Title of Study: Clinical and microbiological efficacy of *Lactobacillus reuteri* in the supportive therapy of periodontitis: a 6 months Randomized Controlled Trial study.

Type of Study: Clinical and microbiological

Protocol No: PROTOCOL #PERIOPRO

Expected study start date: APRIL, 2020 (study initiation)

Study Location/Country: Brescia, Italy

Study Originator(s)/ Contact (name, phone, fax): Magda Mensi, Piazzale Spedali Civili 1, 25123 Brescia, phone: +390303995784, email: magda.mensi@unibs.it

Test Products w/ PDM Numbers:
CE marked Medical Device to be used as an adjunct therapy to standard in patients with moderate to severe periodontal disease (Confidential information).

Study Title:	Clinical and microbiological efficacy of *Lactobacillus reuteri* in the supportive therapy of periodontitis: a 6 months Randomized Controlled Trial study.
Study sites:	Dr. Magda Mensi (lead site) University-Hospital of Brescia Brescia, Italy.
Study Phase:	IV.
Name of Medical Device:	GUM® Periobalance® (SUNSTAR SUISSE S.A. Rte de Pallatex 11, CH-1163 Etoy SWIZERLAND)
Objective:	The objective of the clinical study is to assess the efficacy of the addition of *Lactobacillus reuteri* in the supportive therapy of periodontitis in terms of reduction of PD (Probing Depth).
Patient Population:	44 periodontal patients, systemically healthy, male and female adults (18 – 75 years), with history of periodontitis grade B or C and stage III or IV, with at least 2 residual sites/pockets with probing depth ≥6 mm or pockets of 5 mm with bleeding on probing in two different quadrants.
Structure:	Parallel arms: Number of treatments: 1 (after a session of non-surgical supportive therapy one group takes probiotic (*Lactobacillus reuteri*) lozenges 2 times a day for 21 days, another group takes placebo lozenges 2 times a day for 21 days). Duration of study: 36 months.
Number of Centers:	One.
Blinding:	Patient, Examiner, Operator and Statistician -Blind.
Method of Patient Selection:	Periodontal pockets: at least 2 sites with probing depth ≥6 mm or pockets of 5 mm with bleeding on probing in two different quadrants.
Total Sample Size:	44 adult subjects will be recruited (randomized) to participate in this study, with 40 expected to complete the study.
Primary Efficacy Variable:	Primary outcome measure will be the changing of PD in millimeters.

Clinical and microbiological efficacy of *Lactobacillus reuteri* in the supportive therapy of periodontitis: a 6 months Randomized Controlled Trial study.
PROTOCOL #PERIOPRO

Protocol Date: JAN 13, 2020

Protocol Approval

Signature Page

_____

Dr. Magda Mensi
University-Hospital of Brescia, Italy

Date

ADDRESSES AND RESPONSIBILITIES

Addresses

Study Centres:	Section of Periodontics, School of Dentistry, Department of Surgical Specialities, Radiological Science and Public Health University of Brescia Piazzale Spedali Civili 1 25123, Brescia, Italy Dr. Magda Mensi (Principal Investigator) Tel. +390303995784 Email: magda.mensi@unibs.it
Study coordinator:	Dr. Magda Mensi Section of Periodontics, School of Dentistry, Department of Surgical Specialities, Radiological Science and Public Health University-Hospital of Brescia Brescia, IT
Data Management & Monitoring:	Dr. Magda Mensi Section of Periodontics, School of Dentistry, Department of Surgical Specialities, Radiological Science and Public Health University-Hospital of Brescia Brescia, IT
Statistician:	Prof. Stefano Calza Unit of Biostatistics and Bioinformatics, Department of Molecular and Translational Medicine University of Brescia Brescia, IT
Microbiologist:	Prof. dr. Egija Zaura Head of the Department of Preventive Dentistry Professor in Oral Microbial Ecology Academic Centre for Dentistry Amsterdam Vrije Universiteit Amsterdam and University of Amsterdam Amsterdam, the Netherlands

Responsibilities

The **principal investigator's** responsibilities are described in detail in ISO norm 14155:2011 for the clinical investigation of medical devices for human subjects.

The role of the principal investigator is to implement and manage the day-to-day conduct of the clinical investigation as well as ensure data integrity and the rights, safety and well-being of the subjects involved in the clinical investigation.

