The Procter & Gamble Company
Cincinnati, Ohio USA

Title Page

A CLINICAL STUDY TO MEASURE THE ANTI-EROSION PROPERTIES OF TWO DENTIFRICES

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List of Abbreviations and Definition of Terms

Abbreviation	Definition
AE(s)	Adverse Event(s)
CFR	Code of Federal Regulations
CRF(s)	Case Report Form(s)
GCP	Good Clinical Practices
OST	Oral Soft Tissue Examination
REC	Research Ethics Committee
SOP(s)	Standard Operating Procedure(s)
TMF	Trial Master File

1. Introduction

One of the effects of the growth of the food and drinks industry is the huge increase in the consumption of soft drinks, fruit juices and sport drinks. Many drink products are acidic in nature. Such drinks, if taken in excess, will promote erosion of the tooth surface, where erosion is defined as a loss of tooth substance by a chemical process not involving bacterial action.[1] The incidence of tooth erosion by drinks is becoming an ever-increasing problem[2] and has led to an increased scientific awareness within the dental community. Stannous fluoride has been shown to provide protection for the enamel against acid attack.[3, 4]

This trial will evaluate the protective effects of stannous ions on sections of human enamel that are repeatedly subjected to a citric acid drink challenge using a similar study design to previous erosion studies.[5-8] Loss of enamel will be evaluated and compared by the use of surfometry, also known as profilometry. A profilometer is a precision instrument that allows for accurate measurement of surface characteristics, in this case erosion depths of human enamel sections.

2. Study Objective(s)

To compare the enamel protection efficacy (loss of tooth enamel as measured by surfometry) of two dentifrices in a 10-day in situ erosion model.

3. Identity of Investigational Product(s)

- **Experimental Treatment** - 0.454% Stannous Fluoride (1100 ppm fluoride).The Procter & Gamble Company,US.

- **Colgate® Total** - Sodium Fluoride (1100 ppm fluoride). Colgate-Palmolive, CA.

Challenge Product:

- Orange Juice commercially obtained – appliances in the mouth to be exposed to 250ml x 4 times daily (25ml per minute for a total of 10 minutes per acid challenge).
  (Sainsbury's Supermarkets Ltd, London, UK) – or acidic equivalent

Non-Treatment Dentifrice and Toothbrush:

- Crest® Decay Protection Dentifrice: 0.32% Sodium fluoride (1450 ppm fluoride). The Procter & Gamble Company, UK
- Oral B 35 Manual Toothbrush. Oral B –The Procter & Gamble,UK.

Crest Decay Protection dentifrice and manual toothbrushes will be administered for brushing before and after subjects wear the dental appliance during the treatment phases. In addition, subjects will use these products in place of their normal oral care products during treatment periods – in the morning prior to their study visits and again in the evening, as well as on weekends and on days off.

4. Overall Study Design and Plan — Description

This will be a single center, double-blind, randomized, supervised-usage, two-treatment, four-period crossover study. A sufficient number of subjects will be recruited to enroll approximately 36 subjects in the study and to complete with at least 30 evaluable subjects. Subjects will present for 4 study periods and will be randomized to treatment sequences and receive one of two dentifrice products each period. Each study period will take place over a span of roughly 2 weeks and will be comprised of 10 treatment days which will be conducted on weekdays only (Monday to Friday).

On each treatment day, subjects will brush their teeth at home in their usual manner using the non-treatment toothpaste and manual toothbrush as supplied at the screening visit in the kit box provided. Subjects will then attend the Clinical Trials Unit where they will collect their upper palatal intra-oral appliance fitted with two enamel samples and place it in their mouth. Subjects will wear the appliance for approximately 6 hours total over the course of each study day. While wearing the appliance, subjects will swish twice a day with their assigned treatment toothpaste slurry under the supervision of clinic staff for 60 seconds. The erosive challenge will occur with the appliance in the mouth. The subjects will be required to sip 25mL of orange juice over a timed minute, swishing it around their mouth, then spitting out. This is repeated 10 times so that a total of 250mL of orange juice is exposed to the enamel samples over a 10 minute period. The erosive challenge will occur a total of four times on each treatment day.

