

**Protocol ID: OstiaPhase0**

## **Clinical Trial Study Protocol**

**A Short, Open, Phase 0 Study to Demonstrate the Viability of  
the Lozenge Dosage Form to Deliver SALI-10 Oral Probiotics**

Protocol Date: October 12, 2025  
NCT06819761

Ostia Sciences

### 1. INTRODUCTION

Gingivitis is an oral disease condition affecting 50% to 90% of adults globally (Albandar and Rams, 2002). The gingivitis pathology can be reversed by reduction or removal of microbial plaque that accumulates on hard and soft tissues and is considered standard of care in the industry (Berezow Alex and Darveau Richard, 2010, van der Weijden and Slot, 2011). Regular oral hygiene in combination with therapeutics that delivers an anti-microbial benefit is thought to mitigate the onset of gingivitis (van der Weijden and Slot, 2011, Gunsolley, 2006). However, testing therapeutics for prophylaxis benefit to mitigate development of gingivitis has not been fully examined.

Neutrophils, a type of white blood cell (leukocyte), represent a key component of the innate defence system that protects periodontal tissue from both gingivitis and periodontitis. Not only are they the first line of cellular defence, but they are among the most abundant leukocytes within the periodontal tissues (Hirschfeld, 2020). For example, gingivitis is associated with a significant increase in the number of neutrophils that migrate to periodontal tissue. In contrast, individuals with too few neutrophils brought about by either congenital deficiencies in neutrophil numbers, or transit (LAD 1 and 2) or have an induced neutropenia by chemical induction with antimetabolic agents such as cyclophosphamide invariably develop periodontitis. Likewise, studies in KO mice that are defective in neutrophil transit also develop periodontitis. Consistent with the key contribution of neutrophils to both gingivitis and periodontitis, neutrophil transit to gingival tissue is highly regulated. The periodontium contains a highly orchestrated expression of select innate host defense mediators that facilitate the transit of neutrophils from the highly vascularized gingival tissue to the gingival crevice, where they form a “wall” between the host tissue and the dental plaque biofilm.

Gingivitis is a reversible inflammatory condition caused by the accumulation of dental plaque and the associated disruption of the host–microbial homeostasis. During gingivitis, the microbial community transitions from being dominated by gram-positive health-associated bacteria, such as *Streptococcus* species, to gram-negative periopathogens, including species of the genera *Porphyromonas*, *Tannerella*, *Treponema* and *Prevotella*. This dysbiotic shift triggers inflammatory responses, leading to tissue damage and, in some cases, progression to periodontitis.

A recent study (Kerns, 2023) on human experimental gingivitis identified three distinct host response phenotypes—high, low, and slow responders—based on clinical, inflammatory, and microbial parameters:

1. High Responders: Rapid plaque accumulation accompanied by a significant increase in gram-negative periopathogens and elevated inflammatory markers, such as interleukin-1 $\beta$  (IL-1 $\beta$ ).
2. Low Responders: Similar plaque accumulation to high responders but lower inflammation, suggesting a more muted host response to bacterial dysbiosis.
3. Slow Responders: Delayed plaque accumulation and microbial succession, with prolonged dominance of health-associated *Streptococcus* species. This group exhibited delayed or reduced inflammation, demonstrating a more resilient microbial community and host response.

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The microbial analysis revealed that the persistence of beneficial *Streptococcus* species, such as *S. sanguinis* and *S. oralis*, in slow and low responders correlates with a protective effect against the emergence of periopathogens and the associated inflammatory cascade. Conversely, the loss of these beneficial bacteria in high responders was linked to more severe inflammation, highlighting the critical role of the oral microbiome in modulating gingivitis severity.

Lantibiotic salivaricins are polycyclic peptides containing lanthionine and/or  $\beta$ -methyllanthionine residues that are produced by certain strains of *Streptococcus salivarius*, which almost exclusively reside in the human oral cavity. These molecules importance stems from their antimicrobial activity towards relevant oral pathogens which has been applied through the development of salivaricin-producing probiotic strains. However, salivaricins may also prove to be of great value in the development of new and novel antibacterial therapies in this era of emerging antibiotic resistance (Barbour, Wescombe, & Smith, 2020). In a study by Barbour & Philip 2014, they found that the bacteriocin, levan-sucrase production and basic safety features of *S. salivarius* strains isolated from healthy Malaysian subjects demonstrating their potential for use as probiotics. A new bacteriocin production medium was developed with potential scale up application for pharmaceuticals and probiotics from *S. salivarius* generating different lantibiotics. This is relevant for the clinical management of oral cavity and upper respiratory tract in the human population. Appendix 1 includes a summary table that lists the relevant clinical trials that utilized *S. salivarius*.

### Proposed Solution: *S. salivarius* SALI-10

We propose using a novel strain, *Streptococcus salivarius* SALI-10, as a targeted microbial intervention to modulate the oral microbiome and prevent gingivitis. *S. salivarius* SALI-10 is hypothesized to:

- Maintain a stable population of beneficial streptococci during plaque accumulation.
- Inhibit the growth of periopathogens such as *Porphyromonas*, *Tannerella* and *Prevotella* through competition and production of Salivaricin 10.
- Delay or suppress the dysbiotic shift to gram-negative dominance, thereby reducing the inflammatory response.

By preserving microbial homeostasis, SALI-10 may emulate the microbial resilience observed in slow responders, offering a novel strategy for gingivitis prevention.

Before the therapeutic value of *Streptococcus salivarius* SALI-10 can be assessed, its dosage form must be validated. The classical method of delivering oral probiotics is in a lozenge form, having the individual suck on the lozenge until it dissolves. Once it is verified that this dosage form works for *Streptococcus salivarius* SALI-10, a full clinical trial to determine its therapeutic viability can be conducted.

## 2. STUDY OBJECTIVES

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- 1) The aim of this study is to determine the short-term ability of the Streptococcus salivarius SALI-10 lozenges delivery system to modify the human oral microbiome composition and Oral Inflammatory Load (OIL). Specifically, we aim to:
  - Assess the change in salivary levels of periodontal-disease-associated bacteria (e.g., *P. gingivalis*, *T. forsythia*, *T. denticola*, and *P. micra*).
  - Determine the change in beneficial bacteria levels (e.g., *S. salivarius*, *S. parasanguinis*).
  - Evaluate the change in Oral Inflammatory Load (OIL) by measuring oral neutrophil counts.
- 2) To monitor the short-term adherence of the 8-10 participants to the clinical protocol

### 3. STUDY OVERVIEW

This study is designed as an open-label, single-arm, exploratory pilot study to be completed over 7 days with 8-10 participants. Participants will self-administer one lozenge containing *S. salivarius* SALI-10 (10 billion CFU/lozenge) each night after brushing and before bed. Participants will complete two (2) home sample kits, one before and one after the 7 days.

### 4. RECRUITMENT

#### 4.1 Test Site and Investigator

Site: 124 Edward St, Toronto, ON, M5G 1G6

Study Originator: Abdelahhad Barbour (Ostia Sciences)

Principal Investigator: Dr. Michael Glogauer (Ostia Sciences)

#### 4.2 Study Population

Advertisements (Appendix 2) will be placed at public venues near 124 Edwards inviting volunteers who are interested to participate. All study participants will undergo informed consent process, prior to enrollment in the study. Other than the initial screening questions there is no intention to obtain information from the participants other dental or medical records. Ten (10) qualifying volunteers that agree to participate and sign all corresponding forms will be enrolled.

Volunteers will call a phone number, and be screened into the study over the phone. The first participant will be assigned a number, and all the following enrolled participants will be assigned consecutive numbers by accession. The following inclusion and exclusion criteria will be utilized in this study.

To prevent potential research participants from feeling unduly influenced to participate, it will not be a member of the study team that will be going through the consent documentation with potential study participants; it will be an unrelated employee of Ostia Sciences that will go through the informed consent documentation with any potential study participant. Specifically, it will be a research technician who is working on an unrelated file. In the event of questions, they will be answered by the principal investigator, and he will document the questions asked and answers given so they can be audited at the end of the study to ensure that there was no undue pressure. It

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is also noted for clarity that the individual asking the question will not be identified in this documentation, as its purpose is to review the conduct of the principal investigator.

### 4.3 Inclusion Criteria:

- 1) Male or female volunteers aged 18-70 years
- 2) In good general health, ASA I
- 3) Non-smokers
- 4) Fluent in English
- 5) For sexually active female volunteers, **one** of the following methods must be used to prevent pregnancy during the study:
  - a. hormonal methods or an IUD must be in use at least 30 days prior to first study drug administration;
  - b. barrier methods must be in use at least 14 days prior to study drug administration;
  - c. that vasectomy on the male partner must be completed 3 months prior to first study drug administration;
  - d. or in the alternative that a 0 sperm count for the male partner will suffice

### 4.4 Exclusion Criteria:

- 1) Presence of orthodontic bands.
- 2) Presence of partial removal dentures.
- 3) Dental pain at time of screening.
- 4) History of allergy to a consumer or personal care products or dentifrice ingredients as determined by the dental profession monitoring the study.
- 5) Participation in any other clinical study or test panel within one month before entering the study.
- 6) Current use of anti-inflammatory, antibiotics, or antimicrobial drugs or within the last 30 days of enrolment.
- 7) History of periodontal disease.
- 8) History of systemic inflammatory or immune conditions
- 9) Pregnant or nursing women.
- 10) Use of tobacco products.
- 11) Long-term antibiotic or anti-inflammatory therapy.
- 12) Medical condition or any current usage of medication that the investigator considers may compromise the subject's safety as well as the quality of the study results (note, only Advil and Tylenol are allowed during the study, and all other medications, whether prescription or Over-The-Counter, are not allowed)
- 13) Use of any concomitant medication.

## 5. TEST PRODUCTS/INTERVENTIONS

Each participant will receive seven (7) mint-flavoured SALI-10 lozenges. They will be instructed to take a lozenge daily after brushing by letting it dissolve in the mouth. The participants will be using the SALI-10 lozenges throughout the study. Each participant will also receive four (4) home administered test kits: two (2) Saliva Collection Kits, and two (2) Saline Rinse Collection Kits.