The principal investigator shall be qualified by education, training and experience to assume responsibilities for the proper conduct of the clinical investigation. In particular, the principal investigator is responsible for:

- qualification of investigation site;
- communication with Ethics Committee;
- compliance with Informed Consent process;
- compliance with Clinical Investigation Plan.

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 Site file.

The **Statistician** will be responsible for:

- sample size calculation;
- statistical plan;
- statistical section of the protocol;
- statistical evaluation;
- final statistical report.

The **Microbiologist** will be responsible for:

- sample microbiological processing and analysis;
- final microbiological report.

OBJECTIVE

The study will be performed to assess the efficacy of the addition of *Lactobacillus reuteri* containing lozenges in the supportive therapy of periodontitis in terms of reduction of PD (Probing Depth).

INTRODUCTION

Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'. The influence of probiotics on pathogens flora can derive from three principal modes of action: innate and acquired host defense modulation, production of antibacterial substances and competitive exclusion mechanism. In particular, *Lactobacillus reuteri* has been studied for its antibacterial and anti-inflammatory properties.

It is a heterofermentative bacterium and the distinct strains have different characteristics. In particular it acts as an antibiotic, induces oxidative stress on pathogens, is resistant to proteolytic and lipolytic and present anti-inflammatory properties.

Literature pointed out to potential effectiveness of *L. reuteri* as an adjunct to non-surgical periodontal therapy in initial treatment of periodontitis patients but underlined the limits of their conclusions due to the heterogeneity of the studies and to the few patients included. There is thus a need for more long-term randomized controlled studies. In particular, only one study addressed the use of this probiotic during the supportive therapy and in particular in patients with severe forms of periodontitis but few patients were included.

Patients meeting the criteria of periodontitis stage III and IV, grade C are considered to be affected by severe and advanced forms of periodontitis with a rapid rate of progression. This group of patients could particularly benefit from supplements in the maintenance of periodontal health.

STUDY OUTCOMES

a. Primary outcome measure: change in PD
b. Secondary outcomes:
    - changes in the following clinical parameters: Pocket closure, REC (Clinical Gingival Recession), CAL (Clinical Attachment Level), BOP (Bleeding on Probing), PI (Plaque Index).
    - Risk progression of the periodontitis and the need of periodontal surgery.
c. Microbiological outcomes: changes in microbial composition and proportion of sequences identified as *Lactobacillus reuteri* in the deepest residual pockets.

STUDY POPULATION

Forty-four (44) adults, aged 18-75 years, will be entered into study (randomized). It is expected that forty (40) subjects will complete the study.

Randomized subjects who deviate from the protocol (major protocol deviation) and, for this reason, are excluded from the analysis, will be replaced to guarantee that the sample required for the analysis (40) is reached.
Patients will be treated at the Department of Surgical Specialities Radiological Science and Public Health, School of Dentistry, Section of Periodontics, Brescia, Italy from 04/2020.

Inclusion Characteristics

- Signed Informed Consent Form.
- Male and female subjects, aged 18-75 years, inclusive.
- Good general health (free of systemic diseases such as diabetes, HIV infection or genetic disorder, ongoing malignant disease of any type that could influence the outcome of the treatment and might interfere with the evaluation of the study objectives).
- History of periodontitis staging III or IV grading B or C
- At least 2 sites with probing depth ≥6 mm or pockets of 5 mm with bleeding on probing in two different quadrants.
- Previous periodontal non-surgical treatment at least 3 months maximum 6 months.
- Availability for the 6-month duration of the study for an assigned subject.

Exclusion Characteristics

- Not willing to follow the agreed protocol.
- Presence of orthodontic appliances.
- Smokers more than 10 cigarettes per day
- Chronic obstructive pulmonary disease and asthma.
- Tumors or significant pathology of the soft or hard tissues of the oral cavity.
- Current radiotherapy or chemotherapy.
- Pregnant or lactating women.
- Current or past (within 3 months prior to enrollment) assumption of medications that may influence periodontal conditions and/or interfere with healing following periodontal treatment (i.e., corticosteroids, calcium channel blockers, systemic antibiotics, ...).
- History of allergy to Erythritol or chlorexidine.
- Restorations on the teeth to be treated which may interfere with treatment administration and/or scoring procedures, at the discretion of the examiner.
- Use of systemically administered antibacterial agents or probiotics 3 months prior to enrollment.

