject is considered to have completed the study if they completed all phases of the study, including the last visit or the last scheduled procedure shown in the Schedule of Activities.

The end of this study is defined as the date of the last scheduled procedure shown in the Schedule of Activities for the last subject in the trial

5 STUDY POPULATION

5.1 Type and Planned Number of Subjects

The study will be conducted in male and female subjects in good general and oral health. 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 treatment. Subjects will be recruited by the clinical site.

Subjects may be recruited via letters advertising the study, sent with routine appointment letters from the hospital and/or local general dental practices; via advertisements placed around university and hospital sites, and in local general dentist practices; by advertisements included in university newsletters and e-mails sent to university staff and students; or by word of mouth. Students of, and staff employed by, the investigational site will not be recruited.

An enrolled subject is one who has agreed to participate in the clinical study following completion of the informed consent process and has successfully met eligibility criteria to proceed beyond the screening visit, as applicable for the protocol design.

This study can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom participation in the study is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether a subject is suitable for this protocol.

Subject eligibility to participate in the clinical study should be reviewed and documented by an appropriate member of the investigator's study team before subjects are included in the study.

5.2 Inclusion Criteria

An individual must meet all the following inclusion criteria to be eligible for enrollment into the study.

1. Subject provision of a signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the study before any assessment is performed.
2. Subject is male or female who, at the time of screening, is between the ages of 18 and 65 years, inclusive.
3. A subject who is willing and able to comply with scheduled visits, treatment plan and other study procedures.
4. A subject in good general, mental and oral health with, in the opinion of the investigator or medically qualified designee, no clinically significant or relevant abnormalities in medical history or upon oral examination, or condition, that would impact the subject's safety, well-being or the outcome of the study, if they were to participate in the study, or affect the individual's ability to understand and follow study procedures and requirements.
5. A subject with, in the opinion of the investigator or medically qualified designee, satisfactory oral hygiene based on oral examination.
6. A subject who routinely uses a manual toothbrush for daily oral hygiene.
7. **VISIT 1 (Screening)**

   A subject must have

   a) ≥ 20 natural, permanent teeth

   b) ≥ 40 gradable tooth surfaces (≥ 2/3^{rds} of the tooth surface assessable for RPI)

   Third molars, fully crowned/ extensively restored, grossly carious, orthodontically-banded/bonded or abutment teeth and surfaces with calculus deposits which, in the opinion of the clinical examiner, would interfere with plaque assessment should not be included in the gradable surface count. Third molars can be included if, as a result tooth loss, they are functioning as second molars.

8. **VISIT 2 (Baseline)**

   A subject must have a pre-brushing mean RPI ≥ 0.6 $_{gingival/interproximal}$ (that is, the mean over ten gradable surfaces – sites A,B,C,D,F both facially and lingually)

5.3 Exclusion Criteria

An individual who meets any of the following exclusion criteria will not be eligible for enrollment into the study.

1. A subject who is an employee of the investigational site, either directly involved in the conduct of the study or otherwise supervised by the investigator, or a member of their

immediate family; or a GSK CH employee directly involved in the conduct of the study or a member of their immediate family.

2. A subject who has participated in other studies (including non-medicinal studies) involving investigational product(s) in the 30 days prior to study entry and/or who is participating in other studies during study participation.
3. A subject with, in the opinion of the investigator or medically qualified designee, an acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator or medically qualified designee, would make the subject inappropriate for entry into this study.
4. A subject with any medical/oral condition which, in the opinion of the investigator or medically qualified designee, could impact study outcomes (for example, oral dryness).
5. A subject taking daily doses of medication/having daily treatments which, in the opinion of the investigator or medically qualified designee, could impact study outcomes (for example, is causing oral dryness).
6. A subject with any condition or physical limitation which, in the opinion of the investigator or medically qualified designee, impacts their ability to perform oral hygiene with a manual toothbrush.
7. A subject who is pregnant (self-reported; no pregnancy test required) or intending to become pregnant over the course of the study.
8. A subject who is breastfeeding.
9. A subject with known or suspected intolerance or hypersensitivity to the study materials (or closely related compounds) or any of their stated ingredients.
10. A subject who is unwilling or unable to comply with the Lifestyle Considerations described in this protocol.
11. A subject who has had routine dental prophylaxis within 4 weeks of Screening.
12. A subject who has undergone a tooth bleaching procedure within 8 weeks of Screening.
13. A subject with, in the opinion of the investigator or medically qualified designee, generalized moderate/severe gingivitis.
14. A subject with, in the opinion of the investigator or medically qualified designee, gross periodontal disease.
15. A subject with, in the opinion of the investigator or medically qualified designee, signs of active periodontitis.
16. A subject with a Basic Periodontal Examination (BPE) score ≥ 3 in any sextant.
17. A subject who has undergone treatment for periodontal disease within 12 months of Screening, including scaling and/or root surface debridement.
18. A subject who is currently undergoing treatment for periodontal disease, including scaling and/or root surface debridement.
19. A subject with active caries which, in the opinion of the investigator or medically

qualified designee, could impact study outcomes or the oral health of the subject if they were to participate in the study.

20. A subject with, in the opinion of the investigator or medically qualified designee, evidence of gross intra-oral neglect or the need for extensive dental therapy.
21. A subject with restorations in a poor state of repair which, in the opinion of the investigator or medically qualified designee, could impact study outcomes.
22. A subject with any dental condition (e.g. malalignment, overcrowding) which, in the opinion of the investigator or medically qualified designee, could impact study outcomes.
23. A subject with high levels of extrinsic dental stain or calculus deposits which, in the opinion of the investigator or medically qualified designee, would interfere with plaque assessment or impact study outcomes.
24. A subject with a tongue or lip piercing
25. A subject with multiple dental implants which, in the opinion of the investigator or medically qualified designee, could impact study outcomes.
26. A subject with fixed bridge(s) or removable partial dentures.
27. A subject with fixed or removable orthodontic braces/bands or a fixed orthodontic retainer.
28. A subject who has had fixed or removable orthodontic braces/bands within 3 months of Screening.
29. A subject who is unwilling to forgo use of an orthodontic retainer for the duration of the study, provided there would be no impact on the outcome of any previous, completed orthodontic treatment or the subject's well-being.
30. **VISIT 1 (Screening)**

    A subject who has taken antibiotics within 2 weeks of Screening.
31. **VISIT 2 (Baseline)**

    A subject who has taken antibiotics during the lead-in period (between Screening and Visit 2).
32. Subject with a recent history (within the last year) of alcohol or other substance abuse.
33. A subject who has previously been enrolled in this study.

5.4 Randomization Criteria

Subjects will be randomized into the study provided they have satisfied all subject selection criteria. Subjects will be stratified according to their Day 0 pre-brushing mean RPI score. The stratification factor will give rise to two strata.

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

5.5 Lifestyle Considerations

If, in the opinion of the investigator or medically qualified designee, a subject has not complied with a study restriction prior to a study visit, re-appointment should be considered. The reason for re-appointment will be documented in the CRF. If re-appointment within permitted visit tolerances (Table 1-1 Schedule of Activities) or site availability is not possible, the following visit specific actions should be taken.

- **Visit 2:** the subject will be withdrawn from the study (Section 7.1). AEs will be recorded, and the OST examination completed; no clinical efficacy assessments will be performed. The subject may be replaced.
- **Visit 3:** the subject will continue in the study. AEs will be recorded, and the OST examination completed; no clinical efficacy assessments will be performed. The subject will not be replaced.
- **Visit 4:** the subject will be withdrawn from the study (Section 7.1). AEs will be recorded and the OST/OHT examinations completed; no clinical efficacy assessments will be performed. The subject will not be replaced.

5.5.1 Dental Product/Treatment and Oral Hygiene Restrictions

From Screening (Visit 1) to the Subject's Last Study Visit:

- Subjects should not use any other oral care products (for example, toothpastes, toothbrushes, oral rinses, tongue cleaners, whitening/bleaching products, inter-dental cleaning products) than those provided during the study.
  Note: dental floss (non-antibacterial) can be used to remove impacted food.
- Subjects should not chew gum or consume any confectionery containing xylitol (e.g. sugar-free mints).
- Subjects should delay any non-emergency dental treatment until after study completion (including dental prophylaxis).
- Subjects should delay any tooth whitening treatments (professional and/or home use) until after study completion.

Before Clinical Efficacy Assessment Visits (Visits 2-4):

- Subjects should refrain from oral hygiene procedures for 12-18 hours before their visit.

