NCT number: NCT04810221

Protocol #:	01M2020-CH.LMD
Protocol title:	Postmarket clinical follow-up study (PMCF) to confirm the performance of the wearable pulse-oximeter BrOxy M (SOMBRERO study)
Version:	1.0
Date:	22 December 2020
Medical Device:	BrOxy M (wearable pulse-oximeter for the measurement of cardiac frequency and blood oxygen saturation (SpO_2))
Sponsor:	Life Meter S.r.l. Via N. Vernia 20, 66100 – Chieti (CH), Italy Telephone: (+39) 391 4334 090 Website: www.lifemetersrl.com E-mail: info@lifemetersrl.com

SPONSOR'S SIGNATURE PAGE

Protocol #:	01M2020-CH.LMD
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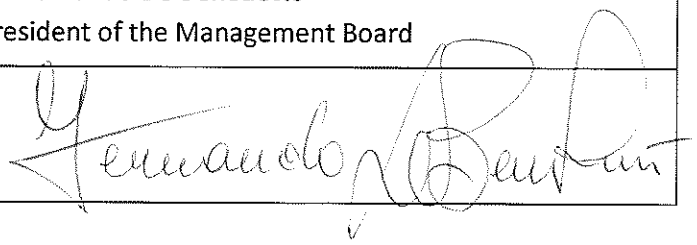
Sponsor Representative:	Dr. Fernando De Benedetto President of the Management Board
Sponsor Representative Signature and Date	

INVESTIGATOR SIGNATURE PAGE

Protocol #:	01M2020-CH.LMD
Protocol title:	Postmarket clinical follow-up study (PMCF) to confirm the performance of the wearable pulse-oximeter BrOxy M (SOMBRERO study)
Version:	1.0
Date:	22 December 2020

I have read and understood this clinical protocol including the appendices and agree to abide by its requirements. I will provide copies of this clinical protocol and all relevant information to the personnel involved in the study, under my supervision. I will discuss this material with them and make sure they are fully informed about the device and the conduct of the trial.

I will conduct the trial in accordance with the clinical study protocol, the latest version of the Declaration of Helsinki (2013), guidelines for good clinical practice, EN ISO 14155:2020 (Clinical investigation of medical devices for human subjects - good clinical practice) and current regulations. I also accept the respective reviews of the clinical study protocol approved by the Sponsor and the relevant Ethics Committee.

Site name and address:

_____

_____

Principal Investigator's Name (block letters)

_____ _____

Principal Investigator's Signature Date

REVISION HISTORY

Version No:	Version Date:	Summary of changes:
1.0	22 December 2020	First release

Table of Contents

ABBREVIATIONS

COPD	Chronic obstructive pulmonary disease
EC	Ethics Committee
HR	heart rate
LTOT	long-term continuous oxygen therapy
NCD	non-communicable diseases
SpO_2	peripheral oxygen saturation
SaO_2	arterial oxygen saturation
YLL	years of life lost

STATEMENT OF COMPLIANCE

This clinical study will be conducted in accordance with the latest version of the Declaration of Helsinki (2013), ISO 14155:2020 Clinical Investigation of Medical Devices for Human Subjects - Good Clinical Practice, Medical Devices Directive 93/42 / EEC, MEDDEV 2.12-1 , and any applicable national regulations relating to the conduct of a post-market clinical study on a medical device. If the study is extended beyond 26 May 2021, vigilance activities will be performed according to the requirements of the 2017/745 Medical Devices Regulation.

The clinical study shall not start until approval from the competent Ethics Committee (EC) and any other approval that may be requested by local regulations has been obtained.

Any additional requirements imposed by the EC must be met when conducting this clinical study.

The Sponsor has obtained a Clinical Study Insurance which will cover expenses in the event of physical injury resulting from study procedures.