The enamel samples will be measured for tissue loss using a calibrated contact surface profilometer. Measurements will be taken at baseline prior to the start of the study and at the end of treatment Day 10. Fresh enamel samples will be placed in the intra-oral appliance at the beginning of each treatment period.

Within two weeks of completing the last treatment period, subjects will attend a follow-up assessment, which will include a brief medical interview and oral exam that will be recorded on site source documents.

Table 1. Study Schedule by Procedure Type and Visit

PROCEDURES	PRE-STUDY SCREENING	PERIODS 1, 2, 3, & 4			POST-STUDY FOLLOW-UP VISIT
		DAY 1	DAYS 2-9	DAY 10	
Informed Consent	X				
Medical History Review	X				
Current/Concomitant Medications	X	X	X	X	
Demographics	X				
Inclusion/Exclusion Criteria	X				
Continuance Criteria		X	**	**	
Oral Status Interview	X				
Oral Soft Tissue Exam	X				
Kit Box Distribution	X				
Treatment		X	X	X	
Erosive Challenge		X	X	X	
Surfometry Measurements				X	
General Comments	X	X	X	X	
AEs		X	X	X	
Subject Accountability				X*	
Follow-up Interview & Exam					X

*At the end of Period 4 only, or as the subject's participation in the study ends.
**Continuance Criteria responses should be recorded on site source documents on Days 2-10 of each period.

Preparation of Enamel Samples
Prior to the beginning of the study, enamel samples will be prepared and a baseline surfometry measurement will be obtained for each sample. For further details of enamel preparation, refer to Appendix I of this protocol.

Prior and Concomitant Therapy
At the beginning of the study, subjects will be instructed to refrain from using any prescription or non-prescription oral care products which are not assigned test articles throughout the duration of the study. Subjects will also be instructed to refrain from receiving an oral prophylaxis or any other elective dental procedures throughout the duration of the study. Emergency dental treatment will be allowed, but continued participation will be at the discretion of the Principal Investigator.

Solicitation of concomitant medications/therapy are not required as part of this protocol with the exception of those that may impact the inclusion/exclusion/continuance criteria; however, the site may collect and retain concomitant medications/therapy information as part of their source documentation.

Detailed Study Plan

Recruiting

Healthy male and female subjects, 18 years of age or older, who potentially meet the entrance criteria will be recruited from Bristol University and the Bristol Dental School and Hospital. A sufficient number of subjects will be recruited to enroll approximately 36 subjects in the study and to complete with at least 30 evaluable subjects.

Screening

Potential subjects will attend a screening visit approximately one month before the start of the study. Prior to receiving any study specific procedures, subjects will be asked to read a participant information sheet and sign an informed consent form, and they will also receive a signed copy of the consent form. Subjects will also be shown a product and ingredient list prior to any product use. Personal medical history and concomitant medication information will be obtained, reviewed, and retained as site source documentation. Demographic information and study entrance criteria will be obtained and documented on the appropriate Case Report Form (CRF). An Oral Status Interview and an Oral Soft Tissue (OST) examination will be conducted and documented on the appropriate CRF. Those subjects who do not have an upper intra-oral appliance will have an impression taken of their upper teeth and palate and an appliance constructed.

Subjects meeting all study entrance criteria will be enrolled in the study and scheduled to return for treatment. In addition, subjects will be issued a kit box containing a manual toothbrush, Crest Decay Protection toothpaste, and written product usage instructions. The Subject Instruction sheet will be verbally reviewed with the subjects. Subjects will be instructed to use these products twice daily, 1) during treatment periods – in the morning prior to their study visits and again in the evening, and 2) on weekends and on days off – both morning and evening. Subjects will be instructed to use these products in place of their normal oral care products.