### 5.1 SALI-10 Lozenges Manufacturer Information

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The probiotics lozenges will be manufactured by  
Lallemand Health Solutions,  
17 975 rue des Gouverneurs  
Mirabel, Québec, Canada  
J7J 2K7  
Tel : 450-433-9139

Content of the probiotic lozenge:

Active ingredient	Content per 1 gram- lozenge (in mg)
<i>Streptococcus salivarius</i>	200* Min. 10 Bi CFU/lozenge at manufacturing
Excipients	
Sorbitol	310
Isomalt	310
Dibasic calcium phosphate	83
Potato starch	QSP (65*)
Mint flavour	20
Glyceryl dibehenate	10
Steviol glycosides	2

### 5.2 Dispensing, Storage, and Accountability

Once pre-screened, all participants will receive all study materials in the mail. This will include the seven (7) lozenges, and the four (4) home administered test kits: two (2) Saliva Collection Kits, and two (2) Saline Rinse Collection Kits. The subjects will follow provided instructions on when and how to brush their teeth. These instructions are explained in the procedure section.

The test kits are self-contained commercially available kits that include all instructions of use. Participants will be provided instructions to mail the samples to Ostia Sciences Inc at 124 Edward St., Toronto, Suit 463, ON Canada M5G 1G6.

Participants will be instructed to throw out all study materials that the end of the 7-day trial.

## 6. STUDY PROCEDURES

### 6.2 Product Dispensing and Use at Home

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The product will be mailed to the participant in a sealed box that will include instructions for at-home use and safety information, including emergency contact details. To monitor patient compliance, clinical study manager will call the participant at the end of the trial for confirmation that all 7 lozenges were consumed. Subjects will be assigned a unique identification number in chronological order (e.g., from 1 to 10) as they enroll in the study. Subjects will be instructed to avoid using any other oral hygiene products. There will be no dietary restrictions during the study.

Subjects will be instructed to brush their teeth twice daily (morning and evening) for two minutes each time with a toothpaste and toothbrush and floss.

The SALI-10 lozenges will be pre-packed when given to each participant.

### **6.3 Telephone Pre-Screening**

Potential study participants who contact the clinical trial coordinator for more information about the study via email will then be contacted via telephone. The email is not to be opened until immediately before phone contact can be made, and the email to be destroyed immediately. At this stage the Recruitment Questionnaire (Appendix 3) will be completed to determine whether they are eligible for the screening phase of the study. If the subject is deemed eligible they will be given the Informed Consent Form to read and ask any questions they may have and sign. The screening will be carried out until ten (10) subjects are enrolled.

There will no in-person visits. Study participants will be mailed all study materials, which included the seven (7) lozenges, and the four (4) home administered test kits: two (2) Saliva Collection Kits, and two (2) Saline Rinse Collection Kits. Subjects will also receive the study product for use at home with directions.

### **6.5 Day 0 (Baseline)**

Participants will utilize one (1) Saliva Collection Kit, and one (1) Saline Rinse Collection Kit. They will mail off the samples to Ostia Sciences as per the given instructions. After sample collection, participants will begin using the lozenges daily for 7 days. The participants will take one lozenge after brushing. They will allow the lozenges to dissolve without biting or swallowing and avoid eating and drinking for one hour. The participants will be instructed on proper oral hygiene and will be instructed to continue their home oral hygiene in addition to use of their assigned product for 7 days (1 week). The participants will be instructed to discard the lozenges' container at the end of the study.

### **6.6 Day 7 (Completion)**

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24 hours after using the seventh (7<sup>th</sup>) lozenge, participants will utilize one (1) Saliva Collection Kit, and one (1) Saline Rinse Collection Kit. They will mail off the samples to Ostia Sciences as per the given instructions. Study participants will discard any remaining study materials they may have. Through phone, the study manager will confirm adherence to protocol and provide post-study instructions. The subjects will be informed that this is the end of the study. They will be told that they may resume their regular oral hygiene. Afterwards, we will ask the participants for their feedback in regards to their experience.

## 7. STUDY PROCEDURES AND CLINICAL REGISTRATIONS

### 7.7 Saliva and Oral Rinse Samples

The Saliva Collection Kit is the SuperSAL2 Universal Saliva Collection Kit, Instructions are found in Appendix 9. The saliva samples will be processed and will be analyzed by DNA

DNA extraction, then qPCR will be used to quantify the full change of specific bacteria in the saliva samples, specifically pathogenic bacteria (*P. gingivalis*, *T. forsythia*, *T. denticola*, *P. micra*) and beneficial bacteria (*S. salivarius*, *S. parasanguinis*).

The Oral Rinse Collection Kit is produced by Ostia Sciences, with instructions found in Appendix 10. There Oral rinse samples will be analyzed for oral neutrophil counts as a marker of Oral Inflammatory Load (OIL).

Since all research projects will be done on de-identified samples, it will not be possible to return individual results to participants. All samples will be destroyed immediately after testing. Because all research will be done on de-identified, aggregated data, it will not be possible to withdraw the data from a specific participant when samples are withdrawn from the study.

## 8.0 STATISICAL ANALYSE PLAN

For statistical analysis, full change of the microbes and the OIL will be compared between Baseline and Day 7 Completion using paired Wilcoxon test. Specifically:

### Design

- Baseline (Day 0) vs. post-intervention (Day 7) comparisons within the same subjects (n = 8). Samples included oral rinses (for OIL quantification) and oral microbiome swabs + saliva (for bacterial quantification).
- Oral Inflammatory Load (OIL): measured via oral neutrophil counts in rinse samples.
- Colonization by SALI-10: assessed with *srnA2* gene (SALI-10 specific) qPCR.

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- Pathogen burden: quantified via qPCR for red- and orange-complex periopathogens, normalized to total bacterial load.

### Statistical test

- Wilcoxon matched-pairs signed rank test.

### Oral Inflammatory Load (OIL):

- OIL was measured by normalizing oral neutrophil counts to baseline (Pre-transplantation, Day 0), followed by calculation of the fold change at Day 7. A 46% reduction in OIL was observed after intervention, consistent with anti-inflammatory activity of SALI-10.

### Quantification of Pathogens:

- Relative abundance was calculated based on reciprocals of the mean normalized cycle threshold (CT) values (CTperiodontal pathogen/CTtotal bacteria) . Significant decreases were observed in:
  - *P. gingivalis* (P<0.05)
  - *T. forsythia* (P<0.01)
  - *P. micra* (P<0.01)
  - *F. nucleatum* (P<0.05)
  - *T. denticola* remained below detection in all samples.

### Colonization:

- *SrnA2* copies were undetectable in most subjects at baseline but became reliably detectable ( $\geq 100$  copies/mL) in all participants post-intervention, confirming engraftment of SALI-10.

## 9.0 REPORT OF ADVERSE EVENTS

Subjects will be told of any possible adverse reactions or side effects from exposure to this method or product. Oral irritation is possible. If side effects occur, it is expected to be mild and temporary. Any event will stop when the subject stops being exposed to the instrument or returns to their normal oral hygiene. The investigators will record any and all adverse reactions and report this documentation to the Product Safety Assurance Department. The nature of the reaction and any correlation with product usage will be assessed. If the evidence indicates that the adverse reaction may be due to product usage, the subject will be instructed to discontinue product use and appropriate treatment will be provided.

In the event of a medical emergency, immediately go to the emergency department or call 911. In the event of an adverse experience, emergency (once stabilized by appropriate medical care) or other problems or questions regarding participation in this study participants can contact the following investigators:

Dr. Michael Glogauer at (905) 973-0664.

### 9.1 Definitions

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Adverse Events (AEs) and Serious Adverse Events (SAEs) are defined by the ICH Guideline for Good Clinical Practice (ICH GCP) as follows:

**Adverse Event:** Any untoward medical occurrence in a patient or clinical investigations subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

AEs include any clinically significant deterioration of a subject's medical status, after being enrolled and signing an Informed Consent Form. The AE may involve any organs or systems and can be represented by the new onset or the deterioration of a disease, a syndrome, a symptom, a physical sign, as well as by findings and results of instrumental examinations and laboratory tests. Any medically relevant and untoward change from study enrollment, including frequency or pattern changes for a fluctuating condition (e.g., migraine), occurring after signing an informed consent is an adverse event. All such occurrences must be recorded and reported accordingly, whether they appear causally related to the study medication, or not.

**Serious Adverse Event:** Any adverse event occurring at any dose that results in any of the following outcomes:

- Death
- Life threatening adverse event
- Inpatient hospitalization, or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Temporary loss of daily function

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such important medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

**IMPORTANT NOTES:** The concepts of Adverse Event / Experience<sup>1</sup> (AE) and Serious Adverse Event / Experience (SAE) represent **regulatory** instruments used to evaluate and monitor the safety of clinical trial subjects. Therefore, these terms only apply in light of their regulatory definition. The term “serious”, in a regulatory sense, does not necessarily mean “severe”. All adverse events (serious and non-serious) reported during a study will be taken into account when analyzing the study data and establishing the safety profile of the investigational drug, and will be included in the final study report. The SAE concept is primarily used to identify, during the

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conduct of the trial, those adverse events that may require an expedited reporting procedure to regulatory authorities.

**Death:** The outcome of death requires that the AE that resulted in death be reported as an SAE. Death, in and of itself, is not an AE; it is only an outcome. The cause of death is the AE; therefore, the investigator should make every effort to obtain and document the cause of death for all subjects who die during the study. If, despite all efforts, the cause of death remains unknown, the AE it should be documented as “unspecified fatal event”.

**Life-threatening Adverse Event:** Any adverse event that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (*i.e.*, it does not include reaction that had it occurred in a more severe form, might have caused death).

**Hospitalization:** It should be noted that hospitalization, in and of itself, does not represent an SAE. It is the adverse event leading to the subject’s hospitalization that becomes “serious” when it requires inpatient care. Consequently, SAE should not be reported in case of pre-planned hospitalizations for pre-existing conditions that did not worsen during the study.