1 SINOPSYS

STUDY TITLE	Postmarket clinical follow-up study (PMCF) to confirm the performance of the wearable pulse-oximeter BrOxy M (SOMBRERO study)
PROTOCOL NUMBER	01M2020-CH.LMD
PROTOCOL VERSION AND DATE	Version 1.0 dated 22 December 2020
STUDY DESIGN	This study is a post market clinical follow up study performed to confirm the performance of BrOxy M pulse oximeter in comparison with a reference, CE marked, pulse oximeter equipment (Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N, Covidien LLC, USA) in a controlled desaturation study over a range between 80% and 100% SpO_2. The study will be performed on a group of healthy volunteers in a controlled clinical setting. Due to the use of a controlled desaturation protocol foreseen by the standard ISO 80601-2-61:2017, this study is classified as an interventional post-market clinical study in accordance with Annex I of EN ISO 14155:2020.
STUDY PHASE	Postmarket clinical follow up study with a CE marked medical device
INVESTIGATIONAL MEDICAL DEVICE	BrOxy M device is a Class IIa, CE marked, wearable medical device with a size similar to that of a watch which, thanks to the sensor placed on the side in contact with the skin of the subject's wrist, is able to acquire photoplethysmographic signals and memorize them. With the use of the proprietary software provided by Life Meter S.r.l., it is possible to download the signals stored in the BrOxy M device, view, archive and export them. Such signals can be used as input for algorithms with diagnostic purposes, provided that they are suitably processed with specifically designed software in order to obtain parameters that describe the physiological state of the patient (such as, for example, heart rate or blood oxygen saturation).
REFERENCE MEDICAL DEVICE	A CE marked pulse oximeter will be used in this study as reference standard (Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N, Covidien LLC, USA). The rationale for the selection of the reference device is that it meets the requirements specified in the Standard ISO 80601-2-61:2017 "2017 "*Medical electrical equipment - Part 2-61: particular requirements for basic safety and*

	essential performance of pulse oximeter equipment", section EE.3. In particular, the Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N has been calibrated against: 1) a Co-Oximeter for measuring SpO_2 and 2) an ECG for measuring cardiac frequency.
NUMBER OF SITES	Single center study conducted in Italy
EXPECTED STUDY DURATION	Around 3 weeks, from enrolment of first patient to study completion/termination of the last patient.
OBJECTIVES AND ENDPOINTS	**Primary Objective:** Confirm the performance of BrOxy M in comparison with a reference, CE marked, pulse oximeter equipment in a controlled desaturation study. **Primary endpoint:** The **primary endpoint** of the study will be a comparison of the accuracy of SpO_2 and heart rate (HR) measurements obtained with BrOxy M with that obtained with the reference pulse oximeter in paired observations over a range between 80% and 100% SpO_2. The study will follow the procedures described in the standard ISO 80601-2-61:2017 "Medical electrical equipment - Part 2-61: particular requirements for basic safety and essential performance of pulse oximeter equipment". **Secondary Objectives:** • To assess BrOxy M performance in subjects with different skin pigmentation (*Note: this is a tentative objective, since few subjects are planned to be enrolled and there is no guarantee that they will have different skin pigmentation*) • to confirm BrOxy M safety **Secondary endpoints**: The following will be verified: • the performance of Broxy M when taking measurements on subjects with varying skin pigmentations as measured with the Fitzpatrick scale • that no adverse events related to the use of the device, besides a temporary slight skin irritation of the wrist, will occur
SAMPLE SIZE	Based on the requirements of the standard ISO 80601-2-61:2017, at least 11 healthy subjects will be enrolled. Data collected on the first subject will not be used for accuracy measurements and will serve to finalize the test set up phase and ensure adequate recording procedures are used by site staff.

	To take into account invalid data and potential drop out samples up to 2 additional subjects may need to be enrolled. For this reason sample size for this study will be between a minimum of 11 and a maximum of 13 healthy volunteers.
STUDY POPULATION	The study population will include adult healthy volunteers that will accept to participate in this oxygen desaturation study in a controlled clinical environment.
INCLUSION CRITERIA	1. Healthy subjects of both sexes with an age ≥ 18 and ≤ 50. 2. ASA classification 1 3. Positive Allen's test 4. Intact and healthy skin on the selected wrist 5. Wrist circumference diameter between 150 mm e 200 mm. 6. Normal ECG obtained at screening or within 2 months prior to screening 7. Ability to understand and execute the required study procedures and provide an informed consent to the study.
EXCLUSION CRITERIA	1. Presence of at least one of the following altered hemoglobin parameters at screening: a. αHb ≤ 10 gr/dl b. COHb ≥ 3% c. MetHb ≥ 2% 2. For pre-menopausal female subjects only: positive pregnancy test performed at screening on urines or capillary blood 3. Presence of any cardiovascular pathology in medical history. 4. Any episodes of respiratory infection during the 30 days prior to screening 5. Any prior experience of Dyspnea 6. Hospitalization during the 2 months prior to screening, for any reason. 7. Chronic drug intake known to interfere with gaseous exchanges or induce changes in cardiac frequency 8. Presence of any medical condition not allowing the subject to perform the required test 9. Known allergy to adhesive tapes 10. Participation in an interventional study with a medicinal product or a medical device in the 30 days prior to screening.
SAFETY	The incidence, causality and severity of adverse events and device deficiencies will be measured for each study subjects and reported according to regulatory requirements