5.5.2 Meals and Dietary Restrictions

Before Clinical Efficacy Assessment Visits (Visits 2-4):

- Subjects should not eat or drink for ≥ 1 hour before their visit and until all visit procedures have been completed.

5.6 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomized. To ensure transparent reporting of screen failure subjects, a minimal set of screen failure information will include demography, screen failure details (e.g. withdrawal of consent), eligibility criteria, and any adverse events or incidents as applicable.

Individuals who do not meet the criteria for participation in this study (screen failure) may not be re-screened.

5.7 Sponsor's Qualified Medical Personnel

Contact information for the sponsor's appropriately qualified medical/dental personnel for the study is documented in the Study Contact List located in the investigator study master file held at the study site.

The contact number is only to be used by investigational staff seeking advice on medical/dental questions or problems in the event that the established communication pathways between the investigational site and the study team are not available.

The contact number is not intended for direct use by study subjects. To facilitate access to appropriately qualified medical/dental personnel on study-related medical/dental questions or problems, subjects will be provided with a contact card. The contact card will provide, as a minimum, protocol identifiers, the subject's study identification number, contact information for the investigational site, and contact details in the event that the investigational site cannot be reached to provide advice on a medical question or problem identified by a healthcare professional other than the investigator.

5.8 Rater/Clinical Assessor Qualifications

The clinical examiners involved in screening and efficacy assessment procedures for this study will be qualified dentists, registered to practice in the UK. Oral examinations to determine subject eligibility and all safety/efficacy assessments of the oral cavity will be performed by appropriately trained/calibrated clinical examiners. No additional qualifications are required for the clinical examiners involved in this study.

6 INVESTIGATIONAL/STUDY PRODUCTS

For the purposes of this study, per International Conference on Harmonization (ICH) guidelines, and GSK policy, investigational product is defined as a pharmaceutical form of an active ingredient, a non-medicinal product (marketed or investigational), or a placebo, being tested or used as a reference (positive or negative control), in a clinical trial. This includes a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.

6.1 Investigational/Study Product Supplies

Study products will be supplied by the Global Clinical Supplies (GCS) group, GSK CH.

Table 6-1 Investigational/Study Product Supplies

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	MFC CCI	MFC CCI
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			

Sundry items will be supplied the GCS group, GSK CH.

Table 6-2 Sundry Items

Item	Pack Design	Dispensing Details	Return/Disposal Details	
			Used Samples	Unused Samples
Lead-In Toothbrush Oral B Sensi-Soft (ultra soft) (US market)	Individual commercial pack	One brush at Visit 1	Dispose at site	Return to 3rd party vendor
Aquafresh Triple Protection toothpaste (UK market) 1450 parts per million (ppm) fluoride MFC CCI	One tube in commercial pack (4 tubes supplied per subject)	Two tubes at Visit 1 for use with lead-in toothbrush Two tubes at Visit 2 for use with assigned study toothbrush	Return to 3rd party vendor	Return to 3rd party vendor
Countdown Timer	Individual commercial pack	One timer at Screening	Subject may keep	Return to 3rd party vendor
Trace Plaque Disclosing Solution (Young Dental)	Individual Commercial pack	As described in Section 9.2.1 Plaque Disclosure	Dispose at site	Return to 3rd party vendor

Item	Pack Design	Dispensing Details	Return/Disposal Details	
			Used Samples	Unused Samples
Dosing cups (for post-brushing rinse and disclosing procedure)	Commercial pack	As described in Section 9.2.1 Plaque Disclosure	Dispose at site	Return to 3rd party vendor
Cotton tipped applicators (for disclosing procedure)	Commercial pack	As described in Section 9.2.1 Plaque Disclosure	Dispose at site	Return to 3rd party vendor
Toothbrush holder	Individual commercial pack	One holder at Visit 1 for use with lead-in toothbrush One holder at Visit 2 for use with assigned study toothbrush	Dispose at site; Subject may keep it	Return to 3rd party vendor

Detailed instructions for the return of study product/study supplies for the accountability checks and subsequent destruction which will be provided by GSK CH during the study in time for study close out visit.

Equipment/supplies required to carry out oral examinations and clinical assessments will be provided by the study site, including petroleum jelly.

6.1.1 Dosage Form and Packaging

Study products are manual toothbrushes and will be used with the regular fluoride toothpaste provided; all are intended for topical oral use.

The lead-in toothbrush will be dispensed at Visit 1 (Screening); study toothbrushes will be dispensed at Visit 2. Each will be supplied with a toothbrush holder for transport to and from the study site. The lead-in toothbrush will be supplied in its' commercial pack; study toothbrushes will be supplied in their commercial packs, in a carton with a study label affixed.

Study subjects will receive sufficient supplies of the regular fluoride toothpaste to cover usage during the lead-in and treatment periods. The regular fluoride toothpaste will be supplied in its' commercial pack with a study label affixed. Sundry items will be supplied in their commercial packaging for dispensing by study staff as required.

The content of the product labels will be in accordance with all applicable regulatory requirements and will be the responsibility of the GSK CH GCS group. Each study label will contain, but not be limited to, protocol number, directions for use and storage requirements.

Care should be taken with the supplied products and their labels so that they are maintained in good condition. It is important that all labels remain intact and legible for the duration of the study. Subjects should be instructed to not remove or deface any part of the study label.

All products supplied are for use only in this clinical study and should not be used for any other purpose.

6.1.2 Preparation and Dispensing

Subjects will be assigned to study product in accordance with the randomization schedule generated by an approved GSK CH vendor, prior to the start of the study, using validated software.

Randomization room will be separate from the clinical examination area. Study product will be dispensed by trained unblinded site personnel per the usage instructions. An additional member of the site staff will verify the dispensing procedures are completed accurately for each subject. These staff members will not be involved in any safety or efficacy assessments, or any other aspects of the study that could be influenced by the knowledge of the product a subject has been assigned to. The clinical examiner will not enter the product dispensing area at the time of dispensing and won't have access to the dispensing log.

A record of product dispensing to each subject will be maintained in the dispensing log; completion of the dispensing procedure will be recorded in the case report form (CRF).

6.2 Administration

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.

To help ensure subjects fully understand the usage instructions, staff will demonstrate dispensing a full ribbon of toothpaste along the length of the toothbrush head to each subject and supervise their first brushing with the lead-in toothbrush/diary completion at Visit 1, after all Screening procedures have been completed and eligibility has been confirmed.

Further on-site brushings (Visits 2-4) will be carried out in the presence of study staff (but will not be supervised) and will be recorded in the dispensing log/CRF.

6.2.1 Medication/Dosing Errors

Product usage/dosage errors may result, in this study, from the administration or consumption of the wrong product, by the wrong subject, at the wrong time, or at the wrong dosage.

Such product usage/dosing errors occurring to a study subject are to be captured in the CRF. In the event of a product usage/dosing error, the sponsor should be notified **immediately and under no circumstance should this exceed 24 hours.**

Product usage/dosage errors are reportable irrespective of the presence of an associated AE, including:

- Product usage/dosage errors involving subject exposure to any of the study products;
- Potential product usage/dosage errors or uses outside of what is foreseen in the protocol that do or do not involve the participating subject.

If a product usage/dosing error is accompanied by an AE, as determined by the investigator, the error and any associated adverse event(s) are to be captured in the CRF AE form.

6.2.2 Overdose

An overdose is a deliberate or inadvertent administration of a product at an amount higher than specified in the protocol.

Overdose is not likely to occur in this study.

Limited quantities of the study product(s) will be supplied, and closely monitored by the site for each subject.

Overdose per se is not an AE. However, any clinical sequelae of an overdose should be reported as an AE (and serious adverse event (SAE), if appropriate). For reporting, follow the AE and SAE reporting instructions.

6.3 Investigational/Study Product Storage

The investigator, or designee, will ensure that all study products/supplies are stored in a secured area with controlled access under required storage conditions and in accordance with applicable regulatory requirements and the product label.

Site systems must be capable of measuring and documenting (for example, via a log), at a minimum, daily minimum and maximum temperatures for all site storage locations (as applicable, including frozen, refrigerated and/or room-temperature products). This should be captured from the time of first product receipt throughout the study. Even for continuous monitoring systems, a log or site procedure that ensures active daily evaluation for excursions should be available. The operation of the temperature-monitoring device and storage unit (for example, a refrigerator), as applicable, should be regularly inspected to ensure it is maintained in working order.