A written informed consent will be obtained from each included patient after explanation of the risks and benefits of participating to this study. No change in the trial design will be made after approval of the Ethical Committee.

1. STUDY DESIGN

The study design was chosen according to the current standards required by ISO, GCP and according to national requirements.

a. A Parallel-Arm, Statistician, Operator and Examiner are blinded, RCT will be established;
b. The RCT will be run as mono-center;
c. A total of 44 subjects will be recruited to participate in the study. It is expected that all of 40 subjects will complete the study in total;
d. Admission into the study will be via rolling admission (estimated recruitment time: 12 months);
e. Each subject must have at least 2 residual sites/pockets with probing depth ≥6 mm or pockets of 5 mm with bleeding on probing in two different quadrants;
f. Each subject will follow the following treatment protocol:
    i. – Visit 1 (Screening): (within 29 days prior to baseline) – consent secured; screening and entry into study; baseline data collection; full periodontal charting (full-mouth PD, REC, BOP, PI); identification the sites for microbiological sample.
    ii. – Visit 2 (T0 – Baseline Visit+Treatment): collection of microbiological samples from the two deepest sites in two different quadrants. All patients receive a full mouth periodontal treatment (GBT) and OHI (oral hygiene instruction). According to the list of randomization the patient will be allocated into one of the two arm of probiotic treatment: study lozenges are given to all patients to consume at home. The patients are instructed to dissolve these on their tongue twice a day, preferably after brushing, for 3 weeks. The patients of the probiotic group will receive probiotic lozenges containing *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 (a minimum of 2×10^8 colony-forming units L. reuteri Prodentis/lozenge, BioGaia AB). The patients of the control group will receive control lozenges without live bacteria. Furthermore, the probiotic and control lozenges will be identical in taste, texture and appearance.
    iii. – Visit 3 (T1) – 3 weeks: the participants will be asked to return the empty packages of the study medication to examine the adherence. At that time, side effects will also be questioned by the examiner by means of an open question. Collection of the

microbiological sample will be performed from the same subgingival sites as during Baseline (T0) and reinforcement of OHI.

iv. – Visit 4 (T2) - 3 months: Collection of the microbiological sample from the same subgingival sites as during Baseline (T0), collection of full periodontal charting (PD, REC, PI, BOP) and reinforcement of OHI.

v. – Visit 5 (T3) - 6 months: Collection of the microbiological sample from the same subgingival sites as during Baseline (T0), collection of full periodontal charting (PD, REC, PI, BOP) and full mouth GBT will be done in both groups. Subjects will either be entered into a routine recall system for treatment or scheduled for any further periodontal treatment as clinically indicated, and exit the study.

g. All treatments are made by the same clinician;

h. Calibration: the examiner is calibrated showing an intra-examiner reproducibility of 96% for duplicate measurement of probing pocket depth (PPD) with a maximum difference of 1 mm in 5 patients;

i. Data collection: A PCP-UNC 15 periodontal probe is used on six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual) with a gentle probing force by the same clinician. Measurements are rounded to the nearest millimeter;

j. Clinical assessment data will be recorded via hard copy CRFs. CRFs will be submitted to a data entry staff member for entry into the statistical database. At the conclusion of the study, such statistical database will be submitted to the statistician for the preparation of the statistical analysis and the final study report.

2. TREATMENTS

Study lozenges (both: test and placebo) will be delivered to the study center prior study start.

Specification of test treatment – Microbiological analysis

Microbiological analysis will assess changes in microbial composition and the proportion of sequences assigned as *Lactobacillus reuteri* in subgingival plaque samples.

Sample for microbiological analysis:

1. Subgingival plaque collection

Subgingival plaque from 2 sites with probing depth ≥6 mm or pockets of 5 mm with bleeding on probing in two different quadrants (the two deepest sites) will be collected using sterile paper point.

After isolating the site with cotton rolls, supragingival plaque was removed using a Gracey curette and the site was dried with airflow. Subsequently, two paper points were inserted in the pocket for 10 s. The paper points with subgingival plaque will be placed in empty sterile Eppendorf vial and stored on ice until transport to the laboratory (max 2 h), and stored at -80°C.

1. Sample processing

Samples will be shipped on dry ice to the Department of Preventive Dentistry, at the Academic Centre for Dentistry Amsterdam (ACTA). Bacterial DNA will be extracted, purified and quantified and prepared for 16S rRNA gene amplicon sequencing using Illumina MiSeq technology. The obtained sequencing reads will be quality filtered and processed into zero radius operational taxonomic units (zOTUs) and assigned taxonomy using HOMD database.