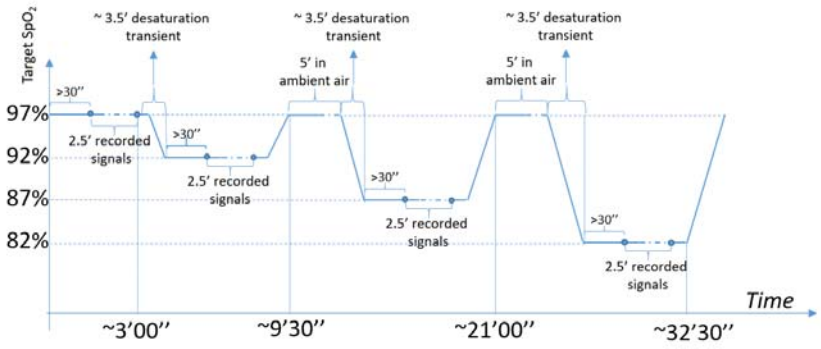
STUDY PROCEDURES

Following subject's acceptance to study participation by providing written informed consent, each subject will be assessed for eligibility.

Eligible subjects will undergo the following test session:

For each subject, four different saturation plateaus will be set up (i.e. at 97%, 92%, 87% and 82% SpO_2), waiting 30'' before starting to keep the SpO_2 level on each plateau for 2.5' in order to obtain 8 measurements in post-processing. These measurements will be at least 20'' away from each other, for a total of 32 measurements for each subject, as summarized in the following table and diagram:

Plateau n.	**Range SpO2 (%)**	**Inhaled solution**	**Plateau duration**	**N. of Measures of** SpO2 and HR **extracted from recordings**
I)	95 – 100 (target 97%)	Ambient air (medical air)	2.5-'	8
II)	90 – 94 (target 92%)	O2 15% + N 85%	2.5-'	8
III)	85 – 89 (target 87%)	O2 13% + N 87%	2.5-'	8
IV)	80 – 84 (target 82%)	O2 11% +N 89%	2.5'	8
			Total	**32 measures**



After completing the study on 10 subjects, collecting around 320 paired measurements it will be verified that, by dividing the desaturation interval into three equal parts, each part contains around a third of the measurements made. Therefore, considering the minimum target number of 200 measurements (per EN 80601-2-61 standard) and the interval between 98% and 82% of SpO_2 to be divided into three parts, at the end of the protocol on all the subjects recruited, it is expected to have:

- At least 67 measurements between 98% and 93% SpO_2;
- At least 67 measurements between 92% and 87% SpO_2;
- At least 67 measurements between 86% and 82% SpO_2;

In the event that this minimum target is not reached to meet the requirements of the EN 80601-2-61 standard, it may be necessary to recruit up to 2 additional subjects.

Study flowchart

Procedures	Screening Visit	Visit 1 - Test session (Within 2 weeks from Screening)
Informed Consent	X	
Medical history	X	
ECG	X*	
Physical exam (including wrist skin evaluation)	X	
Skin Pigmentation assessment (Fitzpatrick scale)	X	
Wrist circumference measurement	X	
ASA classification	X	
Vital signs	X	X§
Allen test	X	
Concomitant medications	X	
Blood test for Hb parameters	X	
Pregnancy test (urinary or capillary blood)	X**	
Inclusion/exclusion criteria assessment	X	
BrOxy M recording		X
Reference device recording		X
Adverse Events		X

§ 5 minutes after end of test session

* ECG required at screening unless a 12-lead ECG has been performed in the last 2 months prior to screening

**pregnancy test only for pre-menopausal women.

STATISTICAL ANALYSIS

Statistical analyses will be performed with SPSS (SPSS Inc, Chicago, IL).

The *accuracy* of the experimental BrOxy M will be evaluated by comparing SpO_2 readings of the BrOxy M to the values obtained with the secondary standard pulse oximeter equipment (Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N, Covidien LLC, USA), that is traceable to co-oximeter SaO_2 values.

The following routines will be used: the paired t test, to evaluate the differences between experimental and standard values; correlation analysis, to quantify the degree to which experimental and standard values are related; concordance correlation coefficient (CCC), to evaluate whether the experimental value is interchangeable with the standard; Bland -Altman (B&A) plot, to evaluate the agreement between experimental and standard values; linear regression analysis to give further information about any potential proportional bias about the mean differences and averages.