Any excursions from the product-label storage conditions should be reported to appropriate site staff upon discovery and communicated to sponsor as soon as possible. The site should actively pursue options for returning the product to the storage conditions as described on the product label, as soon as possible. Excursions from the storage requirements, including any actions taken, must be documented as a protocol deviation and reported to the Sponsor.

Once an excursion is identified, affected product(s) must be quarantined and not used until the sponsor provides documentation of permission to use. Use of any of the affected product(s) prior to sponsor approval will be considered a protocol deviation.

Site staff will instruct subjects on the proper storage requirements for all take-home products.

6.4 Investigational/Study Product Accountability

All products supplied are for use only in this clinical study and should not be used for any other purpose.

All study products/supplies must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only authorized study staff have access. Upon receipt, all study products/supplies should be stored according to the instructions specified on the product labels. Study products/supplies are to be dispensed only to subjects enrolled in the study, in accordance with the protocol, by authorized site staff.

The study site must maintain adequate records documenting the receipt, use, loss or other disposition of all study products/supplies. Study products will be accounted for using the investigational/study product accountability form/record. The investigator is responsible for study product accountability, reconciliation and record maintenance.

The accountability records must be available for inspection by the study monitor during the study. Monitoring of study product accountability will be performed by the monitor during site visits and at the completion of the study.

Subjects will return used and unused lead-in and study products to the investigator site:

- **Visit 2:** lead-in toothbrush and regular fluoride toothpaste;
- **Visit 3:** assigned study toothbrush and regular fluoride toothpaste.

Study product return will be documented using the study product accountability form/record. Accountability records must be available for inspection by the study monitor during the study. Monitoring of product accountability will be performed by the monitor during site visits and on completion of the study.

6.4.1 Destruction of Investigational/Study Product Supplies

At the end of the study, the Principal Investigator (PI) or an appropriate designee, and a representative of GSK CH (study monitor) will inventory all used and unused study products/supplies. The study product accountability record for returned study products will then be completed. Return and disposal instructions for study products/supplies are outlined in Table 6-1 and Table 6-2. Detailed instructions for accountability checks, the return and destruction of study product/supplies will be provided by GSK CH during the study in time for the study close out visit.

6.5 Blinding and Allocation/Randomization

All subjects will be centrally randomized to one of the study treatments using an Interactive Response Technology (IRT). Before the study is initiated, training, login information and directions for use of the IRT will be provided to each site. Study products will be dispensed according to the instruction received through the IRT at the appropriate study visits.

Returned study products should not be re-dispensed to any subject.

This study is described as examiner-blind (the clinical examiner will be blinded to the study treatment received). The site staff, study statistician, data management staff, other employees of the sponsor and vendors acting on behalf of the sponsor, who may influence study outcomes will also be blinded to product allocation.

To ensure the examiner remains blinded throughout the study, staff involved in the dispensing and supervised usage of study products will work in a separate area. The examiner is not permitted in any area where study product is stored, dispensed, or in use.

Subjects will be instructed not to remove study products from the opaque bags provided outside of the dispensing room, while at the study site. Dispensing staff will not be involved in any efficacy/safety assessment procedures during the study.

6.6 Breaking the Blind

At the initiation of the study, the study site will be instructed on the method for breaking the blind. The method will be an electronic process.

The electronic system will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a subject's study product assignment is warranted. Subject safety must always be the first

consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a subject's study product assignment unless this could delay emergency treatment of the subject.

If a subject's study product assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and case report form, as applicable.

Any AE associated with breaking the blind must be recorded and reported as specified in this protocol. The study site may also be required be required to inform the EC if the blind is broken.

6.7 Compliance

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.

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 CRF. Subjects will be re-instructed in the correct usage requirements and diary completion as needed.

6.8 Concomitant Medication/Treatment(s)

Any medications, treatments or vaccine (including over-the-counter or prescription medicines, vitamins and/or herbal supplements) taken during the study, from signing the informed consent, must be recorded in the CRF 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.

Medication/treatments taken in the 30 days prior signing the ICF and before the first use of study treatment (i.e., assigned study toothbrush) will be documented as a prior medication/treatment. Medications/treatments taken after first use of study treatment will be documented as concomitant medication/treatments.

For the Duration of the Study: Screening (Visit 1) to Subject's Last Study Visit

- Subjects should not participate in any other clinical study (including cosmetic studies) or be in receipt of another investigational product.
- Non-emergency, elective dental treatment (including dental prophylaxis) should be delayed until after study completion.

- Should a randomized subject take a medication or start a course of treatment which, in the opinion of the investigator or medically qualified designee, could impact study outcomes, details of that medication/treatment will be recorded in the CRF. The investigator or medically qualified designee will decide if the subject should continue or be withdrawn from the study. Re-appointment can be considered.

  If the subject can be re-appointed, the reason for re-appointment will be documented in the CRF. If re-appointment within permitted visit tolerances (Table 1-1 Schedule of Activities) is not possible, the following visit specific actions should be taken.

  - **Visit 2:** the subject will be withdrawn from the study (Section 7.1). AEs will be recorded, and the OST examination completed; no clinical efficacy assessments will be performed. The subject may be replaced.
  - **Visit 3:** the subject will continue in the study. AEs will be recorded, and the OST examination completed; no clinical efficacy assessments will be performed. The subject will not be replaced.
  - **Visit 4:** the subject will be withdrawn from the study (Section 7.1). AEs will be recorded, and the OST examination completed; no clinical efficacy assessments will be performed. The subject will not be replaced.

7 DISCONTINUATION OF STUDY INTERVENTION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Subject Discontinuation/Withdrawal

A subject may withdraw from the study at any time at their own request or may be withdrawn at any time at the discretion of the investigator or sponsor for safety or behavioral reasons, or the inability of the subject to comply with the protocol-required schedule of study visits or procedures.

The following circumstances require discontinuation of study product and/or premature subject withdrawal.

- Protocol violation that may impact subject safety
- Withdrawal of informed consent
- Subject lost to follow-up
- Unblinding of the subject
- Pregnancy (self-reported)

If a subject is discontinued or prematurely withdraws from the study, the reason(s) for discontinuation or withdrawal and the associated date must be documented in the relevant section(s) of the CRF.

7.2 Lost to Follow up

If a subject fails to return to the site for a required study visit, the site must attempt to contact the subject and reschedule the missed visit within the permitted visit tolerances (Table 1-1 Schedule of Activities), counsel the subject on the importance of maintaining the assigned

visit schedule and ascertain whether the subject wishes to and/or should continue in the study.

A subject will be considered to have withdrawn from the study/lost to follow up if he or she repeatedly fails to return for scheduled visits and cannot be contacted by the study site. Before a subject is deemed lost to follow up, the investigator, or designee, must make every effort to regain contact (minimum 3 telephone calls and/or text messages and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). All contact attempts should be documented.

If contact is made with the subject, the investigator, or designee, should inquire about the reason(s) for non-attendance/withdrawal and ask the subject to return all used/unused study products/supplies to the study site. If appropriate, the subject will be asked to return for a final visit and follow-up regarding any unresolved AEs. Final safety assessments may be carried out when the subject returns to the study site, at the investigator's discretion, which could include the following: an oral examination.

If the subject withdraws from the study and withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

Lack of completion of all or any of the early termination procedures will not be viewed as protocol deviations so long as the subject's safety was preserved.

8 STUDY PROCEDURES

This section lists the procedures to be completed at each planned study visit. The timing of each procedure is listed in the Schedule of Activities section.

Adherence to the study design requirements, including all procedures, is essential and required for study conduct.

8.1 Visit 1/Screening

Screening procedures will be conducted by the Investigator, or suitably qualified designee.

8.1.1 Informed Consent

The investigator, or designee, must obtain informed consent from each subject participating in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study. Two copies of the ICF will be signed and dated by the subject, the subject will retain one copy and the other will be kept at site.

The investigator, or designee, must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time. Appropriate forms for documenting consent will be provided by either the investigator or GSK CH.

The investigator, or designee, should sign and date each copy of the ICF after the subject has signed to confirm that the consent process was completed correctly.

The time the subject signs the ICF will also be captured on the ICF as this is the point at which all Adverse Events will be captured from. The date and time of consent will be transcribed to the CRF.

If, during a subject's participation in the study, new information becomes available that may affect the subject's willingness to participate in the study, each ongoing subject should receive a copy of the new information and be re-consented into the study (2 copies as before). Each subject should be provided with a copy of the signed and dated amended consent form. The date of re-consent will be recorded on the CRF.