Treatment Periods (1, 2, 3, and 4)

Each study period will consist of 10 treatment days. In general, there will be 5 consecutive treatment days per week (excluding weekends). However, this is not a requirement, as accommodations may be made to account for holidays/annual leave and to give subjects some limited scheduling flexibility. Therefore, each treatment period will end and a new study period may not commence until a subject has completed 10 days of treatment with that period's designated study product.

At each treatment day the following will occur:

Table 2: Study Day Schedule

TIMEPOINT (± 30 MINS)	FITTING OF APPLIANCE	TREATMENT	EROSIVE CHALLENGE	REMOVAL OF APPLIANCE	FOOD INTAKE
Baseline (8.30 +/- 30 Mins)	X	X	X		
2 hour (10.30 +/- 30 Mins)			X		
3 hour (11.30 +/- 30 Mins)		X	X		
Lunch, (12.00 – 14.00)* 4-5 hours post baseline				X	X
6 hours (14.30 +/- 30 Mins)			X		
7 hours (15.30 +/- 30 Mins)				X	

*****Subjects are permitted up to a 1 hour lunch break during this time window during which time the appliance can be removed from the mouth and stored in a moist pot.

Day 1

- Intra-oral appliances (containing the enamel samples) will be dipped in Corsodyl® mouthrinse (0.2% w/v chlorhexidine gluconate) for 3 minutes.
- Current/Concomitant medications/therapy will be reviewed and recorded in source documentation.

- Continuance criteria will be assessed and documented on the appropriate CRF.
- Subjects will be randomized according to the randomization schedule
- Subjects will be fitted with the upper intra-oral appliance containing the enamel samples.
- While wearing the appliance, subjects will rinse with a freshly prepared slurry of their assigned dentifrice and water for 60 seconds.
- Subjects will swish with a total of 250 mL of orange juice as instructed for the erosive challenge.
    - Subjects will swish for a total of 10 minutes, using a total of 250 mL of orange juice – subjects will be asked to orally rinse with 25 mL of the orange juice for 1 minute, using a stop clock to keep track of time. After 1 minute is up, subjects will expectorate the juice. This will be repeated until the subject has swished for a total of 10 minutes and ten consecutive times with a total of 250 mL of orange juice.
- Subjects will continue wearing their appliance and will refrain from eating or drinking anything with the appliance in, with the exception of small sips of water.

Days 2 to 10

- Intra-oral appliances (containing the enamel samples) will be dipped in Corsodyl® mouthrinse (0.2% w/v chlorhexidine gluconate) for 3 minutes.
- Concomitant medications/therapy will be reviewed and any changes will be recorded in source documentation.
- Continuance criteria will be assessed and documented on site source documents.
- Subjects will fit their intra-oral appliance into their mouth.
- While wearing the appliance, subjects will rinse with a freshly prepared slurry of their assigned dentifrice and water for 60 seconds.
- Subjects will swish with a total of 250 mL of orange juice as instructed for the erosive challenge.
    - Subjects will swish for a total of 10 minutes, using a total of 250 mL of orange juice – subjects will be asked to orally rinse with 25 mL of the orange juice for 1 minute, using a stop clock to keep track of time. After 1 minute is up, subjects will expectorate the juice. This will be repeated until the subject has swished for a total of 10 minutes and ten consecutive times with a total of 250 mL of orange juice.
- Subjects will continue wearing their appliance and will refrain from eating or drinking anything with the appliance in, with the exception of small sips of water.

2 Hours Post- Baseline

All Days

- Subjects will return to the clinic and will swish with a total of 250 mL of orange juice for the erosive challenge, as previously described.
- Subjects will continue wearing their appliance and will refrain from eating or drinking anything, with the exception of small sips of water.

3 Hours Post-Baseline

All Days

- Subjects will return to the clinic and will rinse with a freshly prepared slurry of their assigned dentifrice & water for 60 seconds.
- Subjects will swish with a total of 250 mL of orange juice for the erosive challenge, as previously described.
- Subjects will continue wearing their appliance and will refrain from eating or drinking anything, with the exception of small sips of water, until their scheduled lunch hour.

