JOHNSON & JOHNSON CONSUMER INC.

SUMMARY CLINICAL STUDY REPORT

PROTOCOL TITLE:	Twelve Week Clinical Efficacy of Virtually Supervised Mouth Rinse and Flossing: Effect on Plaque, Gingivitis, and Microbiome
PROTOCOL NUMBER:	CCSORC004913 Amendment 3 _ v4 _ 20 June 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 (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):	18-APR-2022
STUDY COMPLETION DATE: (Last Subject Completed):	25-JUL-2022

SITE APPROVAL:	

Name	Signature/Date:
[redacted]	

SPONSOR REVIEW AND APPROVAL:	

Name	Signature/Date:
[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	Bacterial plaque is an important factor in dental diseases such as caries and gingivitis. Plaque control methods include a variety of procedures. These cover mechanical removal methods, such as brushing and flossing, or chemotherapeutic methods, such as essential oil containing mouth rinses. Five randomized clinical trials have been published related to the relative efficacy of a mouth rinse containing alcohol and essential oils and dental flossing in the ability to control the accumulation of plaque and subsequently the prevention/reduction of gingivitis.[1-5] Three of these clinical trials were 6-month studies in which subjects used study products but their product use was not observed on a regular basis by study personnel (unsupervised) and two were 12-week studies in which study personnel observed subjects' use of study products on a regular basis (supervised). One of the unsupervised trials indicated that rinsing twice daily with an essential oil mouth rinse was at least as good as unsupervised daily flossing in reducing interproximal plaque and gingivitis. The second unsupervised trial indicated that the essential oil mouth rinse was as good as floss in controlling interproximal gingivitis and significantly more effective than flossing in controlling interproximal plaque. The final unsupervised trial indicated that brushing, flossing and rinsing with an essential oil rinse exhibited statistically and clinically significantly lower mean scores for gingivitis and plaque than brushing, flossing and rinsing with a negative control rinse. In the first supervised trial, subjects conducted their assigned oral care regimen every weekday morning under the observation of study personnel. There were two flossing groups; one group flossed once daily while being observed on weekdays (supervised flossing); the other group had their teeth flossed by a dental hygienist (professional flossing). All groups performed their oral care regimen in the evenings and weekends without being observed by study personnel. Results showed that rinsing with an alcohol-containing, essential oil mouth rinse statistically significantly improved plaque, gingivitis and gingival bleeding compared to negative control rinse after 4 and 12 weeks. While professional and supervised flossing improved gingival health compared to the negative control rinse, it did not significantly reduce plaque at 12 weeks. In addition, the essential oil mouth rinse group reduced plaque and gingivitis significantly more than both floss groups.

	In the second supervised trial, subjects performed their assigned oral care regimen every weekday morning under the virtual observation of study personnel via video call. All groups performed their oral care regimen in the evenings and weekends without being observed by study personnel. Regimens that included an alcohol-containing essential oil mouth rinse significantly reduced plaque, gingivitis and gingival bleeding compared to brushing only and compared to brushing/flossing after 12 weeks. The brush/floss regimen significantly reduced gingivitis and gingival bleeding but did not significantly reduce plaque compared to brushing only after 12 weeks. These previous trials showed that flossing provides a modest benefit in the reduction of gingivitis but does not provide a significant reduction in plaque. Using an alcohol-containing essential oil mouth rinse twice daily provided at least as much benefit, and in some cases more, in reducing plaque and gingivitis than flossing. This included once daily flossing without observation, flossing under the observation of study personnel and even having a dental professional perform the flossing. While these results of trials with an essential oil mouth rinse that contained alcohol, it was not known if an essential oil mouth rinse without alcohol would provide similar results compared to flossing. The clinical trial reported here investigated the effects of regimens that include mechanical methods (brushing and flossing) and chemotherapeutic mouth rinses including a non-alcohol essential oil rinse.
OBJECTIVE	The purpose of this clinical trial was to evaluate the efficacy of brushing, flossing, and rinsing with an essential oil (EO) mouth rinse; brushing and flossing; brushing and rinsing with EO mouth rinses; and brushing only for the prevention and reduction of plaque and gingivitis, in the following four comparisons: • Brushing/flossing/rinsing with an alcohol-free EO mouth rinse versus brushing/flossing • Brushing/flossing/rinsing with an alcohol-free EO mouth rinse versus brushing/rinsing with an alcohol-free EO mouth rinse • Brushing/rinsing with an alcohol-containing EO mouth rinse versus brushing/flossing • Brushing/flossing, brushing/rinsing with an alcohol-containing EO mouth rinse, brushing/rinsing with an alcohol-free EO mouth rinse and brushing/flossing/rinsing with an alcohol-free EO mouth rinse versus brushing only