The zOTU-table will be randomly subsampled to even depth. The Shannon diversity index per sample will be calculated using dedicated software (PAST). This diversity index will be used to characterize the diversity of the microbial community and accounts for both the abundance and evenness of the OTUs present in the sample. Data ordination plots will be obtained using the Principal Component Analysis (PCA) on the $\log_2$ transformed zOTU-data. The statistical difference in microbial profiles between the study groups at each time point will be assessed using permutational analysis of variance (PERMANOVA) with Bray-Curtis similarity distance. Metagenomic biomarker discovery tool LEFSE will be used to identify microbial taxa that significantly discriminate the groups. The Bray-Curtis similarity index will be calculated between the dependent samples collected at different time points and the similarity distances between the study groups will be compared statistically.

zOTUs that taxonomically will be assigned to genus *Lactobacillus*, will be analyzed separately, in order to assess if the probiotic strains have established in the oral cavity. For this, first, a phylogenetic tree will be created based on the representative sequence of each zOTU. Sequences that will be phylogenetically related to *L. reuteri* 16S rRNA gene fragment amplified, will be marked as potential probiotic sequences. Relative abundances of all genus lactobacillus sequences and *L. reuteri* sequences between groups per timepoint will be compared.

Concomitant Therapy

A necessary concomitant medication or therapy is permitted as long as it is not excluded in the exclusion criteria. Every medication has to be reported in the CRF (Appendix 1) and in the

Concomitant Medication form (Appendix 2). All changes during the study are to be reported in the CRF. Usage of prohibited therapy will lead to the exclusion of the subject.

Blinding and Randomization

Blinding: The statistician will be blinded to the group identification. The patient and the operator will not know the treatment. Knowledge of the randomization list will be limited to the persons responsible for creation of the randomization list, provider of randomization numbers to study site, and delivery of the test/control treatment until the last subject (i.e. final examination of the last subject), quality control and verification of the CRFs is completed.

Randomization and allocation concealment: The randomization of the study protocols will be performed by a staff member who is not further involved in this study. This will be done based on a computer-generated table (www.randomization.com) that links each patient to one of the treatment groups. The same staff member will be responsible for blinding the study products. This will be done by labelling the packaging of these products with a letter indicating the treatment group. Additionally, the jars containing the lozenges will be identical in appearance and non-transparent. When a patient will be included in the study, the study medication will be handed out to the researcher according to group to which that patient will be assigned based on the patient number and the randomization list. The similarity of the packaging, and the identical appearance, texture and taste of the study products make the double-blinding of the researcher and patient possible.

Implementation: The Principal Investigator will be informed immediately of Serious Adverse Events (SAEs), regardless of the causality relationship between the administration of the investigational treatment and the SAE.

3. PROCEDURE

Screening and Selection of Subjects

Patients visiting the Department of Dentistry at Brescia University will be asked to participate in this study. Subjects will report to the clinical facility and be screened by the dental examiner to identify those subjects who meet the inclusion/exclusion characteristics. The findings of this initial screening procedure will be recorded on the CRF. Subjects who meet the

inclusion/exclusion characteristics and sign an Informed Consent Form will be entered into the study.

Baseline Oral Soft and Hard Tissue Assessment

All subjects will receive an evaluation of their oral soft and hard tissues. This examination will include an evaluation of the soft and hard palate, gingival mucosa, buccal mucosa, muco gingival fold areas, tongue, sublingual and submandibular areas and the tonsillar and pharyngeal areas. The results of this evaluation will be recorded on the CRF.

Clinical Periodontal Evaluation

All patients receiving treatment will be evaluated by the dental examiner for the following assessments (see Study Flow Chart):

- Collection of subgingival plaque samples;
- Full Mouth Plaque Score (PI);
- Periodontal Inflammation (BOP);
- Probing Pocket Depth (PD);
- Recession (REC);
- Clinical Attachment Level (CAL).

Clinical attachment level (CAL) is the measurement of the position of the soft tissue in relation to the cemento-enamel junction (CEJ) that is a fixed point that does not change throughout life. All parameters will be recorded in the CRF.

Treatment

Patients who have been identified for treatment receive a GBT session and after OHI will be randomized according to a randomization table generated.

