JOHNSON & JOHNSON CONSUMER INC.

SUMMARY CLINICAL STUDY REPORT

PROTOCOL TITLE:	Twelve Week Safety/Clinical/Microbiological Efficacy of Experimental Mouthwashes
PROTOCOL NUMBER:	CCSORC004216 Amendment 2 _ 07 Jan 2022
SPONSOR:	Johnson & Johnson Consumer Inc. [redacted]
STUDY SITE:	All Sum Research Center Ltd. 6635 Kitimat Road, Units 36 & 37 Mississauga, Ontario L5N 6J2 CANADA
PRINCIPAL INVESTIGATOR:	Dr. Chhaju Ram Goyal [redacted]
KEY SITE STAFF	Study Director/Designated Physician Representative (DPR) (until 26 January 2023): Mary Lynn Bosma, RDH, DDS, Director Oral Health, Medical Affairs and Clinical Research [redacted] Study Director/DPR (starting 26 January 2023): Patricia Gorecki, DMD, PhD, MOralSurg, Clinical Strategy Director, Oral Healthcare [redacted] Statistician: Tony McGuire, Director Global Biostatistics [redacted] Study Manager: Alicia DelSasso, CCRP [redacted]
STUDY INITIATION DATE (First Subject First Visit):	15 Nov 2021
STUDY COMPLETION DATE (Last Subject Completed):	14 Apr 2022
SITE APPROVAL:	[redacted]
SPONSOR REVIEW AND APPROVAL:	

The principles of the International Council for Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP E6 (R2)) were applied to this study.

[redacted]

1. STUDY SYNOPSIS

The principles of the International Council for Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP E6 (R2)) were applied to this study.

INTRODUCTION	It has been well documented that the four essential oils (menthol (mint), thymol (thyme), methyl salicylate (wintergreen) and eucalyptol (eucalyptus)) are effective in the reduction of plaque and gingivitis[1]. This study evaluated the safety and efficacy of mouthwash formulations [redacted] compared to a positive and negative control for the prevention and reduction of gingivitis and plaque as an adjunct to tooth brushing when used twice daily as directed during a 12-week treatment period.
OBJECTIVE	The purpose of this clinical trial was to evaluate the safety and efficacy of experimental mouth rinse formulations [redacted] compared to a hydroalcohol control rinse and a positive control mouth rinse for the reduction of gingivitis and plaque when used as an adjunct to tooth brushing during a 12-week product usage period. The primary endpoints were whole mouth mean Modified Gingival Index (MGI) and whole mouth mean Turesky Plaque Index (TPI) after 12 weeks of product use. The secondary endpoints were whole mouth mean TPI and whole mouth mean MGI after one and four weeks of product use, and whole mouth mean Expanded Bleeding Index (EBI) and percent bleeding sites based on the EBI at one, four and 12 weeks. Additionally, the abundances of total live and dead bacteria and select species of interest were measured using quantitative PCR (qPCR) targeting the 16S rDNA at six sites in the mouth (saliva, tongue, buccal mucosa, gingiva, supra- and subgingival plaque) at baseline and after four and 12 weeks.
STUDY DESIGN	This was a 12-week, single center, examiner-blind, randomized, parallel-group controlled clinical trial. The study duration was approximately 13 weeks, with subjects completing four site visits. The trial protocol [redacted] provides the complete trial design.
SUBJECT INFORMATION	Subjects were generally healthy adults, 18 to 59 years of age, in good general and oral health who met the eligibility criteria. The complete eligibility criteria for this study were followed as defined in the study protocol [redacted]
INVESTIGATIONAL STUDY MATERIALS	<table><tr><th>Identification</th><th>UPC/Formula Number</th><th>Product Type</th></tr><tr><td>5% Hydroalcohol control rinse [redacted] abbreviated HC)</td><td>[redacted]</td><td>Investigational Product (IP) Negative Control</td></tr></table>