**Disability:** A substantial disruption of a person’s ability to conduct normal life functions.

## 9.2 Documenting and Reporting Adverse Events

### 9.2.1 General Procedures for All Adverse Events

All clinical complaints, symptoms, or signs that meet the adverse event definition will be recorded on the Adverse Reaction Form (Appendix 5) using a recognized medical term or diagnosis that accurately reflects the event. Source documentation should be maintained that allows for clear identification of each adverse event and the following parameters required for the form:

- AE description
- Date of onset
- Date of resolution
- Outcome
- Severity
- Seriousness
- Relationship to study drug (causality)
- Actions taken

Adverse events will be assessed by the investigator or designee for severity, relationship to the study product, possible etiologies, and whether the event meets the criteria as a serious adverse event and therefore requires immediate notification of the sponsor.

For data collection purposes, the outcome of all adverse events recorded on the Adverse Reaction Form will be designated as of the completion of the final evaluation or examination. However, the investigator is responsible for following all adverse events until resolution or until no longer of clinical concern, and providing these data to the sponsor.

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### **9.2.2 Reporting Procedures for Serious Adverse Events**

Any adverse event that is serious or potentially serious requires additional detailed reports and follow-up. A serious adverse event must be reported via telephone to the sponsor's representative immediately (within 24 hours) so as to facilitate discussion and implementation of necessary follow-up measures, and to enable the sponsor to submit necessary reports to regulatory authorities and other investigators. Following the initial telephone notification, the investigator must complete and submit a Serious Adverse Event Report Form to the sponsor within one calendar day. Serious Adverse Event Report Forms will be provided to the investigator upon initiation of the study. Once the sponsor reviews the Serious Adverse Event Report Form, additional information may be requested from the investigator to allow appropriate medical evaluation and determine the regulatory reporting requirements.

The investigator is responsible for following all adverse events, especially those deemed "serious", until resolution or until the event is no longer of clinical concern, and for providing these data to the sponsor in an agreed-upon format. The investigator is also responsible for reporting all serious adverse events to the Institutional Review Board (IRB) overseeing the conduct of the study at the respective study center, according to the rules and procedures established by the IRB.

Safety will be evaluated by oral examinations, clinical laboratory evaluations, descriptive analysis of AEs (including incidence, severity, seriousness, and relatedness), and immune response, over the entire period of the study.

## **10. COMPENSATION**

Participation in research is voluntary; no financial compensation will be provided. The principal investigator/study team will also provide proper oral hygiene instructions to participants.

## **11. QUALITY ASSURANCE/CONDUCT OF THE STUDY**

This clinical research study will be conducted in compliance with this protocol and U.S. Federal Regulations governing informed consent (21 CFR 50), Institutional Review Board (21 CFR 56), applicable regulations governing Investigator conduct (21 CFR 312) and/or any local regulatory agency (where applicable).

It is the responsibility of the Investigator to ensure that all subject data are collected and reported according to the study protocol. Subject records will indicate subject and data that is unique to this study and the subjects. Proper documentation of all adverse events and final resolutions will be maintained. Case report forms will be used for recording all clinical data. All CRFs used in this study will be provided by the study sponsor. The Investigator will be responsible for maintaining original consent forms, case report forms (CRFs), and other source documentation.

These are examples of case report forms that can be used to collect information/data while conducting this study:

- Subject Assignment Log (Randomization chart)
- Demographic Log
- Inclusion/Exclusion Criteria Form
- Continuance Criteria
- Adverse Event Form

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- Oral Hard & Soft Tissue Exam Form

Forms may be added or deleted, if deemed necessary, by agreement between the PI (principal investigator) and study sponsor prior to the study start date.

### **12. DATA MANAGEMENT RESPONSIBILITIES**

All sample analysis will be conducted by Ostia Sciences within the 124 Edward St Toronto location, and all data will be handled by the clinical research site staff under the direction of the Principle Investigator. The Investigator will keep a copy of every document (clinical and laboratory) related to the research study.

#### **12.2 Subject Termination/Procedures**

A genuine effort will be made to determine the reason(s) why a subject fails to submit the Day 7 Completion Kits. Subjects could be dropped from the study if any of the following occur:

1. Subject fails to substantially comply with the protocol requirements.
2. Subject fails to mail off samples.
3. Subject is treated with medications during the course of the study, which may interfere with the parameters of the study.
4. Subject develops an adverse reaction. The study Investigator will immediately notify the study monitor and information will be recorded on Adverse Reaction Form.
5. Subject receives emergency dental or medical treatment, which may interfere with the parameters of the study.
6. Sponsor elects to terminate the study.
7. Subject elects to terminate participation in the study.

#### **12.3 Removal of Subjects From Study**

Subjects will be dropped from the study if they receive emergency dental treatment, which in the opinion of the monitor could influence the parameters of the study. Any subject treated with an antibiotic during the study will be dropped from the study. Either the investigator or the sponsor may terminate the study at any time for well documented reasons, provided written notice is submitted at a reasonable time in advance of the intended termination.

#### **12.4 Pregnancy**

No pregnant women will intentionally be enrolled in this study. All female subjects being considered for this study will be asked about their pregnancy status. All enrolled female subjects will be required to report to the clinical/research investigator if they become pregnant during the 7-day. In the event a woman enrolled in this clinical research study becomes pregnant at any time during this study, participation in this study will be terminated upon the clinical staff's notification of the event. The subject's medical records used in this study will be updated to reflect the pregnancy and there will be follow-up contact until the end of the pregnancy to record the outcome in the clinical file.

#### **12.5 Ethical and Regulatory Requirements/Human Subject Protection**

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Participants may opt out of the study at any time. All samples and data will be relayed to researchers in the absence of identifying information; therefore, no personal information or results will be relayed back to participants. The testing done in this study is for research purposes only and not intended to make any medical diagnosis.

### **12.6 Protocol Approval, Study Monitoring and Compliance**

Prior to initiation of the study, the Investigator will obtain approval from an Independent Ethics Review Board (IRB) for the study protocol, the informed consent document, study instructions, and any forms of advertising in compliance with regulations. The reviewers will also review any change(s) in the protocol before the change is initiated.

### **12.7 Adherence to Protocol/Amendment(s)**

The Investigator will be required to adhere to the final protocol. Any changes to the protocol, except those necessary to eliminate apparent hazards, will require prior approval by the local reviewers through the submission of a protocol amendment. These changes to the protocol must be implemented only through formal written protocol amendments and only upon joint approval by the sponsor and investigator. If a protocol amendment requires changes to the informed consent form, the revised consent form must also be approved by any local board.

Departures from eligibility requirements may be allowed on a case-by-case basis by the medical monitor or other authorized sponsor representative. Such departures must be medically and scientifically justified, must be pre-authorized, and must be documented in the case report form and tracked as official eligibility waivers.

### **12.9 Advertising**

No newspaper, radio, or television advertising will be used for recruitment purposes. A flyer was created to recruit participants and will be approved by an Institutional Review Board/Ethics Board prior to posting or dissemination (Appendix 2).

### **12.10 Informed Consent Process**

Written informed consent will be obtained from all subjects prior to their enrollment into the study. The purpose and description of the study in lay language, possible adverse reactions, risks and benefits of participation and the subject's right to withdraw without prejudice at any time must be explained to each subject in the presence of a witness. The subject must read, understand and sign the informed consent form provided (Appendix 8). The informed consent form and any other written information for subjects should meet local requirements of language and interpretation (i.e., non-English speaking subjects must be presented with informed consent forms in a language that they can understand). The consent form will comply with all applicable regulations governing protection of the participating subjects in the study, and include basic elements specified in the U. S. Code of Federal Regulations, 21 CFR 50.25(a) and 50.27 and ICH-GCPs, Chapter 4, subpart 4.8. The Informed Consent form will be reviewed and approved by the clinical site's IRB.

Each subject will be given unlimited time to read the consent form and ask questions. Subjects who agree to participate will be asked to sign and date an IRB-approved informed consent form. A copy of the signed and dated consent form will be given to each subject prior to their participation in the study. The original signed and dated informed consent document will be

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retained by the Investigator. All informed consent forms will be documented in a log by date and subject ID; the log will be kept as source documentation. All study procedures must be explained in non-technical terms. Study personnel will assure that participants are clearly informed regarding their roles and obligations to protect vulnerable subjects and ensure they are not under coercion or undue influence. Subjects have the right to withdraw consent at any time.

### **12.11 Confidentiality**

All records of subject participation in this study are confidential and these records are available only to the investigator, specifically trained site personnel, supervising dentist/examiner, potentially the sponsoring company and IRB. In addition, the identity of participating subjects must be protected.

Only investigators (and specifically trained clinical site staff) will collect and have access to a subject's private information (e.g., name, medical records, etc.). Investigators will assign a study number to subjects which will be used to conceal their identity on all case report forms and other documents prior to their sharing with the broader study team, including the sponsor. Documents that identify the subject by name (e.g., the signed informed consent form or health questionnaire) will not be transferred or submitted to the sponsor and will be maintained in strict confidence by the investigator, except to the extent necessary to allow auditing by the sponsor personnel to verify subject, product safety and study compliance. The results of the study may be published in a scientific journal or a government public clinical database. If any publication occurs, only the subject's study number/ID, gender and/or age may be used.

### **12.12 New Findings**

Subjects will be informed of any significant new findings related to study products or procedures when they become known during the course of this clinical research study. Such information may affect the subject's decision to continue participation in the study.

## **ADMINISTRATIVE ASPECTS**

### **12.13 Curriculum Vitae**

The investigator will complete the FDA form 1572 and provide the sponsor with copies of his/her curriculum vitae and those of all sub-investigators listed on the form, at sponsor's request.

### **12.14 Data Collection in the Case Report Form**

All study data will be recorded in the case report form supplied by the sponsor. All entries will be written clearly in black ink. Only the principal investigator, sub-investigators, or study coordinators may make entries in the case report forms. If erroneous data are entered on the case report forms, corrections to the data must be made by crossing out the incorrect entry with a single line (such that the initial entry remains legible) and entering the correction. All corrections on a case report form will be initialed and dated by the investigator, sub-investigator, or study coordinator making the correction.