Follow up

Following completion of GBT and OHI the subject will be given the appropriate number of interproximal cleaning devices and discuss with the operator about the appropriated toothbrush and toothpaste for home use. Subjects will be asked to refrain from using any other adjunctive oral home care products during the study period. Every necessary study lozenges will be given to all patients to consume at home. The patients will be instructed to dissolve these on their tongue twice a day, preferably after brushing, for 3 weeks.

After 3 weeks the compliance will be recorded and microbiological sample will be collected again. At 3 and 6 months the following assessments (see Study Flow Chart) will be recorded:

- Collection of subgingival plaque samples;
- Full Mouth Plaque Score (PI);
- Periodontal Inflammation (BOP);
- Probing Pocket Depth (PD);
- Recession (REC);
- Clinical Attachment Level (CAL).

Study Flow Chart

Study phase	Enrolment	Treatment	Follow-up	Follow-up	Follow-up
Study visit	Visit 1	Visit 2 (T0)	Visit 3 (T1)	Visit 4 (T2)	Visit 5 (T3)
Study Week	-29 days	0	3 weeks	3 months	6 months
Selection criteria	+				
Informed consent	+				
Demographics, ethnics	+				
General health status	+	+	+	+	+
Medical & dental history	+				
Concomitant therapy/treatment	+	+	+	+	+
Safety (adverse events and serious adverse events)		+	+	+	+
Data collection (PD,REC, BOP, PI)	+			+	+
Microbiological Sample		+	+	+	+
Randomisation		+			
GBT		+			+
OHI		+	+	+	+
Dispensing standard interdental floss		+			
Dispensing study lozenges		+			

4. STATISTICAL ANALYSIS

Sample size determination

The sample size was calculated via simulation assuming an expected difference of 1mm pocket probing depth and using a linear mixed effect model considering patients as random effects. We simulated 1000 realizations of pocket probing depth applying the data correlation structure from the AgPro study (fixed effect correlation = -0.664; random effects intercept SD = 0.974; random effects residual SD = 1.65) and assuming an average of 7 experimental sites per patient. A sample size of 20 patients per group allowed for a power of at least 80% at the chosen $\alpha = 0.05$. Taking into account a dropout rate of 10%, we estimated a total sample size of 44 patients. The simulation was performed using R (version 3.6.1).

Statistical Analysis

Data will be described using standard summary statistics such as mean and standard deviations for quantitative variables and proportions for categorical variables. The primary outcome is pocket probing depth (PPD). Only the sites with PPD greater or equal to 6mm at baseline were considered or PPD equal to 5mm and BoP+. For the primary outcome a linear mixed effect model will be used considering patients as random effects.

Secondary continuous outcomes will be modelled using a linear mixed model while generalized mixed models will be applied to categorical outcomes. Data will be modelled both using a 1-level hierarchical model (clustering level: patient) after averaging the outcome variables within subject, and using a 3-level (patient, tooth and site) hierarchical model. Results will be expressed as estimates and relative 95% confidence intervals. A significant level of 5% will be used for all the comparisons and all analysis will be performed using R (version 3.6.1 or higher).

Evaluation of Safety

The following safety variables will be used for the evaluation of safety:

- Adverse events (AE);
- Adverse reactions (AR).

The total number of AEs and the total number of AEs at least possibly related to the study treatment, as well as the total number of patients affected by at least one adverse event will be calculated per treatment group.

The type of AE classified by organ system (according to MedDRA terminology) will be tabulated. Serious and/or unanticipated adverse events and AEs resulting in discontinuation or reduction/withdrawal of the study treatment will be presented separately.

Safety analysis set

Safety summaries will be based on the safety analysis set which will consist of all patients who received the test/control treatment.

5. MONITORING AND AUDIT

Study Monitoring

The study will be monitored by the Principal Investigator at periodic intervals during the course of the study to ensure that the study is being conducted according to Good Clinical Practice.
The Investigators will be contacted by the PI on a regular basis. On this occasion, the progress of the study will be discussed with the Investigator and the CRFs will be checked for completeness and consistency and to verify compliance with the study protocol.

6. SUBJECT TERMINATION/WITHDRAWAL PROCEDURES

All efforts will be made to determine the reason(s) why a patient is withdrawn from the study. Subjects could be withdrawn from the study if any of the following occur:

1. Subject fails to substantially comply with the protocol requirements;
2. Subject develops a serious adverse reaction. The Study Investigator will immediately notify the PI and information will be recorded on a Serious Adverse Event Form (Appendix 3);
3. Subject elects to terminate participation in the study. Participation in the study is voluntary. A subject has the right to withdraw from the study at any time for any reason.