Primary Endpoint

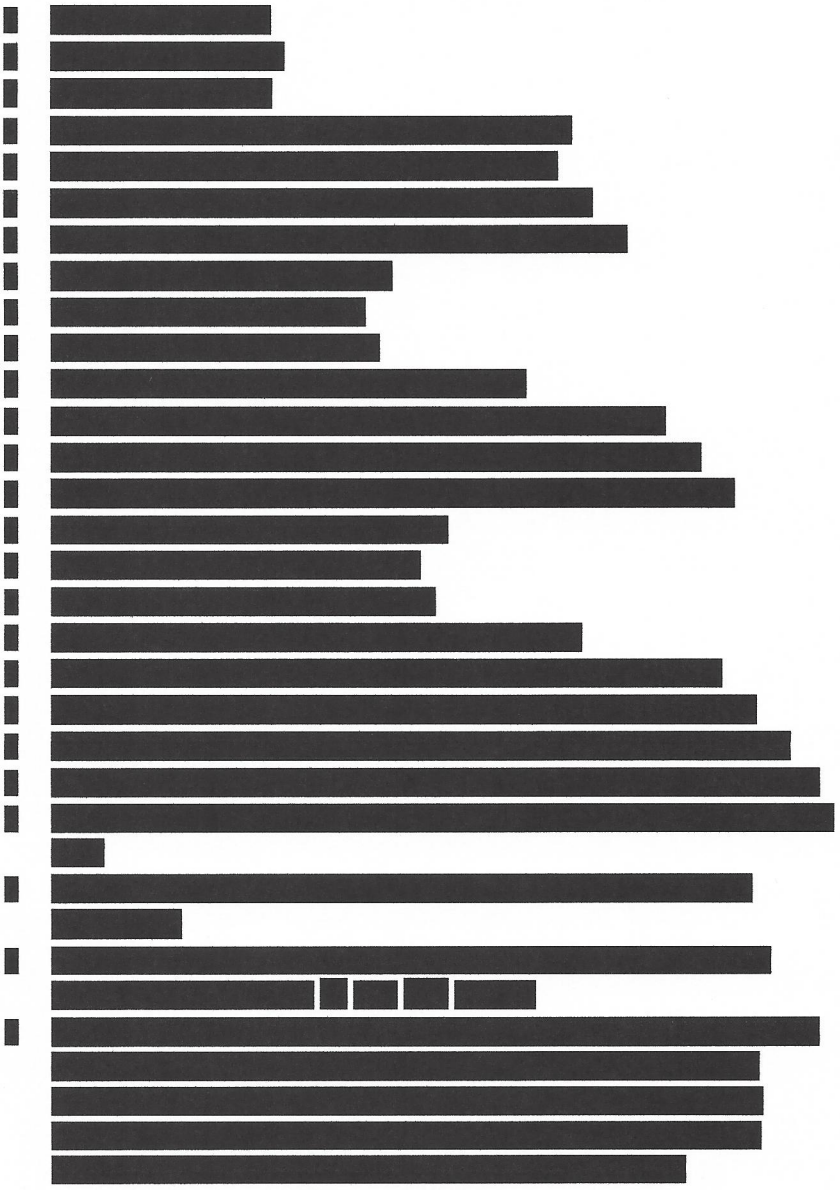
The primary endpoints were interproximal mean Turesky Plaque Index (mean TPI) and interproximal mean Modified Gingival Index (mean MGI) after 12 weeks of product use.

Secondary Endpoints

The secondary endpoints were:

- marginal mean TPI after 4 and 12 weeks
- marginal mean MGI after 4 and 12 weeks
- marginal mean EBI after 4 and 12 weeks,
- interproximal mean TPI after 4 weeks
- interproximal mean MGI after 4 weeks
- whole mouth mean TPI after 4 and 12 weeks
- whole mouth mean MGI after 4 and 12 weeks
- whole mouth and interproximal mean Expanded Bleeding Index (EBI) after 4 and 12 weeks
- whole mouth and interproximal percent bleeding sites after 4 and 12 weeks based on EBI
- whole mouth and interproximal mean pocket depth after 12 weeks
- whole mouth and interproximal mean bleeding upon probing pocket depths after 12 weeks
- microbiome endpoints for supragingival plaque assessment 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, 4 weeks and 12 weeks of product use
- Log10 cell counts of total bacteria and select indicator species (*A. oris, F. nucleatum, P. gingivalis)* measured from supra- and subgingival plaque using quantitative PCR (qPCR), coupled with a propidium monoazide (PMA) treatment done at that study site after collection of the sample, which allowed quantification of the living bacteria.