4-5 Hours Post-Baseline – Lunch Period

All Days

- Subjects will remove their appliance for up to a one hour lunch period and place it in a moist pot to ensure the enamel samples do not dry out. Subjects will return the intra-oral appliance to their mouth after lunch.

6 Hours Post-Baseline

All Days

- Subjects will return to the clinic and will swish with a total of 250 mL of orange juice for the erosive challenge, as previously described.
- Subjects will continue wearing their appliance and will refrain from eating or drinking anything, with the exception of small sips of water.

7 Hours Post-Baseline (see day specific procedures)

Days 1 to 9

- Subjects will return to the clinic and will have their appliance removed and dipped in Corsodyl® mouthrinse (0.2% w/v chlorhexidine gluconate) for 3 minutes.
- Subjects will be reminded of their study obligations and asked to return as scheduled for their next study visit.
- The intra-oral appliance will be placed in a moist pot for overnight storage.

Day 10

- Subjects will return to the clinic and will have their appliance removed and disinfected by being dipped in of 0.5% chlorhexidine and 70% aqueous ethanol for at least 20 minutes.
- New enamel samples will be allocated to the volunteer and placed in the appliance, the appliance containing the samples will then be disinfected by being dipped in of 0.5% chlorhexidine and 70% aqueous ethanol for at least 20 minutes.
- Subjects will be reminded of their study obligations and given verbal instructions of regarding when to next attend the study site.
- Subject Accountability data will be entered into the appropriate CRF (at the end of Period 4 only, or as the subject's participation in the study ends).

Notes

- Treatment periods 2, 3, and 4 will follow the same procedures as described above with the exception that after the completion of period 4, no new samples will be allocated to the participant.
- Fresh enamel samples will be placed in the intra-oral appliance at the beginning of each study period.

Profilometry

- Recordings of each subject-specific enamel sample removed after day 10 will be taken by contact profilometry.
- Following profilometry measurement, enamel samples will be stored in distilled water in eppendorfs.

5. Determination of Sample Size

Approxiamtely 36 subjects will be recruited for this study. There should be at least 80% power to detect a difference between the treatment dentifrices in 2-sided testing at the 5% significance level. This calculation is based on previous research in which a natural logarithm transformation was applied to the data prior to data analysis. This estimate assumes the effect size (mean treatment difference divided by the error standard deviation) is approximately 0.70 or higher in a 2-treatment, 4- period crossover design.

6. Blinding, Labeling, and Shipping Plan

Clinical Site Supplied Product:

Commercially marketed orange juice for this study will be procured by the Investigator.

Sponsor Supplied Product:

Crest Decay Protection toothpaste and a manual toothbrush will be supplied in kit boxes. The shipping containers will be labeled with the "ship to" clinical site address and a "content statement" listing study number and kit box

numbers contained within. Supplemental product will be labeled and issued as described above. In addition, the clinical site will be provided with all treatment dentifrice products, dose cups, and digital timers in bulk.

The kit boxes and dentifrice tubes will be over-tubed (or over-labeled as appropriate) and labeled with the study number, applicable caution and warning statements, usage directions, and other information as dictated by internal regulatory requirements and clinical standard operating procedures (SOPs). Subjects will be instructed not to discuss physical qualities of their assigned test products with other study subjects or site personnel. In addition, the Investigator and the person performing the surfometry measurements should not be present in the dispensing room as subjects are being administered product slurries.

If the study blind needs to be broken, a subject's investigational product may be ascertained by opening the code breaker report (using the appropriate procedure for breaking the blind). The code breaker report will be opened only in the event of a clinically serious AE and if subsequent management of the subject's care requires knowledge of the identity of the investigational product. The date, time, and reason for breaking the code will be recorded in writing.

7. Inclusion Criteria

In order to be included in the study, each subject must:

1) Provide written informed consent to participate in the study, and receive a copy of the signed consent form;
2) Agree not to participate in any other oral/dental product studies during the course of the study;
3) Agree to delay any elective dentistry (including dental prophylaxis) until the study has been completed;
4) Agree to refrain from the use of any non-study dentifrice or other oral hygiene products for the duration of the study;
5) Agree to return for all scheduled visits and follow study procedures;
6) Be at least 18 years of age;
7) Agree to refrain from taking an acidic medication (pH <5.3) during the course of the study; and
8) Be in good general health, as determined by the Investigator/designee based on a review of the health history/update for participation in the study.