After signing the ICF, subjects will undergo the screening assessments to confirm that they meet all the inclusion criteria and none of the exclusion criteria. If the subject is confirmed eligible by the investigator (or designee) to participate in the study the subject is considered enrolled in the study.

8.1.2 Demographics

The following demographic information will be recorded in the CRF: year of birth, gender and race.

8.1.3 Medical History and Prior Medication/Treatment

Details of relevant medical and surgical history, including allergies or drug sensitivity, will be documented in the CRF.

Medications/treatments, including prescription and non-prescription drugs, dietary supplements and herbal remedies, taken in the last 30 days will be documented in the CRF.

8.1.4 Screening Procedures

The following procedures will be completed and the data recorded in the CRF.

- An OST examination will be completed as described in Section 9.3.1.
- An OHT examination will be completed as described in Section 9.3.2.
- Further oral examinations will be completed by the clinical examiner to determine subject eligibility, in accordance with Section 5.2 Inclusion Criteria and Section 5.3 Exclusion Criteria of the study protocol. These examinations will include a BPE and the identification of gradable teeth for RPI assessment.

8.1.5 Inclusion/Exclusion Criteria

Inclusion and exclusion criteria information will be documented in the CRF.

8.1.6 Subject Eligibility

The investigator and/or medically qualified designee will review the inclusion/exclusion criteria, medical history and prior medications to confirm subject eligibility to participate in the study. This will be documented in the CRF.

To prepare for study participation, subjects will be instructed in the Lifestyle Guidelines and any Concomitant Medication/Treatment(s) requirements of the protocol.

8.1.7 Dispensing/Supervised Use of Lead-In Toothbrush

Eligible subjects will be provided with lead-in toothbrush, regular fluoride toothpaste, diary and timer for use between Visits 1 and 2. Toothbrush usage instructions will be described to

the subject and site staff will supervise an on-site brushing with the lead-in toothbrush and completion of first use in the diary.

Spontaneous reporting of adverse events and those elicited by asking subjects to respond to a non-leading question such as "How do you feel?" will be assessed. Any AEs reported on completion of the supervised brushing will be recorded in the CRF.

Stall will remind subjects of the Lifestyle Guidelines and any Concomitant Medication/ Treatment(s) requirements of the protocol before they leave the study site.

Completion of all procedures will be documented in the CRF.

8.2 Study Period

Repeat assessments will be performed for both clinical measures (RPI/TPI) throughout the treatment period (Visits 2-4) to monitor consistency of scoring, as described in Section 9.2.4.

8.2.1 Visit 2/Day 0

Staff will complete visual checks of the returned lead-in toothbrush/toothpaste and review the completed diary. Any suspected over or under use and the number of any missed or additional brushings will be documented in the CRF. *Do not return lead-in toothbrush/toothpaste/diary to subject*.

Changes in health, medication and non-drug treatments/procedures will be documented in the CRF. Spontaneous reporting of adverse events and those elicited by asking subjects to respond to a non-leading question such as "How do you feel?" will be assessed and any AEs recorded in the CRF.

The following procedures will then be completed and the data recorded in the CRF.

Pre-Brushing Procedures:

- An OST examination as described in Section 9.3.1.
- Disclose dental plaque as described in Section 9.2.1.
- Complete pre-brushing RPI and TPI assessments as described in Sections 9.2.2. and 9.2.3 respectively (plaque may be redisclosed between the two assessment types, as described in Section 9.2.1, at the discretion of the clinical examiner).
- Review inclusion and exclusion criteria to confirm subject eligibility to continue in the study.
- Complete stratification and randomization; provide subject with their assigned study toothbrush, regular fluoride toothpaste and a new diary.
- Describe toothbrush usage instructions to the subject. Record first on-site use of study toothbrush in the diary. *Staff should retain study products for return to subject after their post-brushing plaque assessments have been completed.*

Post-Brushing Procedures:

- Post-brushing OST examination as described in Section 9.3.1.
- Disclose dental plaque as described in Section 9.2.1.

- Complete post-brushing RPI and TPI assessments as described in Sections 9.2.2. and 9.2.3 respectively (plaque may be redisclosed between the two assessment types, as described in Section 9.2.1, at the discretion of the clinical examiner).
- Spontaneous reporting of adverse events and those elicited by asking subjects to respond to a non-leading question such as "How do you feel?" will be assessed. Any AEs/incidents reported on completion of the on-site brushing with the assigned study toothbrush will be recorded in the CRF.
- Return study products to the subject; remind the subject of the Lifestyle Guidelines and any Concomitant Medication/Treatment(s) requirements of the protocol.

8.2.2 Visit 3/Day 7

Site staff not involved in any safety or efficacy assessments, or any other aspects of the study that could be influenced by the knowledge of the treatment a subject has been assigned to, will complete visual checks of the returned study toothbrush/toothpaste and review the completed diary. Any suspected over or under use and the number of any missed or additional brushings will be documented in the CRF. *Staff should retain the study toothbrush/toothpaste/ diary until return to the subject for their on-site brushing.*

Changes in health, medication and non-drug treatments/procedures will be documented in the CRF. Spontaneous reporting of adverse events and those elicited by asking subjects to respond to a non-leading question such as "How do you feel?" will be assessed and any AEs recorded in the CRF.

Confirm subject adherence to the requirements of the protocol and continuance.

The following procedures will then be completed and the data recorded in the CRF.

Pre-Brushing Procedures:

- An OST examination as described in Section 9.3.1.
- Disclose dental plaque as described in Section 9.2.1.
- Complete pre-brushing RPI and TPI assessments as described in Sections 9.2.2. and 9.2.3 respectively (plaque may be redisclosed between the two assessment types, as described in Section 9.2.1, at the discretion of the clinical examiner).
- Return study products to subject for on-site brushing with their study toothbrush. On-site use will be recorded in the diary. *Staff should retain study products for return to subject after their post-brushing plaque assessments have been completed.*

Post-Brushing Procedures:

- Post-brushing OST examination as described in Section 9.3.1.
- Disclose dental plaque as described in Section 9.2.1.
- Complete post-brushing RPI and TPI assessments as described in Sections 9.2.2. and 9.2.3 respectively (plaque may be redisclosed between the two assessment types, as described in Section 9.2.1, at the discretion of the clinical examiner).
- Spontaneous reporting of adverse events and those elicited by asking subjects to respond to a non-leading question such as "How do you feel?" will be assessed. Any

AEs/incidents reported on completion of the on-site brushing with the assigned study toothbrush will be recorded in the CRF.

- Return study products to the subject; remind the subject of the Lifestyle Guidelines and any Concomitant Medication/Treatment(s) requirements of the protocol.

8.2.3 Visit 4 /Day 28

Site staff not involved in any safety or efficacy assessments, or any other aspects of the study that could be influenced by the knowledge of the treatment a subject has been assigned to, will complete visual checks of the returned study toothbrush/toothpaste and review the completed diary. Any suspected over or under use and the number of any missed or additional brushings will be documented in the CRF. *Staff should retain the study toothbrush/toothpaste/ diary until return to the subject for their on-site brushing*.

Changes in health, medication and non-drug treatments/procedures will be documented in the CRF. Spontaneous reporting of adverse events and those elicited by asking subjects to respond to a non-leading question such as "How do you feel?" will be assessed and any AEs recorded in the CRF.

Confirm subject adherence to the requirements of the protocol and continuance.

The following procedures will then be completed and the data recorded in the CRF.

Pre-Brushing Procedures:

- An OST examination as described in Section 9.3.1.
- Disclose dental plaque as described in Section 9.2.1.
- Complete pre-brushing RPI and TPI assessments as described in Sections 9.2.2. and 9.2.3 respectively (plaque may be redisclosed between the two assessment types, as described in Section 9.2.1, at the discretion of the clinical examiner).
- Return study products to subject for on-site brushing with their study toothbrush. On-site use will be recorded in the diary. *Retrieve study products from subject.*

Post-Brushing Procedures:

- Post-brushing OST examination as described in Section 9.3.1.
- Post-brushing OHT examination as described in Section 9.3.2.
- Disclose dental plaque as described in Section 9.2.1.
- Complete post-brushing RPI and TPI assessments as described in Sections 9.2.2. and 9.2.3 respectively (plaque may be redisclosed between the two assessment types, as described in Section 9.2.1, at the discretion of the clinical examiner).
- Spontaneous reporting of adverse events and those elicited by asking subjects to respond to a non-leading question such as "How do you feel?" will be assessed. Any AEs/incidents reported on completion of the on-site brushing with the assigned study toothbrush will be recorded in the CRF.