### **12.15 Documentation of Consent and Storage of Study Documents**

All informed consent forms will be documented in a log by date and subject ID; the log will be kept as source documentation. Informed consent forms will be stored in a secure locked room

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designated for research charts storage in the clinical site. The Investigator will provide each subject with a clear and understandable consent letter regarding the processing in connection with the study of personal data (i.e., any information relating to an identified or identifiable individual) by the investigator, sponsor and other persons involved in the study (Appendix 8). Each subject will be given a copy of the consent letter/form which will be referenced as part of the informed consent process.

Any deviation to the consent letter as regards processing by the sponsor (including its vendors, monitors and other representatives) must be agreed with the sponsor. In addition, the Investigator is responsible for ensuring that all study subjects understand and complete the consent letter/form, and that it includes any local requirements regarding data security and privacy laws and regulations applicable to the study and the subjects. Should the consent letter/form fail to meet any applicable local requirements, the Investigator is responsible for amending the consent letter/form to bring it into compliance with local applicable regulations and the Investigator (including Principal and sub-Investigators) agrees to indemnify and be liable to the Sponsor for any damages resulting from such non-compliance. Subjects' study charts will be stored in a secure locked room designated for research charts storage in the clinical site. The chart room is a limited- access area and only delegated study personnel will have access to the study charts and subjects' data.

### **12.16 Study Management**

Under the direct supervision of the Principal Investigator, certain duties may be delegated during the course of the study. These responsibilities will be documented on the Transfer of Responsibilities form maintained in the Investigator's clinical file for the study.

### **12.18 Final Report**

Following the completion of the study, the Investigator shall prepare a final study report. The final report will include a general description of the conduct of the study including protocol deviations, subject withdrawals, discussion of any adverse events, safety and efficacy data, laboratory data, and statistical analysis of the data if available. This report will be approved and signed by the Principal Investigator.

### **12.19 Record Retention and Access to Source Data/Documents**

Source documents must be kept for at least five (5) years after terminating the study. The Investigator will maintain all study documentation for all subjects entered into the study in a secure area ensuring the confidentiality of the information collected. Securing records includes placing written forms in locked file cabinets and/or sealed and labeled storage boxes in a locked room. Access will be denied to all persons with the exception of the Principal Investigator and his designees. The Sponsor will be notified before any destruction of study documents occurs.

### **12.20 Publication**

All manuscripts or presentations based upon this study, including press statements and internal public notices and memoranda must be submitted to the sponsor for review and approval prior to release for publication or presentation. This review period will be up to 60 days in duration. Review of an abstract may be expedited in some circumstances.

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### 13. References

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## APPENDICES

Appendix 1. Table Clinical Trials Utilizing *S. Salivarius*

Study Design	Population	Dose and Duration	Safety-Related Outcomes	Reference
Prospective, randomized, single-blind, placebo-controlled pilot study	Children (M & F; 6 to 11 y) who had at least 3 episodes of microbiologically documented infections with clinical symptoms suggesting Group A beta-hemolytic Streptococcus (GABHS) pharyngitis.  Initial n = 84 Final n = 82	<b>Test:</b> <i>S. salivarius</i> 24SMB and <i>S. oralis</i> . The mix suspension consisted of a minimum of $125 \times 10^9$ (CFU/mL) in 10 mL of saline. <b>Control:</b> matched placebo  2 puffs once a day, with an oral spray that provided $2 \times 10^9$ CFU per puff.  For 3 months	All patients were compliant to the assigned intervention to them (> 80% compliance). Only four patients reported an adverse event—three in the probiotic group and one in the control group. A mild cough was reported by two patients in the probiotic group versus one patient in the control group. Only one patient in the probiotic group reported nausea.	(Andaloro et al., 2019)
Double-blind, randomized, two-arm parallel-group	Healthy adults (M & F; 20 to 24 y)  Initial n = 31 Final n = 30	<b>Test:</b> <i>S. salivarius</i> K12 ( $>1 \times 10^9$ CFU in 1 tablet) <b>Control:</b> matched placebo lozenge  1 lozenge per day  For 4 weeks	One participant from the probiotic group was lost in follow-up due to sickness not related to the intervention (COVID-19). No adverse effects were registered.	(Babina et al., 2022)
Randomized, open-label, controlled, parallel	Children (M & F; 4 to 10 y) with black teeth stains  initial n = 58 (29/group) final n = 54	<b>Test:</b> no less than $1 \times 10^9$ CFU/day <b>Control:</b> no intervention was administered to the control group  For 3 months	Four participants (one in the test group and three in the control group) were excluded from the study	(Bardellini et al., 2020)

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			because they started antibiotic therapy. However, the study authors did not report any adverse events observed by the participants.	
Randomized, triple-blind, placebo-controlled, parallel	Participants (M & F; 10 to 30 y) wearing orthodontic braces  initial n = final n = 64	<b>Test:</b> $7.2 \times 10^9$ CFU/day <b>Control:</b> matched placebo lozenge  For 1 month	According to the study authors, no adverse events were observed or recorded during the trial.	(Benic et al., 2019)
Randomized, double-blind, placebo controlled	Healthy adults performing regular exercise training (M & F; 20 to 25 y)  Initial n = 24 Final n = 20	<b>Test:</b> $5 \times 10^9$ CFU/day <b>Control:</b> matched placebo tablet  For 30 days	The study authors did not report any adverse events observed by the participants. Four subjects abandoned the study for personal reasons. The authors showed an adherence rate of 94%.	(Bertuccioli et al., 2023)
Randomized, parallel. Participants were blinded. Blinding of investigators was not reported.	Healthy adults (18 y and older; average age 19 y; gender not reported)  initial n = 75 final n = not reported	<b>Test 1:</b> $1 \times 10^6$ CFU/day <b>Test 2:</b> $1 \times 10^7$ CFU/day <b>Test 3:</b> $1 \times 10^8$ CFU/day <b>Test 4:</b> $1 \times 10^9$ CFU/day  For 28 days	The study authors did not provide information on any adverse events that may have been experienced by the study participants.	(Burton, Wescombe et al., 2013)
Randomized, double-blind, placebo controlled, parallel	Children (M & F; 5 to 10 y) with a history of dental caries  initial n = 100 final n = 83	<b>Test:</b> $7.2 \times 10^9$ CFU/day <b>Control:</b> matched placebo lozenge  For 3 months	11 participants withdrew for various reasons, including not liking the taste of the lozenges (n=6), protocol deviations (n=1), and being lost to	(Burton et al., 2013)

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			follow-up (n=4). In addition, data from six participants were excluded from the analysis because they did not comply with the study requirements, consuming less than 75% of the prescribed lozenges per month.	
Open-label, single-arm	Healthy adults (M & F) n = 14	4×10 <sup>9</sup> CFU/day, 3 days	No adverse effects	(Burton et al., 2006)
Open-label, observational	Healthy adults (M & F) n = 23	<p><b>Test:</b> &gt;1×10<sup>9</sup> CFU/lozenge</p> <p><b>Control:</b> placebo lozenge</p> <p>3-day regimen of CHX rinsing, followed by intake of lozenges (test, control) at 2h intervals over 8h for 3 days (&gt;4×10<sup>9</sup> CFU/day). Subsequently, subjects in the test group (n=13) took the lozenge twice daily (morning &amp; night) for 2 weeks. Two of these subjects continued to take 2 lozenges/day for 28 days (&gt;2×10<sup>9</sup> CFU/day).</p> <p>For 3 days, 2 weeks (test group only), 28 days (2 subjects in test group only)</p>	The study authors did not report any adverse events observed by the participants.	(Burton, Chilcott et al., 2006)
Randomized, parallel  Blinding not reported	Healthy adults (M & F; mean age 19 y)  initial n = 100 final n was not reported	<p><b>Test 1:</b> 1.5×10<sup>9</sup> CFU/day</p> <p><b>Test 2:</b> 1.1×10<sup>8</sup> CFU/day</p> <p><b>Test 3:</b> 2×10<sup>7</sup> CFU/day</p>	The participants did not report any adverse reactions.	(Burton et al., 2010)

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		<p><b>Test 4:</b> <math>1 \times 10^6</math> CFU/day</p> <p><b>Test 5:</b> <math>7.5 \times 10^4</math> CFU/day</p> <p>for 14 days</p>		
Randomized, double-blind, placebo controlled, parallel	<p>Healthy adults (M &amp; F; age 20 to 60 y)</p> <p>initial n = 56 final n = 53</p>	<p><b>Test:</b> <math>1.1 \times 10^{10}</math> CFU/day</p> <p><b>Control:</b> matched placebo</p> <p>For 28 days</p>	<p>There was no significant difference found between the test and control groups in terms of oral health and gastrointestinal symptoms assessed using a 10-point VAS. No serious adverse events occurred in either group and the proportion of participants reporting any adverse events was similar in both groups. Hematology and clinical chemistry parameters showed no significant difference between the groups. However, the specific gravity of the urine was slightly higher in the placebo group compared to the test group, but still within normal limits.</p>	(Burton et al., 2011)
Double-blind, randomized placebo-control, prospective trial	<p>Healthy adults (M &amp; F; age 21 to 45 y) who practice good oral hygiene</p> <p>Initial n = 25 Final n = 24</p>	<p><b>Test:</b> powder sachet (1.5 g) contained approximately <math>7.77 \times 10^9</math> CFU of <i>L. acidophilus</i> DDS-1®,</p>	<p>The authors found that the treatment was well tolerated by the study population except for one</p>	(Cernioglo et al., 2021)