8. Exclusion Criteria

Subjects may be excluded from study participation if they:

1) Have a susceptibility to acid regurgitation;
2) Have recurrent or regular aphthous ulcers;
3) Have dental erosion or a previous history of being susceptible to high dental erosion after drinking sports drinks or juices;
4) Have excessive gingival inflammation;
5) Have severe periodontal disease, as characterized by purulent exudate, generalized mobility, and/or severe recession;
6) Have any pre-existing oral or medical condition that the examiner determines may place the subject at increased health risk from study participation;
7) Have unremovable mouth or tongue jewelery;
8) Any subject who in the opinion of the investigator (or medically qualified designee) should not participate in the study; or
9) Are personnel* – An employee of the Sponsor, member of the study site or family relative.

*The site for the protocol is the Clinical Trials Unit at the Bristol Dental School and Hospital. Employees of the Bristol Dental School and Hospital or Bristol University not associated with the Clinical Trials Unit are eligible to participate**.**

9. Continuance Criteria

Subjects may be excluded from the entire study, a study period, or the analysis if they:

1) Have used any oral care product other than the assigned study products;
2) Have participated in any other oral/dental product studies since the last visit;

3) Have taken an acidic medication (pH <5.3) since the last visit;
4) Have received any elective dentistry (including dental prophylaxis) since the last visit – emergency dental treatment will be permitted, but in this case, continued participation will be at the discretion of the Principal Investigator;
5) Have been unable or unwilling to comply with product usage instructions for any reason.

Subjects will not necessarily be exited from the study for deviations; however, deviations may impact evaluability therefore, continued participation will be at the discretion of the Investigator, depending on the extent and frequency of the deviations. Should any deviations take place, they must be recorded on the General Comments page within the CRF.

10. Treatment Compliance and Dosing Instructions

Treatment Group	Experimental Dentifrice / Control
Amount	3 g
Dose Form	Slurry
Treatment Duration	1 minute
Frequency Per Day	Twice
Timing	Baseline and 3 hours Post
No. of Days	10

- Subjects will be asked to rinse with their assigned dentifrice slurry orally twice daily for 1 minute prior to erosive challenge with orange juice.
- A site staff-member will prepare the dentifrice slurry for the subject by mixing 3 g of dentifrice with 10 mL of water just prior to product usage.
- Slurry usage will be timed using a digital timer and will be supervised by a site staff-member. Dosing details will be recorded on source documents.
- No modifications to the above-specified dosing schedule are permitted.

11. Efficacy and Safety Variables

Efficacy Assessment

Surface (Contact) Profilometry Measurement

A stainless steel jig constructed to the exact dimensions of the prepared dentine samples will be used to hold the samples in place during the contact profilometry measurement.

The profilometer will be operated in a vibration free environment with the head shielded from draughts and severe temperature changes. The measuring head is fitted with a diamond stylus to follow the surface of the enamel under test. The head transverses the specimen at a constant speed of 10 mm/minute. The signals from the measuring head are processed on an electrical control unit and are displayed on the monitor screen.

The contact profilometer will be calibrated prior to each measuring period using a Precision Reference Specimen which has been milled to accurate, specific dimensions. The result will be recorded and dated. If the reference substrate does not match the required value, recalibration will be necessary and the process repeated.
For each enamel sample, two baseline readings will be taken, saved in the profilometry reading data files and forwarded to the Sponsor prior to the start of the study. The baseline readings are taken across an area which will be exposed to the study treatment. This treatment area will be demarcated on the sample with indelible ink on the surrounding epoxy resin.

An area of the enamel is then delineated by placing PVC tape over the enamel surface on either side of the demarcated area leaving approximately a 2-3 mm zone of enamel exposed. This exposed area is the area of the enamel sample that will be treated with the study treatment whilst the samples are in the upper palatal appliance.