[redacted]

[redacted]	
STUDY DESIGN	This was a twelve-week, single center, examiner-blind, randomized, parallel-group controlled clinical trial. The trial protocol referenced on page 47 of this report provides the complete study design. In brief, there were five groups of subjects who used products, referred to as the Randomized Treatment Groups. There was one group of subjects who did not use any study products, referred to as the Healthy Reference Group, for the purpose of acting as a comparator group for the microbiome exploration.

<table>
<tr><td></td><td>The Healthy Reference Group participation entailed a one-day visit at Baseline / Day 0 with no study products provided.

The Randomized Treatment Groups participation entailed three site visits over 12 weeks (84 days ± 3 days). Subjects were also required to attend once daily weekday virtual visits via video phone calls throughout the study.</td></tr>
<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</td><td>UPC# 312547427357</td><td>Investigational Product (IP)</td></tr>
<tr><td>LISTERINE® COOL MINT® ZERO Alcohol Mouthwash</td><td>UPC# 312547428323</td><td>IP</td></tr>
<tr><td>Reach® Unflavored Waxed Dental Floss</td><td>UPC# 381370092131</td><td>IP</td></tr>
<tr><td>Colgate® Cavity Protection Toothpaste</td><td>UPC# 035000510853</td><td>Auxiliary Product</td></tr>
<tr><td>Colgate® Classic Toothbrush Full Head/Soft Bristles ADA Accepted</td><td>UPC# 035000550101</td><td>Auxiliary Product</td></tr>
</table>
</td></tr>
<tr><td>DOSE AND MODE OF APPLICATION</td><td>The five Randomized Treatment Groups were the following:

Brush Only (Control) Group (abbreviated as B)
Study product: Auxiliary product (Toothpaste/toothbrush)
Subjects used assigned product following the label instructions. Subjects brushed their teeth as directed under virtual supervision once daily for the first use of the day during the week. Subjects brushed a second time unsupervised daily in the evening and twice daily over the weekend/holidays. First product use occurred at the site under supervision.

Brush/Rinse Groups (2 groups)
Study products: Auxiliary product (Toothpaste/toothbrush) + Listerine Cool Mint (LCM) Antiseptic mouthwash (abbreviated as B/LCM)
Study products: Auxiliary product (Toothpaste/toothbrush) + LCM Zero Alcohol mouthwash (abbreviated as B/LZ)
Subjects used their assigned products following the label instructions. Subjects brushed their teeth and rinsed with their IP as directed under virtual supervision once daily for the first product use of the day during the week. Subjects brushed and rinsed a second time unsupervised daily in the evening and twice daily over the weekend/holidays. First product use occurred at the site under supervision.</td></tr>
</table>

	Brush/Floss Group **Study products: Auxiliary product (Toothpaste/toothbrush) + Reach dental floss** (abbreviated as B/F) Subjects used their assigned products following the label instructions. Subjects brushed and flossed as directed under virtual supervision once daily for the first product use of the day during the week. Subjects brushed a second time unsupervised daily in the evening. Over the weekend and holidays, subjects brushed and flossed once daily. Only brushing was performed a second time in the evening. First product use occurred at the site under supervision. **Brush/Floss/Rinse Group** **Study products: Auxiliary product (Toothpaste/toothbrush) + Reach dental floss + LCM Zero Alcohol mouthwash** (abbreviated as B/F/LZ) Subjects used their assigned products following the label instructions. Subjects brushed, flossed and rinsed as directed under virtual supervision once daily for the first product use of the day during the week. Subjects brushed and rinsed a second time unsupervised daily in the evening. Over the weekend and holidays, subjects brushed, flossed and rinsed once daily. Only brushing and rinsing was performed a second time in the evening. First product use occurred at the site under supervision. Subjects in the Floss groups received an ADA How to Floss visual guide. After receiving both written and verbal instructions, subjects in the treatment groups used their assigned study products and completed their oral care regimen at the site under supervision.
METHODOLOGY	For each study visit (Visit 1 for the Healthy Reference Group; Visits 1, 2 and 3 for the Treatment Groups), 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. <u>Visit 1: Day 0 – Screening/Baseline (Healthy Reference Group)</u> Subjects were consented, had their prior and concomitant medications/non-drug therapies, smoking, medical and dental histories and inclusion and exclusion criteria reviewed. The site recorded the subject's brand of toothpaste and mouthwash (if applicable) and how long the subjects had been using them. Female subjects of childbearing potential