	[redacted] Hydroalcohol Prototype mouth rinse [redacted] called Hydroalcohol Prototype [HP] in this CSR)	[redacted]	IP (Experimental)
	[redacted] Avant Prototype mouth rinse [redacted] called Avant Prototype [AP] in this CSR)	[redacted]	IP (Experimental)
	[redacted] Zero Prototype mouth rinse [redacted] called Zero Prototype [ZP] in this CSR)	[redacted]	IP (Experimental)
	LISTERINE® Cool Mint® Antiseptic Mouthwash (abbreviated LCM)	UPC# 312547427357	IP. Positive Control
	Colgate® Cavity Protection Toothpaste	UPC# 035000510853	Auxiliary Product
	Colgate® Full Head/Soft Bristles Toothbrush	UPC# 035000550101	Ancillary Product
DOSE AND MODE OF APPLICATION	Subjects were instructed to brush their teeth twice daily (morning and evening) in their usual manner using the toothpaste and soft bristled toothbrush provided. They were instructed to place a full ribbon of toothpaste across the length of the provided toothbrush. After each brushing, they were instructed to rinse with 20mL of their assigned mouth rinse for 30 timed seconds, following label instructions. The first product use (brushing and rinsing) was conducted at the site under supervision of study personnel. All other brushing and rinsing was unsupervised. Subjects recorded each brushing and rinsing on their subject diary card.		
METHODOLOGY	For each study visit, subjects presented to the clinical site after refraining from oral hygiene for at least eight hours, but no more than 12 hours, and refrained from eating, drinking and smoking for at least four hours prior to the visit. Subjects were allowed to drink water up to two hours before their visit. <u>Visit 1: Day 0 – Screening/Baseline</u> Subjects were consented, had their prior and concomitant medications/non-drug therapies, smoking, medical and dental histories and inclusion and exclusion criteria reviewed. Female subjects of childbearing potential were given a urine pregnancy test. Periodontal pocket depth was measured to ensure subjects had no periodontal pockets of 5 mm or more (an exclusion criterium). Subjects had an oral examination / oral hard and soft tissue assessment. Next, subjects provided an unstimulated saliva sample after sitting quietly for five minutes, and samples from the tongue were taken with a cytology brush from two areas: posterior to the circumvallate papillae and in the middle of the tongue.		

	Samples from the buccal mucosa were taken with a cytology brush from either the right or left surface of the buccal mucosa. Samples from the gingiva were taken with a cytology brush on maxillary and mandibular gingival surfaces. Supragingival plaque was sampled from four teeth. The preferred teeth numbers were 3, 7, 18 and 24. The sampled sites were excluded from plaque grading. The same teeth were sampled throughout the study. If the preferred teeth were not available, suitable adjacent or contralateral substitutes were chosen. Subgingival plaque samples were taken from the same teeth that were sampled for supragingival plaque. After sampling, assessments for MGI, EBI and TPI were conducted. A qualified dental professional performed a complete dental prophylaxis. Another qualified professional checked the teeth to ensure completeness of prophylaxis. Subjects were randomly assigned to one of five treatment groups. Subjects received their assigned mouth rinse product, dose cups, marketed fluoride-containing dentifrice (Colgate® Cavity Protection Toothpaste), a marketed soft bristled toothbrush (Colgate® full head toothbrush) and a diary card/subject instruction sheet. Subjects used their assigned study products following the label instructions under supervision for the first use. Adverse events (AEs) were assessed, and the next visit was scheduled. **Visit 2: Day 7 – 1 Week Post Baseline (± 1 day)** **Visit 3: Day 28 – 4 Weeks Post Baseline (± 2 days)** **Visit 4: Day 84 – 12 Weeks Post Baseline (± 3 days)** Subjects brought all their mouth rinse bottles (empty and full), study toothbrush and toothpaste with them to these visits. Site personnel assessed compliance with use of the IPs by visually inspecting toothpaste for use, weighing mouth rinse bottles, reviewing diary cards and if necessary, reinforcing the usage directions. Inclusion/exclusion criteria, AE assessment and concomitant medications/non-drug therapies were reviewed to ensure subjects were still eligible to participate in the trial. At visit 4 only, female subjects of childbearing potential were given a urine pregnancy test. At each visit, subjects had an oral examination and an oral hard and soft tissue assessment, bacteria sampling for qPCR analysis (except not at visit 2), then MGI, EBI and TPI assessments. AEs were assessed at every visit. Bacteria samples were handled, collected, prepared and packaged as described in the protocol. At visit 4, subject disposition was completed. ----------