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		<p>8.25 x 10<sup>9</sup> CFU of <i>B. lactis</i> UABla-12, and 2 x10<sup>9</sup> CFU of <i>S. salivarius</i> BLIS K12™</p> <p><b>Control:</b> matched placebo</p> <p>one daily dose (one 1.5 g sachet) of probiotic powder mixed with any liquid.</p> <p>For 2 weeks</p>	<p>participant who experienced hives during supplementation which disappeared when supplementation ceased. All other participants were compliant. The overall attrition rate for this study was 4%.</p>	
<p>Randomized, double-blind, placebo controlled, parallel</p>	<p>Infants (M &amp; F, 7 to 13 months) with high risk of acute otitis media</p> <p>initial n = 224 final n = 202 by 2-month visit, 166 by 12-month visit</p>	<p><b>Test:</b> follow-up formula containing proB (<i>S. thermophilus</i> NCC 2496, <i>S. salivarius</i> DSM 13084 [K12], <i>L. rhamnosus</i> LPR CGMCC 1.3724) and preB [Raftilose/Raftiline])</p> <p><b>Control:</b> follow-up formula only</p> <p>Formula contained 2.5×10<sup>7</sup> CFU/g <i>S. salivarius</i> (1×10<sup>9</sup> to 2×10<sup>9</sup> CFU/day according to GRN 807).</p> <p>For 12 months</p>	<p>The study authors reported that both the test and control formulas were well-tolerated. The main reason for discontinuation was non-compliance with the study protocol, specifically, missing three consecutive days per month with less than 300 mL of milk consumed per day. The majority of the reported adverse events (93.1%) were not considered related to the study. There were five adverse events considered likely related, with four in the test group and one in the control group. These included lack of appetite</p>	<p>(Cohen et al., 2013)</p>

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			for milk, regurgitation, dry skin, chronic diarrhea, and abdominal pain. One adverse event, constipation, was considered related, but no further details were provided.	
Open-label  Study was not randomized and not blinded.	Healthy subjects (M & F; 19 to 64 y)  Initial n = Final n = 23	<b><u>Test:</u></b> <i>Streptococcus salivarius</i> 24SMBc and <i>Streptococcus oralis</i> 89a nasal spray (10 <sup>9</sup> CFU/dose)  two bilateral spray injections into each anterior nostril/day  For 7 days.	No severe side effects were recorded in any enrolled patients after the treatment. Only 10% of the subject suffered from allergic cold, and 80% did not show any problems breathing. However, 90% of people reported nasal dripping immediately after the treatment administration.	(De Grandi et al., 2019)
Open-label  Study was randomized but not blinded.	Children (M & F; 6 to 17 y) at high risk for dental caries  initial n = final n = 76	<b><u>Test:</u></b> no less than 1×10 <sup>9</sup> CFU/day <b><u>Control:</u></b> no intervention was administered to the control group  For 90 days	No participants withdrew. <i>S. salivarius</i> M18 was found to be safe with no treatment-related side effects, and no participants dropped out. The majority of participants (35 out of 38) rated the tolerability of the treatment as "good" or "very good," and the remaining	(Di Pierro, Zanvit et al., 2015)

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			three participants rated it as "acceptable."	
Open-label  Study was not randomized and not blinded.	Children (M & F; 3 to 12 y) with and without recurrent streptococcal pharyngitis and/or tonsillitis  initial n = 82 final n = 78	<b><u>Test:</u></b> $5 \times 10^9$ CFU/day <b><u>Control 1:</u></b> no intervention was administered to controls with recurrent illness <b><u>Control 2:</u></b> no intervention was administered to controls without recurrent illness  For 90 days	The test tablet was reported to be well-tolerated, and there were no observed side effects. However, four participants in the test group were excluded from the analysis due to non-adherence to the study protocol, specifically, missing over 20 days of treatment.	(Di Pierro et al., 2012)
Open-label  Study was not randomized and not blinded.	Adults (M & F; 18 to 65 y) with recurrent oral streptococcal pharyngitis  initial n = final n = 40	<b><u>Test:</u></b> $5 \times 10^9$ CFU/day <b><u>Control:</u></b> no intervention was administered to the control group  For 90 days	All 20 subjects who received the test tablets successfully completed the study, and no participants dropped out. The study authors reported that the test tablet was well tolerated, and no side effects related to the treatment were reported.	(Di Pierro et al., 2013)
Open-label  Study was randomized but not blinded.	Children (M & F; 3 to 13 y) with recurrent oral streptococcal disorders  initial n = 61 final n = 60	<b><u>Test:</u></b> no less than $1 \times 10^9$ CFU/day <b><u>Control:</u></b> no intervention was administered to the control group  For 90 days	The study authors reported that the test tablet was well tolerated and did not cause any notable side effects. However, one subject dropped out of the study immediately	(Di Pierro et al., 2014)

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			after enrolment due to the poor taste of the test product.	
Open-label, single-arm	Children (M & F; 3 to 9 y) with recurrent secretory otitis media  initial n = final n = 22	<b>Test:</b> no less than $1 \times 10^9$ CFU/day  For 90 days	The safety profile of <i>S. salivarius</i> K12 was reported as very good, with no treatment-related side effects and no subject drop out. Tolerability was rated as good or very good in 20 of the 22 subjects, with the remaining 2 subjects rating it as acceptable.	(Di Pierro, Di Pasquale et al., 2015)
Open-label  Study was randomized but not blinded.	Children (M & F; 3 to 10 y) with recurrent streptococcal pharyngotonsillitis  initial n = final n = 124	<b>Test:</b> no less than $1 \times 10^9$ CFU/day <b>Control:</b> no intervention was administered to the control group  For 90 days	According to the study authors, the use of <i>S. salivarius</i> K12 was well-tolerated and highly compliant among the participants, and there were no reported side effects observed during the trial.	(Di Pierro, Colombo et al., 2016b)
Open-label  Study was randomized but not blinded.	Healthy children (M & F; 33 to 45 months)  initial n = final n = 222	<b>Test:</b> no less than $1 \times 10^9$ CFU/day <b>Control:</b> no intervention was administered to the control group  For 180 days	All children who were enrolled in the study completed it. The study authors noted that there were no observable side effects in the treatment group, both during the treatment period and the	(Di Pierro, Colombo et al., 2016a)

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			follow-up period.	
Retrospective observational	Children (M & F; 3 to 14 y) with recurrent non-streptococcal infection  n = 133	<b><u>Test:</u></b> no less than $1 \times 10^9$ CFU/day  For 90 days	The study authors reported that compliance and tolerability were excellent. They also noted only one side effect, which occurred in a 6-year-old boy who experienced a mild episode of bronchospasm after a few days of treatment with S. salivarius K12. However, the subject was able to continue with the study without any further incidents.	(Di Pierro et al., 2018)
Randomized, placebo controlled, parallel  Blinding not reported	Children at high risk of acute rheumatic fever (M & F; 5 to 14 y)  initial n = 1314 final n = 1137	<b><u>Test:</u></b> $2.5 \times 10^9$ CFU/day <b><u>Control:</u></b> matched placebo lozenge (*b)  209 days	The study authors did not provide any information about adverse events experienced by the participants. However, they reported that most children found the lozenges to be well accepted, with only two children refusing to take them regularly.	(Doyle et al., 2018)
Retrospective observational	Children (M & F; 3 to 7 y) with recurrent group A beta-hemolytic streptococci pharyngo-tonsillar infections  n = 130	<b><u>Test:</u></b> $1 \times 10^9$ CFU/day <b><u>Control:</u></b> no intervention was administered to the control group  For 90 days	All children were able to complete the study without stopping the test tablet before the	(Gregori et al., 2016)

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			intervention period ended.	
Randomized, double-blind, placebo controlled, parallel	Adults (M & F; 18 y and older) with severe acute pharyngotonsillitis  initial n = 60 final n = 53	<b><u>Test:</u></b> $4 \times 10^9$ CFU/day <b><u>Control:</u></b> matched placebo tablet  10 days	Seven participants were excluded from the study due to noncompliance with the treatment, but the authors did not mention if any adverse events were observed by the participants.	(Gilbey et al., 2015)
Randomized, double-blind, placebo controlled, parallel	Adults (M & F; 23 to 44 y) with tongue-coating associated halitosis  initial n = 33 final n = 28	<b><u>Test:</u></b> $2 \times 10^9$ CFU/day <b><u>Control:</u></b> matched placebo tablet  For 30 days	All participants in the study did not experience any adverse events. However, five participants were excluded from the study, with two participants in the control group and three in the test group, one of which was using antibiotics, and four were lost to follow-up.	(He et al., 2020)
Not applicable (single subject)	Single healthy adult (M), 40 y old	$4 \times 10^{10}$ CFU/day  For 3 days	No adverse events were reported	(Horz et al., 2007)
Randomized, double-blind, placebo controlled, parallel	Adults (M & F; >18 y) with oral candidiasis  initial n = 56 final n = 49 (safety-analyses)	<b><u>Test:</u></b> $\geq 2 \times 10^9$ CFU/day <b><u>Control:</u></b> matched placebo lozenge  For 4 weeks	The study did not report any severe adverse events. However, AEs were reported by 6 subjects in the test group and 8 subjects in the control group. One patient in the K12 group reported borborygmus	(Hu et al., 2019)

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			and pharyngeal discomfort, which was considered a possible drug-related adverse event by the study authors.	
Randomized, double-blind, placebo controlled, parallel	Adults (M & F: 18 y and older) with spondyloarthritis  initial n = final n = 63	<p><b><u>Test:</u></b> powder containing <math>1 \times 10^8</math> CFU/g of <i>S. salivarius</i> K12, <math>4 \times 10^8</math> CFU/g of <i>B. lactis</i> LAFTI B94, and <math>4 \times 10^8</math> CFU/g of <i>L. acidophilus</i> LAFTI L10</p> <p><b><u>Control:</u></b> matched placebo powder</p> <p>Participants were told to take 1 spoonful of powder (ca. 0.8 g) by mouth twice daily, corresponding to ca. <math>1.6 \times 10^8</math> CFU/day of <i>S. salivarius</i> K12</p> <p>For 12 weeks</p>	<p>All participants in the study completed it successfully. Minor and self-limiting adverse events were reported by 43.8% (14/32) of the test group and 38.7% (12/31) of the placebo group, with no significant difference between them. The most common AE in both groups was a change in bowel habit, reported by 7 participants in the test group and 6 in the placebo group. No serious AEs occurred during the study. At the end of the study, there was no significant difference between the test and control groups in terms of fecal calprotectin or change in bowel symptom questionnaire scores.</p>	(Jenks et al., 2010)
Double-blinded,	Healthy adults (M & F; 18 to 75 y)	<b><u>Test:</u></b> $1 \times 10^{10}$ CFU/g BLIS K12	The dose chosen for use	(Laws et al., 2021)