Day 10 contact profilometry readings from this exposed area will be carried out after the PVC tape has been removed. If after the second reading it becomes evident that there has been, in the Investigator's opinion, a significant positioning error resulting in a large difference between readings, then a further contact profilometry reading will be taken. All readings will be kept as source data but only two readings per sample will be transferred to the Sponsor (along with the Baseline readings) in the profilometry reading data files.

Safety Observations and/or Measurements

Safety will be assessed by the absence of irreversible side effects associated with use of the test product. Safety evaluations will include assessment of the oral soft and hard tissues, and dentinal hypersensitivity. Non-serious whole-body AEs will not be collected.

Oral Soft Tissue Examination

Assessment of the oral soft tissues will be conducted via a visual examination of the oral cavity and perioral area utilizing a standard dental light, dental mirror, and gauze. The structures examined include the gingiva (free and attached), hard and soft palate, oropharynx/uvula, buccal mucosa, tongue, floor of the mouth, labial mucosa, mucobuccal/mucolabial folds, lips, and perioral area. All abnormal findings noted after product assignment which were not documented at Screening, or were present at Screening but have worsened during product usage, and which have the potential to be product-related will be recorded on the AE CRF.

Oral Status Interview

Subjects will be interviewed prior to the oral exam with regards to the presence or absence of oral discomfort. This interview should be conducted privately and should not take place in the presence of the Oral Examiner.

12. Statistical and Analytical Plans

Statistical Efficacy Analyses

The primary measure of efficacy in this study will be dental erosion, measured by profilometry at Day 10. For each subject, treatment period, and visit, the average of four erosion measurements will be calculated using two replicate measurements from each of two enamel sections. Since the Day 10 enamel loss distribution is typically right-skewed, the data will be transformed using the natural log function to make the distribution bell-shaped before performing between-treatment analysis that will assume normality. A general linear mixed model will be used to compare treatments with a statistical model that includes period and treatment as fixed effects and subject as a random effect. Other factors such as baseline as a covariate or carryover effect may be investigated although typically these factors are not statistically significant based on past research, and baseline levels are typically near zero. From the statistical model, estimated means on the natural log scale will be back-transformed by using the exponential function (e^{mean}) to obtain the estimated medians or 50^{th} percentiles on the original scale (µm), along with the associated standard errors and/or 95% confidence intervals (CI). Statistical comparisons will be two-sided at a 5% significance level.

Statistical Safety Analyses

All adverse events will be summarized.

13. Method of Assigning Subjects to Treatment

Subjects will be randomly assigned to one of four treatment sequences: AABB, BBAA, ABBA, and BAAB where the letters correspond to the 2 treatments.

14. Research Hypotheses

Hypothesis Testing

The following hypothesis will be tested at Day 10:

Null: The mean dental erosion is equal between the treatment dentifrices.

Alternative: The mean dental erosion is not equal between the treatment dentifrices.

Appendix I:

PREPARATION AND STORAGE OF THE INTRA-ORAL APPLIANCE

Preparation of the appliance
For subjects who do not already have an upper palatal appliance, an upper alginate impression will be recorded in a perforated stock tray. The impressions will then be poured in dental stone within 30 minutes and an upper-oral appliance will be constructed from self curing acrylic. Wire clasps are constructed to fit suitable posterior teeth to aid retention and wire cribs constructed in the anterior and posterior palatal regions to hold the enamel samples in place.

Preparation of the enamel samples
The enamel samples will be prepared at the study site according to the appropriate SOP held by the research laboratories within the Clinical Trials Unit, Bristol Dental School and Hospital. Caries free human third molars that have been recently extracted and donated by patients aged 18 years and over, of either gender will be used for the enamel samples. Prior to donation, each patient will sign an ethically approved informed consent form, allowing their teeth to be used for research purposes within Bristol Dental School and Hospital. To comply with UK law, human molars will be sourced through appropriately licensed Tissue Banks and will be tracked and disposed of in compliance with Human Tissue Legislation. Teeth for this study will be issued from the ethically approved Bristol Dental School & Hospital Tooth Tissue Bank, REC Ref: 11/NI/0145.