	were given a urine pregnancy test. Subjects completed a Habits & Practices questionnaire and Microbiome questionnaire as well as the site's IRB-approved Implementation of COVID-19 Prevention Procedures Consent Form. Subjects had the following assessments in this order: oral hard and soft tissue examination, MGI, EBI, supra- and subgingival plaque sampling for microbiome, pocket depth and bleeding upon probing measurements, TPI and dexterity testing. Adverse events (AEs) were assessed, and subject disposition was completed, and the study concluded for the Healthy Reference Group. <u>Visit 1: Day 0 – Screening/Baseline (Treatment Groups)</u> Subjects were consented, had their prior and concomitant medications/non-drug therapies, smoking, medical and dental histories and inclusion and exclusion criteria reviewed. The site recorded the subject's brand of toothpaste and mouthwash (if applicable) and how long the subjects had been using them. Female subjects of childbearing potential were given a urine pregnancy test. Subjects completed a Habits & Practices questionnaire and Microbiome questionnaire, as well the site's IRB approved Implementation of COVID-19 Prevention Procedures Consent Form. Each subject was given a dexterity test and baseline examinations, which included an oral hard and soft tissue assessment, MGI, EBI, supra- and subgingival plaque sampling for microbiome, EBI, pocket depth and bleeding on pocket depth probing measurements and TPI. After the assessments, subjects received a complete dental prophylaxis. The teeth were checked by another qualified hygienist or dentist for thorough calculus and plaque removal prior to the subject being completed. Qualifying subjects were randomly assigned to one of five treatment groups. Subjects received their assigned floss (if applicable), mouth rinse and dose cups (if applicable), a marketed fluoride-containing dentifrice (Colgate® Cavity Protection toothpaste), soft flat trimmed bristled toothbrush, and a subject instructions/diary card to record their evening and weekend/holiday product use times. Each subject received individualized flossing instructions if they were assigned to a floss group. Subjects used their assigned study products following the label instructions. The first product use - brushing, flossing (if applicable) and rinsing (if applicable) - was conducted at the site under supervision of study personnel.

	AEs were assessed. Subjects were scheduled for their next site visit and their supervised weekday product usage. [redacted] <u>Visit 2: Week 4 (Day 28 ± 2 days)</u> Inclusion/exclusion criteria, AE assessment and concomitant medications/non-drug therapies were reviewed to ensure subjects were still eligible to continue participation in the study. Subjects completed the Microbiome questionnaire. Each subject received assessments/examinations that included an oral hard and soft tissue assessment, MGI, EBI, supragingival plaque sampling for microbiome and TPI. AEs were assessed. Subjects were scheduled for their next site visit and their supervised weekday product usage. Compliance was evaluated by weighing residual volumes of returned mouth rinse and/or floss (if applicable), visually inspecting toothpaste tubes for use and by reviewing the subject diary cards. [redacted] <u>Visit 3: Week 12 (Day 84 ± 3 days)</u> Inclusion/exclusion criteria, AE assessment and concomitant medications/non-drug therapies were reviewed to ensure subjects were still eligible to continue participation in the study. Female subjects of childbearing potential were given a urine pregnancy test. Subjects completed the Microbiome questionnaire. Assessments/examinations included an oral hard and soft tissue assessment, MGI, EBI, supragingival and subgingival plaque sampling for microbiome, pocket depth, bleeding on pocket depth probing, and TPI, and supragingival and subgingival plaque sampling for qPCR.