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placebo controlled human clinical trial	Initial n = 60 Final n = 53	<b><u>Control:</u></b> matched placebo  single 1 g dose mixed with 30 mL water and swallowed directly.  For 1 day	in this study was relatively high, but safe, dose to administer. No adverse reactions were reported by the authors.	
Open-label, single-arm	Patients with rheumatoid arthritis taking stable doses of sulfasalazine (M & F; mean age = 56 y)  initial n = final n = 12	A powder blend (BioRestore™) containing <i>S. salivarius</i> K12 at $1 \times 10^8$ CFU, <i>L. acidophilus</i> L10 at $4 \times 10^8$ CFU, <i>B. lactis</i> B94 at $4 \times 10^9$ CFU.  The powder was taken twice a day for total <i>S. salivarius</i> K12 of $2 \times 10^8$ CFU/day.  For 7 days	During the intervention period, four patients reported adverse events (AEs), with three reporting gastrointestinal disturbances and one experiencing a flare-up of rheumatoid arthritis. The reported AEs were of mild to moderate severity.	(Lee et al., 2010)
Randomized, non-blinded, controlled, parallel	Adults with oral lichen planus (M & F; 22 to 79 y)  initial n = final n = 40	<b><u>Test:</u></b> no less than $2 \times 10^9$ CFU/day <b><u>Comparator:</u></b> topical 0.1% triamcinolone acetonide dental paste  4 weeks	No adverse reactions were observed.	(Li et al., 2020)
Randomized, double-blind, placebo-controlled study	Healthy adults (M & F; 18 to 65 y) Initial n = 64 Final n = 60	Test: <i>S. salivarius</i> DB-B5 at $10^9$ CFU Control: matched placebo  1 sachet in approximately 4 ounces of bottled water daily  For 4 weeks.	The safety was confirmed. 4 individuals did not complete the study: One participant in the test group discontinued from the study because they changed their mind about participation. The remaining 3 participants were from the placebo group, and they were discontinued	(Li et al., 2021)

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			<p>from the study for the following reasons: physician decision due to elevated eosinophils in the baseline blood sample (n = 1), occurrence of adverse event (n = 1), and lost to follow-up (n = 1). There was a total of 15 adverse events reported in 6 participants in the study. In the test group, 2 participants reported a total of 5 adverse events throughout the study that were considered “possibly related” to the interventions. All the events were mild in nature and resolved on their own. Moreover, a similar frequency of adverse events was reported in the placebo group, in which 4 participants reported a total of 10 adverse events, including one individual discontinued from the study due to the</p>	
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			occurrence of mild urticaria.	
A prospective, single-open, multi-centre study	Caucasian children with recurrent respiratory infections (RRIs) (M & F; 1 to 12 y)  Initial n = 100 Final n = 91	<b><u>Test:</u></b> <i>Streptococcus salivarius</i> 24SMBc and <i>Streptococcus oralis</i> 89a nasal spray (10 <sup>9</sup> CFU/dose)  2 puffs for nostril twice/day for 7 days/months.  For 3 months.	Although a good tolerability profile was reported, 9 children experienced burning nose, leading to interruption of therapy. However, none of the children were withdrawn from the study because of adverse events. The authors suggest that this treatment is safe and seems to be effective on short-term in the treatment of RRIs	(Manti et al., 2020)
Open-label  Study was randomized. Blinding not reported.	Adults (M & F; 67 to 83 y) who are denture wearers.  initial n = final n = 50	<b><u>Test:</u></b> BactoBlis™ containing <i>S. salivarius</i> K12 (10 <sup>9</sup> CFU/day) <b><u>Control:</u></b> no intervention was administered to the control group  For 30 days	The study authors did not provide information on whether any adverse events were reported by the study participants.	(Passariello et al., 2020)
Open-label, single-arm	Infants (age and sex not reported) prone to otitis media scheduled to undergo ventilation tube placement  n = 19	<b><u>Test:</u></b> powdered formulation with <i>S. salivarius</i> K12 (reported as 1×10 <sup>10</sup> to 3.4×10 <sup>10</sup> CFU/day in GRN 581)  1 teaspoon was placed on the child's tongue twice daily.  For 10 days	The study authors did not provide information on whether any adverse events were reported by the study participants.	(Power et al., 2008)
Open-label  Study was	Children (M & F; 1 to 6 y) attending daycare centers	<b><u>Test (children ≤3y old):</u></b> <b><u>powdered</u></b>	The administration of <i>S. salivarius</i>	(Sarlin et al., 2021)

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randomized. Microbiologic al analyses were blinded.	initial n = 121 final n reported as number of biological samples collected at 1-month and 2- month time period	<u>formulation with <i>S. salivarius</i> K12</u> <u>(<math>5 \times 10^9</math> CFU/sachet)</u> <b><u>Test (older children):</u></b> <u>Chewable tablet containing <i>S. salivarius</i> K12</u> <u>(<math>1 \times 10^9</math> CFU/tablet)</u> <b><u>Control: no intervention was administered to the control group</u></b>  <u>Daily dose provided was</u> <u><math>5 \times 10^9</math> CFU/day (powder) and</u> <u><math>1 \times 10^9</math> CFU/day (tablet).</u>  For 30 days	K12 did not cause any changes in the diversity of the nasopharyngeal or saliva microbiome. However, there was a temporary increase in the proportion of <i>S. salivarius</i> in the saliva of children who received the product containing <i>S. salivarius</i> K12.	
Randomized, double-blind, placebo controlled	Adults (M & F; mean age 53.5 y in placebo, 53.3 y in test) who had received radiotherapy in the previous 6 months  initial n = 17 final n = 13	<b><u>Test:</u></b> $3.5 \times 10^9$ CFU/day <b><u>Control:</u></b> matched placebo lozenge  For 4 weeks	Four participants dropped out of the study: three from the placebo group and one from the test group. Two participants were lost to follow-up, one received antibiotics, and one did not take the lozenges as instructed. The study authors did not mention whether any adverse events were reported by the participants.	(Vesty et al., 2020)
randomised double-blind				

# **RECRUITING HEALTHY ADULT MEN AND WOMEN FOR A SHORT RESEARCH STUDY LOOKING AT PROBIOTIC LOZENGES**

## **Why are we doing this?**

Gum disease is a common condition in the mouth.

This study is looking to see if lozenges can be used to provide oral probiotics, which may help treat gum disease in adults. All Participation is voluntary.

## **What participants must do?**

**Using at home test kits, while being on oral probiotic lozenges for 7 days**

## **Where will this study take place?**

124 Edward St Toronto

## **Will I be compensated?**

**No compensation will be paid.**

## **How do I sign up?**

Please email us at [contact@ostia-sciences.com](mailto:contact@ostia-sciences.com) expressing participants interest in taking part in this study

# Protocol ID: OstiaPhase0

## Appendix 3. Recruitment Questionnaire

### Recruitment Questionnaire

1. Name \_\_\_\_\_

2. Birth Date (Month/Day/Year) \_\_\_\_\_

3. Profession \_\_\_\_\_

**4. Oral Hygiene Habits:**

Do you brush your teeth? Yes No

If so, how many times a day? \_\_\_\_\_

Do you use dental floss? Yes No

If so how often? \_\_\_\_\_

**5. Dental Visit History:**

Have you visited a dentist before? Yes No

When was your last visit to the dentist (if applicable)? \_\_\_\_\_

At your last dental visit what was done (if applicable)? \_\_\_\_\_

**6. Smoking History:**

Do you smoke cigarettes? Yes No

Do you use any other tobacco product(s)? Yes No

Do you smoke anything other than cigarettes? Yes No

**7. General Health:**

Do you suffer from any major illness? Yes No

If so what are they? \_\_\_\_\_

Do you take any medications? Yes No

If so what are they? \_\_\_\_\_

Do you have any allergies?

If so what are they? \_\_\_\_\_

8. Are all your teeth missing? Yes No

9. Do you wear a complete denture? Yes No

10. Do you wear a partial denture? Yes No

11. Are you pregnant (if applicable)? Yes No

12. Are you currently nursing (if applicable)? Yes No

**13. English Language Proficiency:**

Do you require English translation? Yes No

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Are participants able to read in English? Yes No

Eligible for the study? Check One	
<b>YES</b> <input type="checkbox"/>	<b>NO</b> <input type="checkbox"/> <b>Reason(s) (violation of inclusion/exclusion criteria):</b>

Name of Assessor: \_\_\_\_\_

Signature of Assessor: \_\_\_\_\_

Date: \_\_\_\_\_

# Protocol ID: OstiaPhase0

## Appendix 4. Personal Information Form

Participants Trial Number

*Office use only*

### **Personal Information**

Surname		First Name	
Middle Name	Date of birth	Male <input type="checkbox"/>	Female <input type="checkbox"/>
Occupation			

### **Mailing Address**

Address (#, street, unit)			
City	Province	Postal Code	Country

### **Phone Numbers/E-mail Address**

Home Phone	Cell Phone
E-mail Address	

# Protocol ID: OstiaPhase0

## Appendix 5. Adverse Events Form

### Ostia Sciences Non-Serious Adverse Event/ Adverse Reaction Form

**Instructions:** Do not leave any field blank. Please indicate if information is unknown, not provided or not available (refused). Please note that this form is expandable.