The Investigator may terminate the study at any time if the risk-benefit ratio is no longer favourable. Before discontinuation, the Investigator should inform the PI and ask for advice. If the Investigator is concerned about continuation of the study, his/her concerns should be transmitted immediately to the PI.

7. DOCUMENTATION AND DATA MANAGEMENT

Data collection in the case report form (CRF)

All study data will be recorded in the case report forms identified by the subject number. Only the principal investigator, co-investigators, or designated study personnel may make entries in the case report forms.
The Investigator has to identify all data that were directly recorded into the CRF and to be considered to be source data.
The CRFs will be checked for completeness and plausibility by the PI. The Investigator will resolve any queries.

Investigator Site File (ISF)

The ISF includes all documents that are required for the clinical study. During monitoring, the ISF will be checked regularly for completeness and actuality. After the clinical trial is finished or stopped, the ISF has to be stored 15 years in the study center.

Data Management

Data extraction from CRFs into a single, electronic database is performed by examiners. Discrepancies are to be clarified and corrected by authorized persons by means of documented data queries between Statistician and Investigator(s).
After the study is finished and before data are analyzed, a blind data review meeting will be held between the investigator and the statistician. When the database has been declared to be complete and accurate, it will be locked. Any changes to the database after this procedure can only be made by joint written agreement among the clinical trial leader, the trial statistician, and the co-investigators.

8. ADVERSE EXPERIENCES/EVENTS

Subjects will be informed of any possible adverse reactions which they could experience and will be instructed to immediately report any event to the investigator. The investigators will record any and all adverse reactions and report this documentation to the Principal Investigator. In the event of an adverse experience, emergency or other problems or questions regarding participation in this study, the subject can contact the following investigators:

for Brescia Site: Dr. Magda Mensi (Principal Investigator) – tel. +39 030 3995784.

Adverse Events (AEs) and Serious Adverse Events (SAEs) are defined by the ICH Guideline Medical Device Directive 93/43/EEC and Guidelines on Medical Devices MEDDEVG 2.7/3rev. 3,

May 2015 Clinical Investigations: Serious Adverse Event Reporting under Directives 90/385/EEC and 93/42/EEC for Good Clinical Practice (ICH GCP) as follows:

An adverse event (AE) is any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the investigational medical device.

With respect to intensity, adverse events are classified as follows:

- Mild Some awareness of symptoms, but easily tolerated;
- Moderate Symptoms causing enough discomfort to interfere with usual activity;
- Severe Incapacitating event causing inability to work or to perform usual activity.

Adverse events are classified as either non-serious or serious.

Serious Adverse Event (SAE) is an adverse event that:

a) led to a death;

b) led to a serious deterioration in health of the subject, that either resulted in:
   - a life-threatening illness or injury, or
   - a permanent impairment of a body structure or a body function, or
   - in-patient hospitalization or prolongation of existing hospitalization, or
   - in medical or surgical intervention to prevent life threatening illness or
   - injury or permanent impairment to a body structure or a body function.

c) led to fetal distress, fetal death or a congenital abnormality or birth defect.

Device deficiency: inadequacy of an investigational medical device related to its identity, quality, durability, reliability, safety or performance. This may include malfunctions, use error, or inadequacy in the information supplied by the manufacturer.

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 baseline, including frequency or pattern changes for a fluctuating condition (e.g., migraine), occurring after the administration of investigated treatments

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 Device Effect (SADE) is an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Unanticipated Serious Adverse Device Effect (USADE) is a serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report or investigator's brochure (IB).

NOTE: Anticipated SADE (ASADE): an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report or IB.

The following events are considered reportable:
- any SAE;
- any Device Deficiency that might have led to a SAE if:
  a) suitable action had not been taken or
  b) intervention had not been made or
  c) if circumstances had been less fortunate;
- new findings/updates in relation to already reported events.

Reportable events have to be reported by clinical investigators.