Staff will remind subjects to inform the site if they experience any untoward medical occurrence in the 5 days following study completion (or early discontinuation).

8.3 Diary Review

Site staff not involved in any safety or efficacy assessments, or any other aspects of the study that could be influenced by the knowledge of the study treatment a subject has been assigned to, will review the diary at Visits 2-4 with the subject. Any subject comment captured in the diary which is considered an AE will be assessed and reported as per the defined procedure in this protocol. AE reporting procedures are summarized in Adverse Event and Serious Adverse Events.

Any subject comments relating to medications/treatments provided in the diary will be reviewed by the investigator or medically qualified designee with the subject and entered into the CRF as appropriate.

Additional and missed brushings with study product will be considered deviations from the protocol and will be recorded on the Deviations Log and entered into the CRF as appropriate.

8.4 Study Conclusion

The Study Conclusion page of the CRF will be completed for all subjects whether they complete all study procedures or if they are discontinued from the study early. If the subject is discontinued early, at any point during the study, the primary reason for withdrawal should be recorded on the Study Conclusion page.

If a subject has any clinically significant, study-related abnormalities or AEs at the end of the study, the GSK CH medical monitor (or designated representative) should be notified. The subject may be asked to remain at the clinical site or be asked to return for a follow-up visit to ensure any issues are resolved or deemed not clinically significant.

8.5 Follow-up Visits/Phone Calls

The study site may contact a subject to follow up an AE post-study completion/withdrawal and, in some circumstances, request they return to the site for additional follow-up visits (final safety assessments of the oral cavity). If needed, additional examinations may be carried out at such visits.

9 STUDY ASSESSMENTS

Every effort should be made to ensure that protocol-required procedures are completed as described. However, it is anticipated that from time to time there may be circumstances, outside the control of the investigator that may make it unfeasible to complete an assessment. In these cases, the investigator must take all steps necessary to ensure the safety and well-being of the subject. When a protocol-required assessment cannot be performed, the investigator (or designee) will document the reason for the missed assessment as a protocol deviation and any corrective and preventative actions that he or she has taken to ensure that required processes are adhered to as soon as possible. The Sponsor must be informed of any missed assessments in a timely manner.

To ensure the clinical examiner/staff involved in safety and efficacy assessments remain blind to treatment received, throughout the study: site staff involved in the dispensing of study treatment and the supervision of on-site study treatment usage will work in a separate area; the examiner will not be permitted in any area where study product is stored anddispensed, or when it is in use; study subjects will be instructed not to remove study product from the

opaque bags provided outside of the dispensing room, while at the study site; dispensing staff will not be involved in any safety/efficacy assessment procedure during the study.

All clinical examinations should be conducted using a standard dental light to illuminate the oral cavity.

9.1 Screening Assessments

Oral examinations to determine subject eligibility will be completed, in accordance with Section 5.2 Inclusion Criteria and Section 5.3 Exclusion Criteria of the study protocol, by appropriately trained clinical examiners at the times and in the order defined in Section 8 Study Procedures. These examinations will include a BPE and the identification of gradable teeth for RPI assessment.

9.2 Efficacy Assessments

Clinical efficacy assessments will be performed by a single appropriately trained clinical examiner, at the times and in the order defined in Section 8 Study Procedures section of this protocol. Dental plaque will be disclosed prior to the clinical efficacy and repeatability assessments by an appropriately trained clinician (dental hygienist, dental therapist, dentist).

9.2.1 Plaque Disclosure

Dental plaque is colorless and so is usually disclosed ('stained') prior to assessment. The disclosing solution will be used according to the manufacturer's instructions.

- At the request of the subject, the clinician may apply a thin layer of petroleum jelly to the subject's lips, as a barrier to help minimize staining by the disclosing solution. Care should be taken to ensure no petroleum jelly comes into contact with the labial surfaces of the anterior teeth as this could impact clinical assessments in this region.
- The subject will rinse their mouth with 10 mL tap water for 10 seconds to remove any food debris and expectorate.
- The clinician will use a cotton tipped applicator dipped in Trace Plaque Disclosing Solution (ex Young Dental) to paint the disclosing solution on to the teeth, thereby disclosing the plaque. Care will be taken not to dislodge the plaque during this process. The subject will then rinse with 10 mL tap water for 10 seconds and expectorate to remove excess solution.
- Plaque may be redisclosed between the NPI and the TPI assessments at the discretion of the clinical examiner.

9.2.2 Rustogi-modification of the Navy Plaque Index (RPI)

Supra-gingival plaque will be assessed on the facial and lingual surfaces of the teeth (7-7 in each arch) using the RPI (Rustogi et al. 1992). Third molars can be included in the assessment if, as a result tooth loss, they are functioning as second molars and gradable 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 (as described in Section 9.2.1) and the presence or absence of plaque in each zone recorded, generating a total of 18 scores per tooth.

Score	Description
0	Plaque absent
1	Plaque present

9.2.3 Turesky Modification of the Quigley Hein Index (TPI)

Supra-gingival plaque will be assessed on the facial and lingual surfaces of the teeth (7-7 in each arch) using the TPI (Lobene et al. 1982, Turesky et al. 1970). As with RPI, third molars can be included in the assessment if, as a result tooth loss, they are functioning as second molars and gradable 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 (as described in Section 9.2.1) and scored for each site as follows.

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

9.2.4 Repeatability Assessments

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 in previous studies and/or calibration exercises. 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 2-4) 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 (plaque will be disclosed as described in Section 9.2.1). 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.

9.3 Safety and Other Assessments

The following safety assessments will be performed by appropriately trained clinical examiners at the times, and in the order defined in Section 8 Study Procedures of this protocol.

9.3.1 Oral Soft Tissue (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 CRF 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.

9.3.2 Oral Hard Tissue (OHT) Examination

Where possible, this procedure 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

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9.3.3 Pregnancy Testing

For GSK CH studies in which no drug is utilized or studies of single-use marketed products that are classified as a non-medicinal product in the market where the testing is occurring and there is no pregnancy warning on labelling, a pregnancy test will not be required.

Subjects will be asked to provide verbal confirmation of negative pregnancy status; this will be documented as part of the exclusion criteria.

10 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

10.1 Definition of an Adverse Event (AE)

An AE is any untoward medical occurrence in a clinical study subject, temporally associated with the use of a study product including any washout or lead-in product (or medical device), whether or not considered related to the study product, including any washout or lead-in product (or medical device).

NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study product including any washout or lead-in product (or medical device).

Events Meeting the AE Definition:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g. electrocardiogram (ECG), radiological scans, vital sign measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study product administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study product or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
- "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE if they fulfill the definition of an AE.

Events NOT meeting the AE definition:

- Any clinically significant abnormal laboratory findings (if applicable) or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
- Medical or surgical procedure (e.g. endoscopy, appendectomy) is not the AE. The condition that leads to the procedure is an AE (e.g. appendicitis).
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.2 Definition of a Serious Adverse Event (SAE)

An SAE is a particular category of an adverse event where the adverse outcome is serious. If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g. hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A serious adverse event is any untoward medical occurrence at any dose that:

- **Results in death**
- **Is life-threatening**
  - The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe;
- **Requires inpatient hospitalization or prolongation of existing hospitalization**
  - In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred, or was necessary, the AE should be considered serious.
  - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- **Results in persistent or significant disability/incapacity**
  - The term disability means a substantial disruption of a person's ability to conduct normal life functions.
  - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption
- **Results in congenital anomaly/birth defect**
- **Other situations:**
  - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
  - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Note: Classification of an AE as 'serious' is based on the outcome of the event and is a factor in determining reporting requirements.

10.3 Reporting of Adverse Events

10.3.1 Reporting Period

All AEs, and therefore all SAEs, will be collected immediately after a subject consents to participate in the study by the completion (signature) of the ICF and until 5 days following last administration of the study product (or last procedure).

Medical occurrences that began before obtaining informed consent will be recorded in the Medical History/Current Medical Conditions section of the CRF not the AE section.

Details recorded by the subject on a diary or similar document that meet the definition of an AE must also be discussed with the subjects and transcribed in the AE section of the CRF.

10.4 Reporting Procedures

The investigator and any designees are responsible for detecting, documenting and reporting events that meet the definition of an AE and remain responsible for following up on AEs that are serious, considered related to the study product(s), participation in the study, or a study procedure, or that caused the subject to discontinue the study product or study.