Upon donation to the Tissue Bank, the teeth are soaked for 24 hours in a solution containing 20,000 ppm available chlorine for at least 24 hours. The teeth are then scraped clean of any remaining tissue with a scalpel and the root sectioned from the crown to enable dental pulp removal and disposal, then soaked for a subsequent 24 hours in a solution containing 20,000 ppm available chlorine. The teeth are then washed in distilled water and stored in the tooth tissue bank in a solution containing 5,000 ppm available chlorine until use. They will be issued from the Tooth Tissue Bank after the study has received a favourable opinion from the Research Ethics committee.

Tooth crowns obtained from the tissue bank under approval are sectioned into 1 mm slices using a microslice to produce the enamel samples. The enamel slices are then cut into samples using a high-speed hand piece fitted with a diamond bur. Each enamel sample is then placed with the test surface facing down in a polyurethane mould 6 mm x 8 mm x 2 mm (width, length and depth respectively) and filled with epoxy resin. After 24 hours, when the epoxy resin has cured, the sample will be removed.

When set, the back of the sample is flattened using a stainless steel jig on a lapping and polishing unit fitted with p600 silicon carbide paper. The surface that will be exposed for treatment then has any resin flash removed on the lapping and polishing machine with p1200 grit silicon carbide paper.

The samples are then polished by hand using a slurry of 1200 grit silica powder on a glass slab. The hand polishing is carried out using small rotational figure of eight movements until the sample is deemed smooth by eye. The sample is then ultrasonicated in deionised water to remove any powder debris. The final stage of polishing is carried out by hand using a glass slab and a slurry of 0.3µm alpha alumina powder on a felt cloth. The samples are polished again using figure of eight movements until the sample surface is shiny and smooth. The samples are finally ultrasonicated in deionised water to remove any powder debris.

Two baseline readings of each enamel sample will be taken using a contact profilometer.

Samples will be masked with PVC tape on either side of a 2-3 mm wide window of enamel.

Each enamel sample will be identified with a unique number on the reverse of the enamel sample using a permanent marker.

DISINFECTION OF THE APPLIANCES AND ENAMEL SAMPLES

Start and end of day

The palatal appliances (containing the enamel samples) will be dipped in Corsodyl® mouthrinse (0.2% w/v chlorhexidine gluconate) twice daily for approximately 3 minutes and briefly rinsed in tap water, at the start of the treatment day and upon removal from the mouth at the end of each treatment day.

Prior to taking enamel sample profilometry reading and analysis

Prior to making profilometry measurements, the appliance will be disinfected by soaking in a mixture of 0.5% chlorhexidine and 70% aqueous ethanol for a period of at least 20 minutes, and then the samples removed.

Storage of the enamel sample and intra-oral appliance

The upper palatal appliances (containing the enamel samples) will be removed for 1 hour over lunch and also overnight until the next day. When removed at these times, the appliances will be stored in a 'moist pot' (a pot containing a damp cotton wool pad, moistened with water).

Appendix II:

Note: This language is standard for U.S. studies, and this section may be subject to change per regional regulatory requirements.

Adverse Event (AE) Reporting

A *serious event* is defined as an event, which suggests a definite hazard or handicap to the subjects. Serious events are any events resulting in death, life threatening situations, permanent disability, hospitalization or prolonged hospitalization, or congenital anomaly.

When an Investigator is notified of a serious AE, the Investigator must promptly (within 24 hours) notify Procter & Gamble (P&G, the Clinical Trial Manager or the Medical Monitor) of the serious or unexpected event, regardless of causality. Additionally, the REC (Research Ethics Committee) must be notified of the serious or unexpected event at the time they are reported. Within 5 working days, a written report describing the circumstances of the event must be submitted to P&G. Any AEs (serious or non-serious) continuing at study end must be followed up to resolution unless documented as "not clinically significant" or the subject is lost to follow-up by the Investigator.

