JOHNSON & JOHNSON CONSUMER INC.

SUMMARY CLINICAL STUDY REPORT

PROTOCOL TITLE:	Twelve Week Safety, Clinical and Microbiological Efficacy of Experimental Zinc Containing Mouth Rinses
PROTOCOL NUMBER:	CCSORC005014 Amendment 1 (v2): 08 Aug 2022
SPONSOR:	Johnson & Johnson Consumer Inc. [redacted]
STUDY SITE:	Salus Research, Inc. 1220 Medical Park Drive, Building 4 Fort Wayne, Indiana 46825 [redacted]
PRINCIPAL INVESTIGATOR:	Jeffery Milleman, DDS, MPA [redacted]
KEY SITE STAFF	Sub-Investigator: Kimberly Milleman, RDH, PhD [redacted] 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):	31 Aug 2022
STUDY COMPLETION DATE (Last Subject Completed):	08 Dec 2022

SITE APPROVAL:	[redacted]
SPONSOR REVIEW AND APPROVAL:	[redacted]

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

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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]. Zinc [redacted] has been added to formulations in oral care products for additional benefits such as plaque and malodor control and calculus reduction[2, 3].
OBJECTIVE	The purpose of this clinical trial was to evaluate the efficacy and safety of two zinc and essential oil-containing experimental mouth rinse formulations and a non-zinc essential oil-containing mouth rinse compared to a hydroalcohol negative control 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 (mean MGI) after 12 weeks • whole mouth mean Turesky Plaque Index (mean TPI) after 12 weeks The secondary endpoints were: • whole mouth mean TPI after 1 and 4 weeks • whole mouth mean MGI after 1 and 4 weeks • whole mouth mean Expanded Bleeding Index (mean EBI) and percent bleeding sites, based on the EBI after 1, 4 and 12 weeks • whole mouth bleeding on probing and probing depth at 12 weeks • microbiome endpoints for supragingival plaque assessment, which included the observed species richness, the Shannon-Weaver diversity index, the total species microbial load, the categorical species microbial load and sample clustering analysis for composition changes at baseline, 1, 4 and 12 weeks • log10 cell counts of total bacteria and select species of interest measured from supra- and subgingival plaque using quantitative PCR (qPCR), coupled with a propidium monoazide (PMA) treatment at baseline and 12 weeks, which allowed quantification of the living bacteria Additionally, post hoc analysis of percent healthy sites, in which both MGI and EBI = 0, was conducted to compare differences in healthy sites between the mouth rinse groups and the negative control.
STUDY DESIGN	This was a twelve-week, single center, examiner-blind, randomized, parallel-group controlled clinical trial. The trial protocol [redacted] provides the complete trial design.