The investigator (or medically qualified designee) is to report all directly observed AEs and all AEs spontaneously reported by the study subject. In addition, each study subject will be questioned about AEs.

Each AE is to be assessed to determine if it meets the criteria for a SAE. If an SAE occurs, expedited reporting will follow local and international regulations, as appropriate.

When an AE occurs, it is the responsibility of the investigator (or medically qualified designee) to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) related to the event.

The investigator or site staff will then record all relevant information regarding an AE in the CRF and all details relating to an SAE in the paper SAE Form provided.

It is **not** acceptable for the investigator (or medically qualified designee) to send photocopies of the subject's medical records to GSK CH in lieu of completion of the AE CRF page/SAE form.

There may be instances when copies of medical records for certain cases are requested by GSK CH. In this instance, all subject identifiers, except for the subject number, will be redacted on the copies of the medical records prior to submission to GSK CH.

The investigator (or medically qualified designee) will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. The diagnosis will be the documented as the AE/SAE where known and not the individual signs/symptoms. (e.g. upper respiratory tract infection, seasonal allergy, etc. instead of runny nose).

AEs elicited by the investigator (or medically qualified designee) in a standard manner at the study visits should also be recorded in the AE section of the CRF and/or using the SAE form (subject to the classification of the AE). Care will be taken not to introduce bias when questioning a subject about any changes in their health. Open-ended and non-leading verbal questioning should be used.

10.4.1 Reporting of an Adverse Event

All AEs will be reported on the AE page of the CRF by the investigator or site staff. It should be noted that the form for collection of SAE information is not the same as the AE CRF. Where the same data are collected, the AE CRF page and the SAE form must be completed in a consistent manner. For example, the same AE term should be used on both. AEs should be reported using concise medical terminology on the CRF as well as on the form for collection of SAE information.

10.4.2 Reporting of a Serious Adverse Event

In addition to recording the details of each AE on the AE CRF page, an SAE form should be completed, as fully as possible. Hard copies of the 'paper' SAE form will be provided in the investigator study master file. Original SAE forms will be retained in the investigator study master file.

It is essential to enter the following information:

- Protocol and subject identifiers
- Subject demography
- Description of events, with diagnosis if available
- Investigator opinion of relationship to study product (or study procedure, if appropriate)
- Criterion for seriousness.

The following are desirable and are of particular relevance for investigator and GSK CH assessment of the SAE report:

- Date of onset of AE
- Date AE stopped, if relevant
- Study product start date
- Study product end date if relevant
- Action taken in relation to the study product
- Outcome if known

The SAE form, completed as fully as possible, must be scanned and e-mailed to the GSK CH Clinical Operations Safety Reporting email box with the study number and subject number in the subject line of the email **immediately and under no circumstance should this exceed 24 hours** after study site personnel learn of the event. The investigator will submit any updated SAE data to the sponsor, **immediately and under no circumstance should this exceed 24 hours** of it being available. The GSK CH Study Manager should also be notified of the situation by telephone or email.

Email Serious Adverse Events to:

PPD

The GSK CH Study Manager or designee will be responsible for forwarding the SAE form to the Case Management Group, Global Clinical Safety and Pharmacovigilance mailbox (PPD).

The initial report will be followed up with more information as relevant, or as requested by the GSK CH study manager.

10.5 Evaluating Adverse Events

10.5.1 Assessment of Intensity

The investigator or medically qualified designee will make an assessment of intensity for each AE reported during the study and will assign it to one of the following categories:

- **Mild:** An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- **Moderate:** An event that is sufficiently discomforting to interfere with normal everyday activities
- **Severe:** An event that prevents normal everyday activities.

NOTE: An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both non-serious AEs and SAEs can be assessed as severe. For example, a headache may be severe (interferes significantly with the subject's usual function) but would not be classified as serious unless it met one of the criteria for SAEs, listed above. An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

10.5.2 Assessment of Causality

The causality assessment is one of the criteria used when determining regulatory reporting requirements. For each AE (serious and non-serious), the investigator (or medically qualified designee) **must** provide an assessment of causality on the AE CRF page and the SAE form (subject to the classification of the AE). The investigator will also document in the medical notes that he/she has reviewed the AE and assessed causality, where applicable.

A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. Generally, the facts (evidence) or arguments to suggest a causal relationship should be provided.

The investigator will use clinical judgment to determine the relationship and will also consult the Safety Statement and/or Product Information for marketed products, in the determination of his/her assessment. Alternative causes, such as underlying disease(s), concomitant therapy, other risk factors, and the temporal relationship of the event to the study product will be considered and investigated.

For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

The investigator's assessment of causality must be provided for all AEs (serious and non-serious); the investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the SAE reporting requirements if applicable.

There may be situations when an SAE has occurred, and the investigator has minimal information to include in the initial report to GSK. **However, it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK CH.** The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.

10.6 Follow-up of Adverse Events

After the initial report, the investigator is required to proactively follow up with each subject and provide further information on the subject's condition.

All AEs (serious and non-serious) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK CH to elucidate as fully as possible the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

New or updated information will be recorded on the AE CRF page and on the SAE form (subject to the classification of the AE).

The investigator will submit any updated SAE data to GSK CH within 24 hours of receipt of the information.

Investigators are not obliged to actively seek AEs in former subjects. However, if the investigator learns of a SAE, including death, at any time after a subject has been discharged from the study, and considers the event reasonably related to the study product or study participation, the investigator will promptly notify GSK CH by emailing the information to the GSK CH Clinical Operations Safety Reporting email box (PPD). The GSK CH Study Manager or designee will be responsible for forwarding the information to the Case Management Group, Global Clinical Safety and Pharmacovigilance group mailbox at GSK (PPD).

The investigator will submit any updated SAE data to GSK CH within the designated reporting time frames.

10.7 Withdrawal Due to an Adverse Event

Withdrawal due to AEs should be distinguished from withdrawal due to other causes, according to the definition of an AE noted earlier, and recorded on the appropriate AE CRF page.

When a subject withdraws because of an SAE, the SAE must be reported in accordance with the reporting requirements defined.

10.7.1 Sponsor's Reporting Requirements to Regulatory Authorities and Ethics Committees

GSK CH has a legal responsibility to notify, as appropriate, the local regulatory authority and other regulatory authorities about the safety of a product under clinical investigation. Prompt

notification of SAEs by the investigator to GSK CH is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met.

GSK CH will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, EC and investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE or other specific safety information from the sponsor will review and file it in the investigator study master file, and will notify the EC, if appropriate, according to local requirements.

10.8 Pregnancy

10.8.1 Time Period for Collecting Pregnancy Information

Pregnancy information will be collected on all pregnancies reported while a female subject is participating in the study from the signing of informed consent until 5 days after last administration of study product.

10.8.2 Action to be Taken if Pregnancy Occurs

The investigator will record pregnancy information on the appropriate form scan and e-mail it to the GSK CH Clinical Operations Safety Reporting email box (PPD) within 24 hours of learning of the subject becoming pregnant. The GSK CH Study Manager or designee will be responsible for forwarding the pregnancy form to the Case Management Group, Global Clinical Safety and Pharmacovigilance mailbox (PPD). Original pregnancy information forms will be retained in the investigator study master file.

The subject will be followed to determine the outcome of the pregnancy. Information on the status of the mother and infant / neonate (including concomitant medications taken by the mother during the pregnancy) will be forwarded by the investigator to the GSK CH Clinical Operations Safety Reporting email box and the GSK CH Study Manager or designee will forward this information to the Case Management Group, Global Clinical Safety and Pharmacovigilance group mailbox at GSK (PPD). Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE, abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are, and should be recorded as an SAE.

Any female subject who becomes pregnant while participating will discontinue study treatment and/or be withdrawn from the study.

11 DATA MANAGEMENT

As used in this protocol, the term CRF is understood to refer to either a paper form or an electronic data record or both, depending on the data collection method.

For this study, subject data will be entered into an electronic CRF (eCRF), using a validated system. Data relating to SAEs and pregnancy will also be collected on paper forms.

The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The source documents (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subject diaries, questionnaires, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files and records kept at the pharmacy, at the laboratory and at the medico-technical departments involved in the clinical study) which contain the source of data recorded in the CRF should be specified. The CRF and diary can be used as a source document at the discretion of data management.

Each subject will be assigned and identified by a unique Screening Subject Number. Any reference made to an individual subject within the study must be done using their unique Screening Subject Number.