Assessment of Causality

The following criteria are to be used for the assessment of the causal relationship to the test/control treatment. For classification, all criteria of one of the following categories must be met:

related, if

- a timely correlation exists and
- dechallenge and/or rechallenge and
- a biological plausibility exists and
- other factors are clearly excluded
- a medical report or clinical proof exists;

possibly related, if

- a timely correlation exists and
- dechallenge and/or rechallenge and
- a biological plausibility exists and
- a medical report or clinical proof does not exist;

unrelated, if
- a timely correlation does not exist or is uncertain and
- dechallenge and/or rechallenge does not exist and
- a biological plausibility exists and
- a medical report or a clinical proof does not exist;

unknown, if
- not enough information exists for evaluation
- no further contact with reporter possible.

Sound medical/clinical judgment will be applied when assessing the causality and seriousness of the adverse events.

Adverse Event Reporting

The study center will provide the study participants with emergency telephone numbers for study related support and feedback. The emergency telephone number is operated by either an investigator of the study or by a qualified person designated by the principal investigator. The schedule of reachability of the emergency telephone number will be defined prior to start of the study.

Serious Adverse Events

The Investigator shall immediately after awareness (and in any event, not later than within 24 hours after awareness) inform the PI. The Investigator will send the SAE report to the following email address: magda.mensi@unibs.it. Appendix 3 has to be used for SAE reporting. Form has to be completed electronically and in English.

Adverse Events

Adverse events will be assessed by the investigator or designee within 24 hours 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 to the PI. For data collection purposes, the outcome of all adverse events recorded on the Adverse Reaction section of the CRF 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 PI. At the end of the study, the investigator will report all adverse events (serious and non-serious) to the PI on CRFs. Forms have to be completed electronically and in English.

Pregnancy

No pregnant women (according to medical history) will intentionally be enrolled in this study. In the event a woman enrolled in this clinical research study becomes pregnant during the course of the 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.

9. 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. In the event of emergency, the Investigator shall engage any medical procedures that he/she deems appropriate. However, all such procedures must be promptly reported to the PI and Ethical Committee.

The Ethical Committee which granted approval for the study must be notified of all changes in the protocol and must provide written approval if changes are substantial (e.g. increase the risk to the subject, and/or affect the rights of the subject or validity of the investigation, change of/within study population, number of participants or changes of patients' age group).

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 CRF and tracked as official eligibility waivers.

10. ADMINISTRATIVE ASPECTS

Final Report

Following the completion of the study, the PI 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, and statistical analysis of the data. This report will be shared with the Co-investigator at the Participating Centre by the Principal Investigator, and agreed upon parts before being sent to the Sponsor.

Data Retention

The information in this and any further document contains trade secrets and commercial information that are privileged or confidential and may not be disclosed unless such disclosure is required by law or regulations.

In any event, persons to whom the information is disclosed must be informed that the information is privileged or confidential and may not be further disclosed by them.

The files pertaining to this study will be kept by the University-Hospital of Brescia for a period of 15 years from the day of delivery of the final report and will be available for consultation by competent authorities at any time.

The Investigators will retain originals of the approved study protocol, copies of completed CRFs, subjects' participation agreements, relevant source documents and all other supporting documentation related to the study for a period of 5 years. These files must be made available for inspection upon reasonable request by an authorized representative of EMS or the competent authorities.

11. ETHICAL ASPECTS AND REGULATIONS

Ethical Conduct of the Study

This study is to be conducted in accordance with the ethical principles of the Declaration of Helsinki and according to local laws and regulations.

Independent Ethics Committee (IEC) and Relevant Authorities

Before starting the study, the study protocol will be subject to review by the Ethical Committee of the University-Hospital of Brescia. As required by law, the study will be notified to authorities if applicable. No subject should be admitted to the study before the Ethical Committees issue their written favourable opinion of the study. Periodic status reports must be reported to the ethics committee at least annually as well as notification of the completion of the study. The investigator must maintain an accurate and complete record of all reports and documents submitted to and received from the ethics committee according to the ISO 14155:2011 Clinical Investigation of Medical devices for human subjects.

Subject information and Informed consent form

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. Each subject must read, understand and sign the informed consent form provided before any study-related procedure.

Subject data protection and Confidentiality

The name of the subject as well as all other personal data will be kept strictly confidential by the Investigator. If due to medical reasons, it is necessary to identify the subject during the study course, this will be done under medical secrecy.

The subject has given his/her consent before the beginning of the study. In case of withdrawal of this consent subject has to leave the study.

All subjects will agree to verify, by letter, that they participated in this study, if called upon to do so.

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.