Date of Awareness (dd/mmm/yyyy):

Protocol #:

Protocol Title:

Indication of use protocol (if applicable):

Investigator:

Study Originator/Director:

**Type of study:**

<input type="checkbox"/> Clinical	<input type="checkbox"/> Consumer	<input type="checkbox"/> Panel
-----------------------------------	-----------------------------------	--------------------------------

**Product Category:**

<input type="checkbox"/> Fabric care	<input type="checkbox"/> Household Surface care	<input type="checkbox"/> Oral Care
<input type="checkbox"/> Personal care	<input type="checkbox"/> Other:	

**Day of study the earliest event (s) occurred (Day 0 – Day 7):**

		<input type="checkbox"/> Other:

**Subject/Patient information:**

ID	Initials	Sex	Age	Weight	Ethnic group

**Product information:**

Product name (PIM#):	
Start date (dd/mmm/yyyy):	Stop Date or Duration (dd/mmm/yyyy):
Dosage:	Frequency:
Randomization group:	

**Reaction/Event information:**

Onset date (dd/mmm/yyyy)	Stop date or duration (dd/mmm/yyyy):	Severity (mild, moderate, severe)	Relationship to product (Possibly related, Related, Unrelated, Unknown)

Describe reaction(s)/event(s) in detail:

--

## Protocol ID: OstiaPhase0

### Outcome:

<input type="checkbox"/> Resolved, Date (dd/mmm/yyyy):	<input type="checkbox"/> Resolving	<input type="checkbox"/> Unknown
<input type="checkbox"/> Not resolved	<input type="checkbox"/> Resolved with sequelae	<input type="checkbox"/> Other:

### Action taken with the product:

<input type="checkbox"/> Continued	<input type="checkbox"/> Reduced
<input type="checkbox"/> Discontinued	<input type="checkbox"/> Unknown
<input type="checkbox"/> Temporarily discontinued	<input type="checkbox"/> Other:

Did the event(s) abate after product was stopped or dose reduced (yes/no)?

Did the event(s) reappear after product was reintroduced (yes/no)?

☐ Protocol Continued

☐ Protocol Discontinued

### Treatment rendered for the event(s):

**Relevant Medical History Data:** ☐ Yes (List below) ☐ None ☐ Not provided ☐ Unknown  
(Medical history with onset dates)

**Relevant Concomitant Medications:** ☐ Yes (List below) ☐ None ☐ Not provided ☐ Unknown  
(Medication name, dose, frequency, start/stop dates [dd/mmm/yyyy] or duration of therapy)

**Relevant Lab data:** ☐ Yes (List below) ☐ None ☐ Not provided ☐ Unknown  
(Lab test, results, dates [dd/mmm/yyyy])

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## Serious Adverse Events Report Form for Studies on Humans

**Instructions:** Please complete all fields of this form. Please indicate if 'Not provided,' 'Not Available' or 'Unknown.'  
(Date eg: dd/mmm/yyyy 01/Dec/2009)

Case-ID-No:

### A) Study site details

Study No.:	Centre Name:
Study type : clinical / consumer test / panel test	Investigator:
If clinical, please define:	Address:
Study product:	
Dosage form and strength:	Country of occurrence:
Indication if appropriate:	

### B) Reporter Information

Sender / Reporter	
Name:	Health professional: YES <input type="checkbox"/> NO <input type="checkbox"/>
Address:	Profession (Speciality):
Tel.:	Date of Awareness (dd/mmm/yyyy):
Fax:	Date of report:(dd/mmm/yyyy)
e-mail:	

### C) General information

#### Which product is concerned?

☐ Test Product    ☐ Comparator (Control product)    ☐ Placebo    ☐ Unknown

#### Specify the day that the event occurred during the study (between 0 – 7)?

#### Subject concerned:

Subject's ID-number:

Sex: ☐ Male    ☐ Female    Year of birth (dd/mmm/yyyy):

In case of presumption or intoxication: Weight (kg):

If Female, Pregnancy: ☐ NO    ☐ YES, For how many months?

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D) Information on SAE		
<b>Reason for serious adverse event report</b> <input type="checkbox"/> Death <input type="checkbox"/> Hospitalisation/Prolonged Hospitalization <input type="checkbox"/> Life Threatening <input type="checkbox"/> Congenital Anomaly <input type="checkbox"/> Disability/Incapacity <input type="checkbox"/> Other, specify: <input type="checkbox"/> Suspected transmission via a medicinal product of an infectious agent  If hospitalisation, give dates (dd/mmm/yyyy) From: To: <input type="checkbox"/> Hospitalization ongoing		
<b>Diagnosis / Symptoms</b> Please, indicate diagnosis or main symptom (s) and list serious most significant adverse event first: 1. 2. 3. 4.		
<b>Date of primary symptom (dd/mmm/yyyy) :</b>		
<b>Duration of taking the product:</b>		
<b>Outcome of serious adverse event :</b> <input type="checkbox"/> Not resolved <input type="checkbox"/> Resolving <input type="checkbox"/> Resolved with sequelae <input type="checkbox"/> Resolved, date: (dd/mmm/yyyy) <input type="checkbox"/> Unknown/ lost to follow up <input type="checkbox"/> *Death, date: (dd/mmm/yyyy)		
Cause of death if known:		
Autopsy: <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> Unknown <input type="checkbox"/> Outcome:		
<b>Action taken:</b> <input type="checkbox"/> Protocol continuation <input type="checkbox"/> Clinical study product dose reduced, new dose: <input type="checkbox"/> Clinical study product discontinued <input type="checkbox"/> Medical intervention <input type="checkbox"/> Exclusion of the patient <input type="checkbox"/> Other:		
<b>Event abated after drug stopped or dose reduced?</b> <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> N.A.		
<b>Event reappeared after drug reintroduction?</b> <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> N.A.		

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### Causality

☐ Related ☐ Possibly related ☐ Not related\* ☐ Unknown

\*If not related, please provide an alternative causality:

Code broken (unblinded)

☐ YES ☐ NO ☐ N.A.

### E) Case narrative

Please provide full details of the serious adverse event, dechallenge/rechallenge information and vital signs.

Attach any relevant reports from the source document or hospitalisation file. In case of death, report cause and attach a copy of the autopsy report, if performed.

Information enclosed: ☐ NO ☐ YES, specify:

### F) Relevant medical history

Description	Start Date (dd/mmm/yyyy)	Stop Date (dd/mmm/yyyy)

### G) Concomitant medication

Please report the medication taken in the last 4 weeks prior to the SAE(s)

Drug	Dose [unit]	Route	Frequency	Indication	Start Date (dd/mmm/yyyy)	Stop Date (dd/mmm/yyyy)

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### H) Clinical study products

Please indicate if Test product, Comparator (control product) or Placebo

Product	Lot. No.	Dose [unit]	Route administration	of Frequency	Start Date (dd/mmm/yyyy)	Stop Date (dd/mmm/yyyy)

### I) Relevant laboratory data & other test procedure for SAE

Test	Date (dd/mmm/yyyy)	Result (normal, abnormal, clinically significant)

This report has to be sent within **1 Calendar day (No later than 1 business day)**:

- By the Investigator to the Study Manager:
  - Address
  - Phone
  - E-mail
- By the Study Manager to **contact@osia-sciences.com**
  - Email:

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### Appendix 6. Subject Withdrawal Form

#### Subject Withdrawal Form

*This form should be completed for all subjects who do not complete full participation of the study.*

Subject ID Number	
Date of Withdrawal	
Person completing withdrawal form	
Date informed of withdrawal	
Name of person reporting the withdrawal	

Withdrawal was made at the request of (tick one :)

Subject \_\_\_\_\_ Investigator \_\_\_\_\_ Study Dentist \_\_\_\_\_ Assessor \_\_\_\_\_

REASON FOR WITHDRAWAL (Tick all that Apply)

	PROTOCOL VIOLATION (Give details)
	NON-COMPLIANCE (Give details)
	ADVERSE EVENT (Please attach copy of completed AE form)
	MEDICAL REASONS AND/OR EXCLUSION CRITERIA (Give details)
	SUBJECT'S PERSONAL REASONS (Give details)
	OTHER (Give details)

Person reporting withdrawal \_\_\_\_\_ Date \_\_\_\_\_

Investigators signature \_\_\_\_\_ Date \_\_\_\_\_

Product returned      Yes              No              NA              (circle as appropriate)

COMMENTS: \_\_\_\_\_

## Protocol ID: OstiaPhase0

### Appendix 7. Study Termination Form

#### Study Termination Form

**SUBJECT ID No** \_\_\_\_\_

#### MEDICATION HISTORY

Does the patient currently take any medications (including OTC products)? Yes No (Circle One)

If **YES**, please list all medications below:

Medication	Total Daily Dose	Date Started	Date Stopped (circle C if continuing)	Indication
			C	
			C	
			C	
			C	

\_\_\_\_\_  
Date

\_\_\_\_\_  
Clinical Examiner's Signature

# Protocol ID: OstiaPhase0

## Appendix 8. Informed Consent Form

### CONSENT TO PARTICIPATE IN A RESEARCH STUDY (CLINICAL TRIAL)

<b>Title</b>	A Short, Open, Phase 0 Study to Demonstrate the Viability of the Lozenge Dosage Form to Deliver SALI-10 Oral Probiotics
<b>Principal Investigator</b>	Dr. Michael Glogauer Tel: (905) 973-0664
<b>Sponsor</b>	Ostia Sciences Tel: (647) 643-7547

### **INTRODUCTION**

You are being invited to take part in a type of research study called a clinical trial. The information below will tell you about the study, what will be asked of you and if there are any risks and benefits to you. It is up to you to decide whether you would like to participate in the study. Please read the consent form very carefully and make sure that all of your questions are answered before you consent to take part. Before you make your decision, feel free to talk about this study with anyone you wish. Participation in this study is voluntary.

### **BACKGROUND AND PURPOSE**

Clinical trials are necessary to help us learn more about the safety of medicinal products, as well as how well they work. The clinical trial, which we are presenting to you here, has been reviewed and approved by the Veritas Independent Review Board (IRB), to be conducted in Canada. Veritas IRB is an independent Research Ethics Board (REB) based on Canada. The study was initiated and is funded by Ostia Sciences the sponsor of this study.

We are doing this research study to figure out how well a new oral probiotic in the form of a dissolvable oral lozenge works to improve a person's oral health. Probiotics are bacteria that protect human health by taking up space in our bodies. This leaves less places inside our bodies for harmful bacteria to grow. Gingivitis, or the inflammation of the gums, is commonly due to infection of the gum tissues that is caused by bacteria that attach to the teeth. When these bacteria are not properly removed (by brushing and flossing), the bacterial accumulation may cause the inflammation of the gums. Clinical signs of gingivitis include (but are not limited to) redness or swelling of the gums, and bleeding from the gums, usually after irritation such as brushing or flossing. Gingivitis is a completely reversible condition, with no long-term complications if adequate daily hygiene is performed. However, it is currently estimated that 80% of adolescents and adults have gingivitis. Once bacteria are attached to teeth, they are much harder to damage or kill, and common ingredients in commonly available toothpastes aren't always effective against all of them.