11.1 Case Report Form

A CRF is a printed, optical, or electronic document designed to record the protocol required information to be reported to the sponsor on each trial subject.

For each subject who has given informed consent the CRF must be completed and signed by the PI (or authorized designee) to certify the data are complete and correct. The investigator must maintain accurate documentation (source data) to support the information entered in the CRF.

Management of clinical data will be performed in accordance with Third Party Biostatistics and Data Management (BDM) Vendor applicable standards and data cleaning procedures with oversight by GSK CH to ensure integrity of the data, for example, to remove errors and inconsistencies in the data.

To protect the privacy of subjects, no Personal Information (including the subject's name or initials or full birth date) is to be recorded in the CRF or as part of the query text.

All CRF pages should be completed during a subject assessment when the CRF has been designated as the source. Data that is sourced elsewhere should be entered into the CRF in an agreed upon timeframe between the Investigator and Sponsor.

GSK CH will obtain and retain all CRFs and associated study data as applicable at the completion of the study.

11.2 Data Handling

Documentation of all data management activities should allow step-by-step retrospective assessment of data quality and study performance.

Any changes or corrections to data will be performed in the Electronic Data Capture (EDC) System, and it will include rationale for changes. The EDC system has an audit trail, which will provide a complete record of the changes and corrections endorsed by the Investigator.

AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and any concomitant medication terms (if applicable) using an internal validated medication dictionary, GSKDrug.

11.2.1 Data Queries

Programmed edit checks will be generated automatically, as the data are being entered into the system. Reports and listings on the CRF data will also be run, in addition to the queries already programmed and generated by the system, to raise manual queries as needed for site clarification or correction. The Clinical Dictionary Development and Management Group will raise queries as needed on safety data to code the terms (AEs and Drugs or concomitant medication) appropriately.

The study monitor will perform ongoing review the of the CRFs in accordance with the monitoring plan, to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Any queries will be generated in the EDC System to the Investigator or designee, enabling the errors to be addressed in parallel with Data Management review. The study monitor can also run reports and listings on the CRFs, to raise manual queries as needed for site clarification or correction.

11.3 Processing Patient Reported Outcomes

Paper based patient reported outcome (PRO) data may be collected from a diary, questionnaire, or other specified document, etc. and entered into the data management system (DMS).

Electronic patient reported outcome (ePRO) data may be collected using electronic devices and transferred electronically to GSKCH or Third-party Data Management vendor.

All PRO source data should be reviewed by the study staff and the study monitor to ensure accurate transcription of data and that any potential AEs or concomitant medications reported on these documents are discussed with the subject and transcribed accurately to the CRF and/or DMS. PROs that are classed as source data will be retained by the investigator and true/certified copies may be sent to a designated vendor or GSK CH as required.

To protect the privacy of subjects, no PI (including the subject's name or initials or birth date) is to be recorded on any PRO/ePRO that will be forwarded to GSK CH or Third-Party Vendor.

12 STATISTICAL CONSIDERATIONS AND DATA ANALYSES

12.1 Sample Size Determination

No formal sample size has been produced. However, based on similar studies evaluating the plaque removal efficacy of manual toothbrushes (He et al. 2009, Nathoo et al. 2004), approximately 20 evaluable subjects per treatment 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. Approximately 80 subjects will be randomized to study treatment. No allowance will be made for dropouts; none is expected for the primary objective given the clinical assessments will be made shortly after randomization.

12.2 Statistical Methods and Analytical Plan

Additional details of the proposed statistical analysis will be documented in the statistical reporting and analysis plan (RAP), which will be written following finalization of the protocol and prior to study unblinding/analysis (as appropriate).

In this study, baseline is defined as the Day 0 pre-brushing mean plaque score for each index (RPI/TPI). Day 7 and Day 28 pre-brushing plaque levels will also be used to assess plaque removal after single brushing events at these timepoints, noting these values may be influenced by treatment used.

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.

12.3 Definition of Analysis Populations

The Safety population will comprise all randomized subjects who complete at least one use of study treatment. This population will be based on the treatment the subject actually received.

The mITT population will comprise all randomized subjects who have a post-baseline plaque assessment. This population will be based on the treatment to which the subject was randomized. Any subject who receives a randomization number will be considered to have been randomized.

A Per Protocol population will not be defined due to the exploratory nature of the study.

The Repeatability population will comprise all subjects who have a repeat clinical efficacy assessment at any visit.

12.3.1 Exclusion of Data from Analysis

Exclusion of any data from the analyses will be determined during a Blind Data Review (BDR) Meeting prior to database lock. Any reasons for exclusion from an analysis population will be listed, if applicable.

12.3.2 Demographic and Baseline Characteristics

Descriptive statistics (number of subjects, mean, SD, median, minimum and maximum for continuous variables, and frequency and percentage for categorical variables) will be provided for demographic and baseline characteristics. Demographic and baseline characteristics will be summarized by treatment group.

12.3.3 Study Drug/Product Compliance and Use of Other Therapies

12.3.3.1 Study Drug/Product Compliance

A summary of product use will be tabulated by treatment group and time across the duration of the study. Data will be listed.

12.3.3.2 Prior and Concomitant Medications

Prior medications, concomitant medications and non-drug therapies taken during the study will be listed for the safety population.

12.3.4 Primary Analysis

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 $TPI_{overall}$.

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.

Change from baseline in mean $RPI_{overall}$ and mean $TPI_{overall}$ will be analyzed using ANCOVA. For RPI, the model will include treatment as a fixed effect and the baseline $RPI_{overall}$ value as a covariate ($RPI_{gingival/interproximal}$ stratification level (lower/higher) will not be used in the analysis as the actual value will be used as a covariate). For TPI, $RPI_{gingival/interproximal}$ stratification level (lower/higher) will be included as an extra factor. Change from baseline will be summarized for each study treatment (adjusted mean change, 95% CIs and p value for reduction from zero). No formal sample size has been generated for this methodology development study, thus the p-values will be interpreted with caution. 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).

12.3.5 Secondary Analyses

Secondary outcome variables include change from baseline in:

- Mean $RPI_{overall}$ (Day 7, Day 28)
- Mean $TPI_{overall}$ (Day 7, Day 28)
- Mean $RPI_{gingival\ margin}$ (Day 0, Day 7, Day 28)
  Sites A, B and C will be used for calculations
- Mean $RPI_{inter\text{-}proximal}$ (Day 0, Day 7, Day 28)
  Sites D and F will be used for calculations
- Mean $TPI_{inter\text{-}proximal}$ (Day 0, Day 7, Day 28)
  Mesial and distal sites will be used for calculations

Secondary outcome variables will be derived, based on relevant tooth sites, as the primary outcome variables, with the following summaries generated and analyses performed.

Variable Calculation	Timepoint	Type of Analysis
Actual value	Day 0 (pre- and post-brushing)	Summary statistics

	Day 7 (pre- and post-brushing) Day 28 (pre- and post-brushing)	Plot of pre- and post-brushing values for Days 0, 7 and 30 Box plots
Change from baseline [Day 0 (pre-brushing)]	Day 7 (pre- and post-brushing) Day 28 (pre- and post-brushing)	Summary statistics Plot of change from baseline for Days 7 and 30 ANCOVA for within treatment comparisons

Summary statistics (number of subjects, mean, median, SE, SD, minimum, maximum) will be presented for each outcome variable at each assessment time point by treatment group.

For change from baseline calculations, baseline will be the mean Day 0, pre-brushing plaque score; percent change will also be presented and calculated as 100 x (Value - Baseline Value)/ Baseline Value. This percent change will be calculated from the aggregated mean levels not at the individual subject level.

Actual mean plaque scores (± SE) for both indices will be plotted over time by treatment group. Pre-brushing values will be set at the nominal time recorded (Days 0, 7 and 28); post-brushing values will be set at a small nominal time after (Days 0.5, 7.5 and 28.5). Box plots will also be presented for each outcome variable, over time by treatment group

For variables to be formally analyzed to test for a significant change from zero, change from baseline will be analyzed using an ANCOVA model, with treatment as a fixed effect and the appropriate baseline value as a covariate, as per the primary analysis. For TPI analyses, an extra factor will be included for the RPI $_{gingival/interproximal}$ stratification level (lower/higher). Adjusted mean changes, 95% CIs and p values for change from zero will be presented.

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).

12.3.6 Safety Analysis

All AEs will be coded using MedDRA. AEs will be categorized as oral and non-oral by the PI prior to database lock. The number of AEs and number of subjects with AEs will be listed and tabulated by treatment group. The results of OST and OHT exams will be tabulated.