Advertising

Any advertisements used in recruitment of subjects must receive prior approval from P&G and the Investigator's REC. A copy of the REC-approved advertising and the documentation thereof must be provided to P&G.

Data Collection

Case Report Forms

The Data Manager will supply the paper and/or electronic CRFs to be used in this study. It is the responsibility of the Investigator to maintain and submit accurate and timely CRFs to the Sponsor. All hard copy CRFs will be filled out legibly in ink.

All questions should be answered. For paper CRFs, if an entry requires correction, a single line will be placed through the entry so as not to obscure the original record, the corrected entry will be initialed and dated by the individual making the change, and a reason will be given for the change. There will be no whiteouts or erasures. For electronic CRFs, if an entry requires correction, the change is made directly to the CRF in the database, the user is prompted to provide a reason for the change, and the correction is logged in by an electronic audit trail.

As necessary, the Data Manager may make specified allowable changes to the database without issuing a query to the site, as agreed upon by study site per this protocol. Examples of allowable changes include incorrect date formats, incorrect current year recorded (as in the start of a new year), and unambiguous spelling errors. Changes to common abbreviations and symbols to equivalent text to meet system or coding constraints (e.g., @ = at, ~ = approximately), may also be allowable. Values that are ambiguous or open to interpretation will be queried to the sites. It is the responsibility of the Data Manager to ensure all changes are supported by information contained elsewhere and/or are unambiguous.

Source Documents

The Investigator has the responsibility for ensuring that all source documents (i.e., study and/or medical records) and CRFs are completed and maintained according to the study protocol and are available at the site. Any CRF used as a source document must be identified as such in the Investigator Notebook.

Protocol Amendments/Changes

Changes to the Protocol following REC approval affecting the safety of subjects, scope/objectives of the investigation, or the scientific quality of the study are documented as amendments. Such changes require P&G, Investigator, and REC approval prior to implementation, unless immediate action is required to safeguard subject safety. Administrative/minor changes (e.g., typos, changes in P&G personnel [excluding medical monitor], etc.)

are documented as revisions but do not have to be submitted as amendments unless required by the REC. Any change in P&G's monitoring staff, Clinical Trial Manager or Medical Monitor during the conduct of the study, must be reported to the Investigator.

Good Clinical Practices

The Principal Investigator will ensure that this study is conducted in full conformance with the laws and regulations of the country in which the research is conducted and the Declaration of Helsinki.

During the course of the trial, the clinical site is monitored by P&G staff (Clinical Trial Manager or designee) to ensure compliance with the Protocol, regulations and guidelines, adequacy of the equipment and facilities, and satisfactory data collection.

It is the understanding of P&G that this protocol (and any modifications) as well as appropriate consent procedures, will be reviewed and approved by an REC. This body must operate in accordance with the current local requirements. A letter or certificate of approval will be sent by the Investigator to the Sponsor prior to initiation of the study, and also when subsequent modifications to the protocol are made.

Obligations of the Investigator

Following completion of the study, the Investigator shall submit a final report within 30 days to P&G describing the conduct of the study, deviations from planned conduct, early withdrawals and subject accountability, adverse events, and other information on study conduct necessary for full interpretation of collected data.

Study Medication Dispensing, Storage and Accounting

Study products are stored in a secure area, under environmental condition as required by label instructions or as described in the Protocol, and dispensed only under the authorization of the Investigator. The storage condition shall be properly documented. Both the receipt and dispensation of all test products (used and unused) are documented using forms provided by P&G or suitable forms provided by the site. Study products are returned to P&G following the trial, or alternatively, they are destroyed at the clinical site provided the site has an existing SOP for the destruction of clinical materials and prior written approval from P&G.

Subject Consent

It is the responsibility of the Investigator, or designee, to obtain written (signed and dated by the subject) informed consent from each individual participating in this study. Major/substantial amendments to the protocol that affect the scope of the study at the subject level and/or updates to the safety profile of the investigational product (Investigator Brochure) should be reflected in the consent form and active subjects reconsented.

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