The purpose of the study is to evaluate an oral probiotic named SALI-10, and determine whether delivering it through a dissolvable lozenge can improve the bacteria composition in the human oral cavity.

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The results from this study will provide additional information for the use of SALI-10 oral lozenge probiotic in patients with gum disease.

### **STUDY DESIGN**

Everyone enrolled in this research study will receive SALI-10 oral lozenge probiotic to use according to instructions at home. There will be approximately 10 people enrolled in this study. Once you are enrolled over the phone, the principal investigator / study team will mail you 7 lozenges and 4 sample collection kits. On the first day you will do two sample collection kits at home and mail them to the principal investigator / research team. You will take one lozenge a day at home for 7 days. On the 7<sup>th</sup> day you will do two sample collection kits at home and mail them to the principal investigator / study team. When the kits are delivered to the principal investigator / study team, you will get a phone call to confirm that you followed the protocol.

### **STUDY VISITS AND PROCEDURES**

There will be no visits during this study.

If you decide to participate, you'll be asked to sign the consent form. At the start of the study, you'll be asked questions about your mouth, teeth and gums to determine if you are eligible for entry into the study. If you are accepted into the study, you will be required to follow a specific set of instructions. You will agree to use only the oral lozenges provided to you by the principal investigator / study team for the duration of the study. You will perform standard oral hygiene during the study. If you must receive emergency dental treatment, you will notify the principal investigator Dr. Michael Glogauer at (905) 973-0664. There will be no restrictions regarding your dietary habits.

Your participation in the clinical study will last up to 7 days as described below:

#### **Day 0**

You will complete two (2) at home test kits: one Saliva Collection Kit, and one (1) Oral Rinse Collection Kit. You will mail the sample kits to Ostia Sciences Inc, 124 Edward St., Toronto, Suit 463, ON Canada M5G 1G6, in the special envelopes provided. You will then take one (1) lozenge each day for 7 days. As per the instructions for the Saliva Collection Kit and the Oral Rinse sample collection, once the tubes are sealed, the tubes are to be placed in the provided special envelopes and sent to Ostia Sciences.

#### **Day 7 (Completion)**

24 hours after using the seventh (7<sup>th</sup>) lozenge, you will complete two (2) at home test kits: one Saliva Collection Kit, and one (1) Oral Rinse Collection Kit. You will mail the samples to Ostia Sciences Inc, 124 Edward St., Toronto, Suit 463, ON Canada M5G 1G6, in the special envelopes provided. As per the instructions for the Saliva Collection Kit and the Oral Rinse sample collection, once the tubes are sealed, the tubes are to be placed in the provided special envelopes and sent to Ostia Sciences.

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After completion of the study, study personnel will ask for your feedback with regards to your experience in the study. Any questions or concerns you may have will be discussed at that time and you will then be formally exited from the study.

While participating as a subject in this study, you cannot participate as a subject in any other clinical studies. In addition, you cannot use drugs, other than over the counter analgesics during the course of the study. Specifically, only Advil and Tylenol are allowed, and all other medications, whether prescription or Over-The-Counter, are not allowed. You agree to inform the investigator about any new medications you are planning to take, including but not limited to antibiotics, antiseptics, decongestants and antihistamines.

Instructions for the Test Kits are attached.

### **RISKS RELATED TO PREGNANCY**

Pregnant women must not take part in this study, neither should women who plan to become pregnant during the study. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her study doctor. In the event you are a woman enrolled in this clinical research study and you become pregnant during the course of the study, your participation in this study will be terminated upon the clinical staff's notification of the event. Your medical records used in this study will be updated to reflect the pregnancy and you will have follow-up contact until the end of the pregnancy to record the outcome in your file.

To ensure that no pregnant women are involved in the clinical trial, for sexually active female volunteers, one of the following methods must be used to prevent pregnancy during the study:

- hormonal methods or an IUD must be in use at least 30 days prior to first study drug administration;
- barrier methods must be in use at least 14 days prior to study drug administration;
- that vasectomy on the male partner must be completed 3 months for the male prior to first study drug administration;
- or in the alternative that a 0 sperm count for the male partner will suffice

### **VOLUNTARY PARTICIPATION**

You are under no obligation whatsoever to participate in this study and your participation in this study is strictly voluntary. You may withdraw or discontinue participation at any time and you may decline to answer any question or participate in any parts of the procedure. There will be no consequences if you choose to withdraw from the study. You do not waive any legal rights by participating in the study.

You may also withdraw consent for the use of your data, but you understand that you must do this in writing. You understand that the investigator has the right to withdraw you from the study at any time if they determine it would not be in your best interest to stay in it. Your study doctor can stop treatment even if you are willing to stay in the study. Any data collected may not be withdrawn because there may not be any identifiers with the data.

If you decide to pull out of the study for any reason, you may be contacted for safety reasons.

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## **COMPENSATION**

Participation in research is voluntary. You will also receive proper oral hygiene instructions. You will not be compensated for participating in this study.

## **BENEFITS**

Participation in this study may not benefit you personally. The results of the study may allow improved methods to dose probiotics in the oral cavity, and the principal investigator would like to thank you for considering participating in this study.

## **DISCOMFORTS AND RISKS**

Oral irritation is possible and surface staining of the teeth may occur as a side effect. These events are expected to be mild and temporary and will stop when you stop using the study product. Professional oral hygiene will be provided in case of tooth staining. There may be some additional risks that are unknown at this time. At the end of the study you will be asked if you have experienced any discomfort or oral irritation. The investigators will record any and all adverse reactions. The nature of the reaction and any correlation with product usage will be assessed. If the evidence indicates that the adverse reaction may be due to product usage, you will be instructed to discontinue product use and appropriate treatment will be provided. In the event of an adverse experience, emergency or other problems or questions regarding your participation in this study you can contact the principal investigator Dr. Michael Glogauer at (905) 973-0664. In the event of a medical emergency, please go to the emergency room or call 911. Once the situation is stable, contact the principal investigator Dr. Michael Glogauer at (905) 973-0664.

No discomfort or side effects are expected from the collection of saliva or oral rinse.

## **NEW FINDINGS**

Sometimes during the course of a research study, new information becomes available about the treatment/drug that is being studied. You will be informed of any significant new findings related to study products or procedures as soon as the new information is known. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study, you will be asked to sign an updated consent form.

Also, on receiving new information your study doctor might consider it to be in your best interests to withdraw you from the study, and explanations will be given accordingly.

## **CONFIDENTIALITY**

If you agree to join this study, the study doctor and his study team may look at your Recruitment Questionnaire collected during the initial phone call, solely for the purpose of determining if you meet inclusion criteria. No other personal health records will be requested.

If inclusion has been confirmed you will be issued a study number/ID, and the Recruitment Questionnaire will be de-identified (name, birth date and profession deleted and replaced with your Study ID). The de-identified Recruitment Questionnaire will be destroyed after you formally exits the study or has been removed from the study. All remaining records with identifiable information will be destroyed after you formally exits the study or have been removed from the study. The only exception is the signed informed consent forms, which will be

## **Protocol ID: OstiaPhase0**

kept for 15 years by the sponsor and maintained in strict confidence in a locked filing cabinet in a locked room.

All information collected during this study, including your personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law. You will not be named in any reports, publications, or presentations that may come from this study. If you decide to leave the study, the information about you that was collected before you left the study will still be used. No new information will be collected without your permission.

The results of this study may be published and/or submitted to Health Canada and/or local regulatory agencies in other countries, including US FDA. Your identity will be kept confidential, only your subject number/ID, gender and/or age may be used you in connection with any such publication of the study results. All documents that identify you by name (e.g., the signed informed consent form and health questionnaire)

However, the investigator must allow auditing/monitoring to verify subject enrollment, product safety and study compliance. If any monitoring occurs, study documents can be looked at, but no private information will be copied or removed from the clinical site. The following people may come to the Ostia Sciences location to look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines:

- The study sponsor or its representatives/partner companies;
- Health Canada
- Veritas IRB

All samples collected during the study will be destroyed after analysis. If you decide to leave the study, the information about you that was collected before you left the study will still be used. No new information will be collected without your permission.

### **What will happen to the results of the research study?**

The results of this study may be published online, in a medical journal and shown at medical meetings. You will not be identified (by name or any other personal means) in any of these publications.

If you would like to know the study results, the study will be published on the Ostia Sciences website when it is available.

### **CONFLICT OF INTEREST**

Ostia Sciences is sponsoring this clinical trial. The principal investigator is a co-founder of the company and has a financial interest in the success of the trial. All other individuals you will interact with during the trial are paid employees of the sponsor.

All of these people have an interest in completing this study. Their interests should not influence your decision to participate in this study. You should not feel pressured to join this study.

### **QUESTIONS ABOUT THE STUDY**

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If you have any questions, concerns or would like to speak to the study team for any reason, please contact Dr. Abdelahhad Barbour through email : [contact@ostia-sciences.com](mailto:contact@ostia-sciences.com).

'This study has been reviewed by Veritas Independent Review Board (IRB). If you have any questions about your rights as a research participant or the Investigator's responsibilities, you may contact the Manager of Veritas IRB 24 hours per day and 7 days per week at 514-337-0442 or toll-free at 1-866-384-4221. An IRB is a group of scientific and nonscientific individuals who perform the initial and ongoing ethical review of the research study with the subject's rights and welfare in mind. If you have any study-related comments, complaints or concerns, you should first contact the study investigator. Please call the IRB if you need to speak to a person independent from the Investigator and the research staff, and/or if the Investigator and the research staff could not be reached.'

### **INFORMED CONSENT**

You will be asked to sign the consent form at the end of this patient information sheet. If you take part in this study, you should follow the study procedures.

I, \_\_\_\_\_ have read and understand the  
(PRINT NAME)  
information provided above and have been given an opportunity to ask questions and my questions were satisfactorily answered. I have been provided with a copy of this informed consent. By my signature below, I consent to participate in the study.

\_\_\_\_\_  
Participant's Name (Print)

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Person Obtaining Consent  
(Print)

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date