<table>
<tr><td>SUBJECT INFORMATION</td><td>The complete eligibility criteria for this study were followed as defined in the study protocol [redacted]</td></tr>
<tr><td>INVESTIGATIONAL STUDY MATERIALS</td><td>
<table>
<tr><th>Identification</th><th>UPC/Formula Number</th><th>Product Type</th></tr>
<tr><td>LISTERINE® Cool Mint® Antiseptic Mouthwash (abbreviated LCM)</td><td>UPC# 312547427357</td><td>Investigational Product (IP)</td></tr>
<tr><td>5% Hydroalcohol (Negative Control, abbreviated NC) rinse</td><td>[redacted]</td><td>IP</td></tr>
<tr><td>[redacted] Zinc-containing mouth rinse with alcohol [redacted]</td><td>[redacted]</td><td>IP (Experimental)</td></tr>
<tr><td>[redacted] Zinc-containing mouth rinse, zero alcohol [redacted]</td><td>[redacted]</td><td>IP (Experimental)</td></tr>
<tr><td>Colgate® Cavity Protection Toothpaste</td><td>UPC# 035000510853</td><td>Auxiliary Product</td></tr>
<tr><td>Colgate® Full Head/Soft Bristles Toothbrush</td><td>UPC# 035000550101</td><td>Ancillary Product</td></tr>
<tr><td>Colgate® Waxed Floss</td><td>UPC# 890134076755</td><td>Ancillary Product</td></tr>
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</td></tr>
<tr><td>DOSE AND MODE OF APPLICATION</td><td>Subjects were instructed to brush their teeth twice daily for one timed minute 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.</td></tr>
<tr><td>METHODOLOGY</td><td>For each study visit, subjects presented to the clinical site after refraining from oral hygiene for at least eight hours, but no more than 18 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 but were instructed not to "swish" water around their mouths. In addition, subjects were instructed to refrain from drinking any alcoholic beverages and consuming yogurt for at least 12 hours prior to the 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. Subjects completed a Habits & Practices</td></tr>
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	questionnaire prior to the oral and soft tissue assessment. Subjects were required to fill out the site's IRB approved Implementation of COVID-19 Prevention Procedures Consent Form. Subjects were asked to bring a list of their current medications and supplements and their current toothpaste and mouthwash (if applicable) with them. Site personnel recorded the brand/toothpaste and how long they had been using that specific brand/toothpaste. Site personnel also recorded the brand and name of mouthwash used (if applicable) and how long they had been using that specific brand/mouthwash. Assessments / examinations included an oral hard and soft tissue assessment, digital photo of anterior teeth, MGI, supragingival plaque sampling for microbiome, EBI, pocket depth for all gradable teeth, bleeding on pocket depth probing, TPI, and supragingival and subgingival plaque sampling for qPCR. • Microbiome: Supragingival plaque was sampled from four teeth. The preferred teeth numbers were 3, 7, 18 and 23, if they exhibited the following criteria: MGI scores from 1 to 3, with at least two sites with MGI score of 2 or 3; one bleeding site; no pocket depth greater than 4 mm. The sites sampled for microbiome plaque were excluded from plaque grading. The same teeth were sampled throughout the study. If the preferred teeth did not exhibit the criteria, suitable adjacent or contralateral substitutes were chosen. • qPCR: Supragingival plaque was sampled from four teeth. The preferred teeth numbers were 10, 14, 24 and 31, if they met the criteria of pocket depth of 4 mm or less. If the preferred teeth did not meet the criteria, suitable adjacent or contralateral substitutes were chosen. • qPCR: Subgingival plaque was sampled from the same teeth chosen for supragingival qPCR teeth. 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 four 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 began use of their assigned study products following the label instructions. 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 three 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, MGI, plaque sampling for microbiome, and qPCR if applicable, EBI and TPI assessments. Digital imaging of subject's anterior teeth were taken at visits 1, 2 and 4. No image was taken at visit 3. Supragingival plaque sampling for microbiome was done at all visits. Supragingival and subgingival plaque sampling for qPCR was done at visits 1 and 4 after TPI (note: qPCR plaque sampling was not done at visit 2 or 3). Samples were handled, collected, prepared and packaged as described in the protocol. AEs were assessed and 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, and they were permitted to use the floss provided to them at the start of the trial. --- Statistical Analysis: 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 within-subject correlation structure was specified as unstructured. The superiority of zinc and essential oil-containing mouth rinse formulations (Alcohol) vs. negative control, zinc essential oil-containing mouth rinse formulation (Zero alcohol) vs. negative control, and LISTERINE® COOL MINT® Antiseptic Mouthwash (LCM) vs. negative control, were tested at a significance level of 0.05 two-sided, separately. 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.

MEASUREMENT AND/OR EVALUATION SCHEDULE	Oral tissue tolerance, MGI, supragingival plaque sampling for microbiome, EBI, and TPI were assessed at all site visits. Pocket depth, bleeding on pocket depth probing were assessed at Visit 1 (Screening/Baseline) and Visit 4 (Week 12 ± 3 days) only. Supragingival and subgingival plaque sampling for qPCR was done at visit 1 and visit 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 Jul 2022 Applicable Amendments: Protocol Amendment 1 (v2) dated 08 Aug 2022 Approval date: 10 Aug 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 mouth rinses containing zinc with essential oils, with or without alcohol, provided clinically and statistically significant reductions in plaque, gingivitis and bleeding compared to a negative control of a 5% hydroalcohol rinse. Listerine Antiseptic Cool Mint, a marketed rinse containing alcohol and essential oils, also provided clinically and statistically significant reductions in plaque, gingivitis and bleeding compared to the negative control. The mouth rinses appeared to be generally safe and well tolerated, with a small percentage of subjects experiencing single incidents of mucosal exfoliation and/or dry mouth that were mild and resolved without treatment. This low irritation potential was similar among all investigational mouth rinses. Analysis of supragingival plaque microbiome by shotgun metagenomic sequencing and live bacterial enumeration by PMA-qPCR confirmed mouth rinses containing zinc and essential oils, with or without alcohol, provided significant reductions in microbiome diversity, richness, abundances of total bacteria as well as those species associated with commensals, gingivitis and malodor above and below the gumline. These results may be useful to oral healthcare practitioners when recommending oral care regimens to control plaque, gingivitis and gingival bleeding.

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