	Compliance was evaluated by weighing residual volumes of returned mouth rinse and/or floss (if applicable), visually inspecting toothpaste tubes for use and by reviewing the subject diary cards. AEs were assessed, and subject disposition was completed. --- Subjects were instructed to use only the products given to them throughout the study. No other oral hygiene products or procedures were permitted while participating in this study, including teeth cleaning, whitening or dental procedures except for an emergency treatment. The decision to withdraw a subject due to emergency dental treatment occurred at the discretion of the investigator. **Microbiome Plaque Sampling** Supragingival plaque sampling was done at all visits. Subgingival plaque sampling was done at Visits 1 and 3 only. Plaque samples were placed in tubes prefilled with buffer and frozen immediately or soon after collection. All samples were logged using the sample manifest form. **qPCR Plaque Sampling** On Visit 3 only, qPCR samples were collected then treated with PMA for live/dead germ-kill evaluation. The sample manifest form was sent to the Sponsor for review. Once approved, the Sponsor provided the site with sample submission form to be printed and included in the sample shipment. Site shipped the samples on dry ice [redacted]. --- Statistical Analysis: For endpoints assessed at Visits 1 (Baseline), 2 (Week 4) and 3 (Week 12), investigational products were compared based on a mixed model for repeated measures analysis (MMRM), including terms for treatment and visit, and the corresponding baseline value as a covariate. Treatment-by-visit and baseline-by-visit terms were included to make treatment comparisons and estimate treatment differences at specific visits. For endpoints assessed at Baseline and Week 12, comparisons were based on analysis of covariance with the corresponding baseline value as a covariate. For endpoints assessed at Week 12 only, comparisons were based on an analysis of variance. For interproximal mean TPI, MGI, and BI at Week 12, comparisons for B/LZ vs. B/F and B/F/LZ vs. B/F at Week 12 in parallel each followed a fixed

	sequence testing procedure strongly controlling the familywise error rate at 2.5%. Therefore, based on the Bonferroni method, the familywise error rate for all comparisons included in this sequential procedure is strongly controlled at the 5% significance level. In parallel, for interproximal mean TPI, MGI, and BI at Week 12, comparisons for B/LCM vs. B/F at Week 12 followed a fixed sequence testing procedure strongly controlling the familywise error rate at 5%. The number and percentage of subjects experiencing AEs and treatment-related AEs during the clinical study was presented by MedDRA System Organ Class preferred term and treatment. Oral exam findings were summarized.
MEASUREMENT AND/OR EVALUATION SCHEDULE	Oral tissue tolerance, MGI, EBI, and TPI were assessed at all site visits. Pocket depth and bleeding on pocket depth probing was assessed at Visits 1 and 3. **Plaque Sampling:** For microbiome plaque, sampling of supragingival plaque was done at all visits; subgingival plaque was done at Visits 1 and 3. For qPCR, supra- and subgingival plaque sampling was done at Visit 3.
INSTITUTIONAL REVIEW BOARD (IRB)	This study was reviewed and approved by the following IRB: - Veritas IRB Inc. - (866) 384-4221 - info@veritasirb.com - Approval date: 04 April 2022 Applicable Amendments: Approval dates: - Protocol Amendment 1 dated 11 Apr 2022 approved 11 Apr 2022 - Protocol Amendment 2 dated 18 Apr 2022 approved 19 Apr 2022 - Protocol Amendment 3 dated 20 Jun 2022 approved 28 Jun 2022
SAFETY AND ADVERSE EVENTS	All AEs / Serious AEs (SAEs) were collected regardless of causal relationship to the subject's participation in the study. The information was collected/reported within the reporting timelines specified in the protocol. Oral tissue tolerance was monitored through oral exams and the collection of AEs at each visit. 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.

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 a combination of essential oils, with or without alcohol, provided clinically and statistically significant reductions in plaque, gingivitis and bleeding compared to brushing only, and compared to brushing and flossing. While flossing provided a benefit to gingival health with respect to bleeding and pocket depth, it did not provide significant reductions in plaque or gingivitis. The mouth rinses were deemed to be overall safe and well tolerated, with a small percentage of subjects experiencing single incidents of mucosal exfoliation that were mild, except for one incident classified as moderate, and resolved without treatment. Observations of the brush, floss and rinse procedures showed that familiarity over time increased the overall speed at which subjects could complete their oral healthcare regimen. Further research is needed to evaluate the association between time spent flossing and clinical results. Analysis of supragingival plaque microbiome by shotgun metagenomic sequencing confirmed mouth rinses containing a combination of 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. The reductions observed did not show complete eradication of bacteria but showed maintenance of thinner dental plaque biofilms. Flossing, on the other hand, was not effective at controlling plaque above the gingival margin and no significant differences in microbiome diversity, richness, total and categorical bacterial abundances were observed compared to brushing only. These results were further corroborated by qPCR analysis using supragingival plaque. qPCR Analysis of subgingival plaque showed flossing and rinsing by themselves are not effective at controlling plaque below the gingival margin. Instead, the results showed synergy when the combination of flossing and alcohol-free rinse was applied and indicated a potential role for flossing in selectively inhibiting *P. gingivalis.* The results of this trial align with previous study results noted in the introduction and indicate the benefit of twice daily brushing, daily flossing, and twice daily rinsing with an essential oil mouth rinse to control plaque, gingivitis and gingival bleeding. These results may be useful to oral healthcare practitioners when recommending oral care regimens.

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