	Subjects were instructed to use only the products given to them throughout the trial. Subjects were permitted to continue to use an interdental cleaning device only to remove impacted food between the teeth if it was part of their usual oral care regimen. --- Statistical Analysis: In general, comparisons between investigational products were based on a mixed-effects model for repeated measures analysis (MMRM), including terms for investigational product and visit, and the baseline value as a covariate. Investigational product-by-visit and baseline-by-visit terms were included to compare investigational products at specific visits. The only exception to this general approach was for qPCR measurements from subgingival plaque, for which laboratory equipment failure resulted in loss of a large number of observations, including more than 40% of the baseline values. For this reason, analysis of subgingival plaque data was performed using separate analyses of variance by visit (1, 4, and 12 weeks), with investigational product as a factor. For each primary and secondary endpoint, each prototype mouth rinse and LCM was tested for superiority versus the hydroalcohol negative control rinse (HC). For MGI and TPI assessments at week 12, each prototype mouth rinse was assessed for non-inferiority vs. LCM, provided the prototype mouth rinse was significantly better than negative control for both MGI and TPI. Non-inferiority was assessed by a one-sided test at the 2.5% significance level, comparing each prototype mean to the average of the placebo and LCM mean. For additional information, 97.5% one-sided Fieller confidence intervals were provided for the ratio of the effect of the prototype rinse of each prototype rinse (vs. HC) versus LCM (vs. HC). The number and percentage of subjects with AEs and those experiencing investigational product-related AEs were tabulated by MedDRA System Organ Class, preferred term, and investigational product. Oral exam findings were summarized. Note that subjects were randomized among the five treatment groups in a 2:2:2:2:1 allocation ratio, with the "1" representing the HC group.
MEASUREMENT AND/OR EVALUATION SCHEDULE	Oral tissue tolerance, MGI, EBI and TPI were assessed at all site visits. Bacterial sampling on supragingival and subgingival plaque, buccal mucosa, gingiva, tongue and saliva was done at visits 1, 3 and 4.
INSTITUTIONAL REVIEW BOARD (IRB)	This study was reviewed and approved by the following IRB: - Veritas IRB Inc. - +1 (866) 384-4221 - info@veritasirb.com - Approval date: 28 Oct 2021 Applicable Amendments approval dates: – Protocol Amendment 1 dated 11 Oct 2021 approved 21 Oct 2021

	– Protocol Amendment 2 dated 07 Jan 2022 approved 10 Jan 2022
SAFETY AND ADVERSE EVENTS	Oral tissue tolerance was monitored through oral exams and the collection of AEs. Safety was assessed through observation and query of each subject at each visit for any new or continuing symptoms since the previous visit and through the tabulation of AEs. Details of AEs, including resolution, were captured. Any intra-oral adverse events were photographed and sent to the Sponsor.
MONITORING, QUALITY CONTROL, AND QUALITY ASSURANCE	The study monitoring was conducted as per the Sponsor's requirements. The study site was subject to review by the IRB, quality assurance audits performed by the Sponsor, and/or to inspection by appropriate regulatory authorities.
CONCLUSION	The results of this 12-week clinical trial show that experimental mouth rinses [redacted] provided statistically significant reductions in plaque, gingivitis and bleeding compared to a 5% hydroalcohol negative control rinse. LISTERINE® Cool Mint® Antiseptic, a marketed rinse containing alcohol and an established combination of four essential oils, also provided statistically significant reductions in plaque, gingivitis and bleeding compared to the negative control. The experimental mouth rinse [redacted] (HP), was shown to be non-inferior to LISTERINE® Cool Mint® Antiseptic in reducing plaque and gingivitis. One of the experimental rinses [redacted] (AP) was shown to be non-inferior to LCM in reducing gingivitis (but not plaque). AP was not demonstrated to be non-inferior to LCM in reducing plaque and ZP was not demonstrated to be non-inferior to LCM in reducing plaque or reducing gingivitis. The mouth rinses appeared to be generally safe and well tolerated, with a small percentage of subjects in three of the groups experiencing single incidents of mild oral mucosal exfoliation. The qPCR technique used in this trial lacks the sensitivity to differentiate between live and dead bacteria and was not able to measure germ kill efficacy of the mouth rinses on various sampled sites. Therefore, the qPCR technique used in this trial was not adequate to reliably assess whole mouth protection against oral bacteria. For future studies, additional improvement in differentiating live versus dead bacterial DNA in the qPCR technique is needed. These results may be of interest in further research on the experimental formulations and their effect on oral health, e.g., plaque, gingivitis and bleeding, in comparison to LISTERINE® Cool Mint® Antiseptic.

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