12.3.7 Other Analyses

Not applicable.

12.3.8 Repeatability Analyses

The repeat dental plaque assessments 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. All subjects who have repeatability data will be included in this analysis.

12.3.9 Handling of Dropouts and Missing Data

Subjects who withdraw from the study early will be included in the study analysis up to the point of withdrawal. Subjects who withdraw will not be replaced. No data will be imputed in the case of dropouts or missing data.

12.3.10 Interim Analysis

No interim analysis is planned for this study

13 STUDY GOVERNANCE CONSIDERATIONS

13.1 Quality Control

In accordance with applicable regulations including ICH GCP, and GSK CH procedures, GSK CH or designee (i.e. third-party vendor) monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK CH requirements.

When reviewing data collection procedures, the discussion will include identification, agreement and documentation of data items for which the CRF will serve as the source document.

GSK CH or designee will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

The extent and nature of monitoring will be described in a written monitoring plan on file at GSK CH. The investigator (or designee) agrees to allow the monitor direct access to all relevant documents and agrees to co-operate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

13.2 Quality Assurance

To ensure compliance with ICH GCP and all applicable regulatory requirements, GSK CH may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study.

In the event of an assessment, audit or inspection, the investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any

findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

The investigator(s) will notify GSK CH or its agents immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with GSK CH or its agents to prepare the study site for the inspection and will allow GSK CH or its agent, whenever feasible, to be present during the inspection. The investigator will promptly apply copies of the inspection finding to GSK CH or its agent. Before response submission to the regulatory authority, the investigator will provide GSK CH or its agents with an opportunity to review and comment on responses to any such findings.

The sponsor will be available to help investigators prepare for an inspection.

13.3 Regulatory and Ethical Considerations

13.3.1 Ethics Committee (EC)

It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, informed consent documents, safety statement (including any updates) and other relevant documents (e.g. recruitment advertisements, if applicable) from the EC. All correspondence with the EC should be retained in the investigator file. Copies of EC approvals should be forwarded to GSK CH prior to the initiation of the study, and also when subsequent amendments to the protocol are made.

The only circumstance in which an amendment may be initiated prior to EC approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the investigator must notify the EC and GSK CH in writing immediately after the implementation.

13.3.2 Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), International Ethical Guidelines for Health-Related Research Involving Humans (Council for International Organizations of Medical Sciences 2016), guidelines for ICH GCP (ICH 2016), and the Declaration of Helsinki (World Medical Association 2013).

In addition, the study will be conducted in accordance with the protocol, ICH GCP guidelines, and applicable local regulatory requirements and laws.

13.3.3 Subject Information and Consent

All parties will ensure protection of subject personal data and will not include subject names or other identifiable data in any reports, publications, or other disclosures, except where required by laws.

When study data are compiled for transfer to GSK CH and other authorized parties, subject names, addresses, and other identifiable data will be replaced by numerical codes based on a numbering system provided by GSK CH in order to de-identify study subjects.

The study site will maintain a confidential list of subjects who participated in the study, linking each subject's numerical code to his or her actual identity. In case of data transfer,

GSK CH will maintain high standards of confidentiality and protection of subjects' personal data consistent with applicable privacy laws.

The informed consent documents must be in compliance with ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy laws.

The informed consent documents used during the informed consent process must be reviewed and approved by the sponsor, approved by the EC before use, and available for inspection.

The investigator must ensure that each study subject is fully informed about the nature and objectives of the study and possible risks associated with participation.

The investigator, or a person designated by the investigator, will obtain written informed consent from each subject before any study-specific activity is performed. The investigator will retain the original of each subject's signed informed consent document.

13.3.4 Subject Recruitment

Advertisements approved by the EC and investigator databases may be used as recruitment procedures. Use of EC approved, generic, pre-screening questionnaires to assess basic subject characteristics to determine general eligibility for this study is allowed. This generic questionnaire may be used by the study site as a phone script and/or to review internal databases to identify subjects.

GSK CH will have an opportunity to review and approve the content of any study recruitment materials directed to potential study subjects before such materials are used.

13.3.5 Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

Within GSK CH a serious breach is defined as a breach likely to affect to a significant degree the safety and rights of a subject or the reliability and robustness of the data generated in GSK CH-sponsored human subject research studies.

In the event of any prohibition or restriction imposed (i.e., clinical hold) by an applicable competent authority in any area of the world, or if the investigator is aware of any new information that might influence the evaluation of the benefits and risks of the investigational product, GSK CH should be informed immediately.

In addition, the investigator will inform GSK CH immediately of any urgent safety measures taken by the investigator to protect the study subjects against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the investigator becomes aware of.

13.4 Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins in accordance with applicable GSK CH processes.

GSK intends to make anonymized subject-level data from this trial available to external researchers for scientific analyses or to conduct further research that can help advance medical science or improve patient care. This helps ensure the data provided by trial participants are used to maximum effect in the creation of knowledge and understanding

13.5 Provision of Study Results to Investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the Clinical Study Report (CSR). The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK CH site or other mutually-agreeable location.

GSK CH will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK CH Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

13.6 Records Retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location.

The records (study/ site master file) must be maintained to allow easy and timely retrieval, when needed (e.g. for a GSK CH audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.

Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.

The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

The investigator must assure that the subject's anonymity will be maintained. On CRFs or other documents submitted to GSK CH, subjects should not be identified by their names or initials, but by an identification code. The investigator should keep a separate log of subjects' codes, names and addresses. Documents not for submission to GSK CH, e.g. subjects' written consent forms, should be maintained by the investigator in strict confidence.

Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final CSR or equivalent summary, unless local regulations or institutional policies require a longer retention period. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK CH standards/procedures, and/or institutional requirements.

No study document should be destroyed without a prior written agreement between GSK CH and the investigator. The investigator must notify GSK CH of any changes in the archival

arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

13.7 Conditions for Terminating the Study

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the EC, or study product safety problems, or at the discretion of GSK CH. In addition, GSK CH retains the right to discontinue development of GSK CH study products at any time. For multicenter studies (if applicable), this can occur at one or more or at all sites.

If a study is prematurely terminated, GSK CH will promptly notify the investigator. After notification, the investigator must promptly contact all participating subjects and should assure appropriate therapy/ follow-up for the subjects. As directed by GSK CH, all study materials must be collected and all CRFs completed to the greatest extent possible. Where required by the applicable regulatory requirements, GSK CH should inform the regulatory authority(ies) and the investigator should promptly inform the EC and provide the EC a detailed written explanation of the termination or suspension.

If the EC terminates or suspends its approval/favorable opinion of a trial, the investigator should promptly notify the GSK CH and provide GSK CH with a detailed written explanation of the termination or suspension.

Upon completion or premature discontinuation of the study, the GSK CH monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including ICH GCP, and GSK CH Standard Operating Procedures.

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15 APPENDICES

15.1 ABBREVIATIONS

The following is a list of abbreviations that may be used in the protocol.

Table 15-1 Abbreviations

Abbreviation	Term
ADA	American Dental Association
AE	Adverse Event
ANCOVA	Analysis of Covariance
BDR	Blinded Data Review
BPE	Basic Periodontal Examination
CI	Confidence Interval
CRF	Case Report Form
CSR	Clinical Study Report
DMS	Data Management System
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ePRO	Electronic Patient Reported Outcome
EudraCT	European Clinical Trials Database
GCS	Global Clinical Supplies
GSK CH	GlaxoSmithKline Consumer Healthcare
ICF	Informed Consent Form
ICH GCP	International Conference on Harmonisation Good Clinical Practice
IND	Investigational New Drug
IRT	Interactive Response Technology
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
mL	Milliliter
mm	Millimeter
N/A	Not Applicable
OHT	Oral Hard Tissue
OST	Oral Soft Tissue
PBT	Polybutylene Terephthalate
PI	Principal Investigator
ppm	Parts Per Million
PRO	Patient Reported Outcome
RAP	Statistical Reporting and Analysis Plan
RPI	Rustogi Modified Navy Plaque Index
SAE	Serious Adverse Event
SD	Standard Deviation
SE	Standard Error
SOP	Standard Operating Procedure
SRSD	Single Reference Study Document
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event

Abbreviation	Term
TPE	Thermoplastic Elastomer
TPI	6-site Turesky Modified Quigley and Hein Plaque Index
UK	United Kingdom
US	United States

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