2 INTRODUCTION

Currently over 70% of deaths are due to non-communicable diseases (NCDs); the growing weight of these NCDs makes their prevention and adequate treatment of primary importance (1). In particular, 73.4% of deaths, corresponding to over 41 million deaths worldwide and 53% of years of life lost (YLL) due to disease, are caused by NCDs, including chronic obstructive pulmonary disease (COPD) that is responsible for 4 million deaths and more than 50 million YLLs (2). Heart failure is also one of the major causes of morbidity and mortality in the industrialized world with an incidence of 20 per-thousand in individuals of advanced age and 50% mortality within 5 years after diagnosis (3). It also represents a very frequent comorbidity (from 70 to 90% in various studies), together with other cardiovascular disorders, in COPD patients (4-7), who, as a consequence of this, show a marked impairment in health and quality of life, and worsening prognosis (8-10).

The natural history of COPD is full of episodes of flare-up of symptoms, generally on an infectious basis, which represent critical events for the life of patients, as they determine an increase in the speed of respiratory functional decline and the risk of cardiovascular complications, worsening of the health state and related quality of life, increased frequency of emergency medical consultations and hospital admissions and increased mortality (11-16). COPD patients who experience two or more exacerbations per year have a greater risk of subsequent exacerbations and death (17, 18). In addition, cardiac arrhythmias, ischemic heart disease and heart failure are frequent causes of hospitalization and death in COPD patients (19-22).

Dyspnea is a distinctive feature of COPD, first arising from heavy physical strain and then also from milder strains. Sometimes dyspnea proves to be disproportionate to the patient's clinical-functional conditions (dyspnea out of proportion) and must be investigated to highlight the possible causes, often cardiovascular, also with continuous monitoring of the patient's vital parameters and clinical conditions (23, 24). Patients with COPD, especially those that experience frequent episodes of exacerbation, can undergo severe functional respiratory alterations that are at the basis of the onset of respiratory failure, with the need to undertake long-term continuous oxygen therapy (LTOT) and, in the most severe cases, non-invasive or invasive mechanical ventilation therapy. The clinical conditions of patients suffering from chronic respiratory and cardiovascular diseases can change significantly from one day to another or within the same day, so they must be strictly monitored for various vital parameters such as the level of oxygenation, respiratory rate, heart rate and rhythm and others, both during the day while the subject carries out normal daily activities, and during the night. This is required in order to promptly detect clinical and functional signs of deterioration and promptly initiate adequate treatment, thus decreasing the need for emergency consultations and hospitalization, as demonstrated in a recent systematic review and meta-analysis (25).

Non-invasive monitoring of these vital parameters also allows to identify the need for LTOT and to verify its need over time (26). Furthermore, clinical-functional monitoring throughout the day, while the patient carries out his/her normal activities or is at rest, appears essential for a correct definition of the causes of dyspnea, especially if this is disproportionate to the clinical and functional state (27, 28). In addition, the continuous measurement of parameters, in particular the level of oxygenation and heart

rhythm during the night is essential for the identification of possible respiratory alterations during sleep such as OSA (obstructive sleep apnea) (29) .

In this context, albeit with a significantly lower prevalence than the aforementioned chronic diseases, but reported in widespread increase, idiopathic pulmonary fibrosis (IPF) also deserves to be mentioned. IPF causes a serious reduction in respiratory function and patient's health state, characterized by dyspnea following minimal physical strain, secondary to the development of marked respiratory insufficiency, and by rapid and progressive impairment of the state of health which leads to a very severe prognosis despite the currently available pharmacological treatments (30, 31).

Reliable measurement of oxygen saturation and heart rate are generally obtained at certain predetermined times and with the use of equipment that is not always easy to use, that is burdensome for the patient and is usually obtained in a hospital or outpatient setting, imposing a limitation in subject mobility and activity. There is currently no availability on the market of a non-invasive and easy to use instrument capable of providing the clinician with reliable measurements of oxygen saturation and heart rate recorded continuously both during the day, while the subject carries out his normal activities, and during the period of night sleep.

BrOxy M is a new, CE marked, pulse oximeter that is wearable like a watch and as such is not burdensome for the patient. BrOxy M is a medical device that can continuously record oxygen saturation and heart rate and, potentially, also other parameters during a 24-hour period, while the subject carries out his/her daily physical activity and even at night. This allows a more precise diagnosis and allows a more appropriate therapy with considerable advantages for the subject's health and quality of life and, at the same time, with a reduction in costs at the expense of the public health system.

3 STUDY DESIGN

The study is a post market clinical follow up study performed to confirm the performance of BrOxy M pulse oximeter in comparison with a reference, CE marked, pulse oximeter equipment (Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N, Covidien LLC, USA - from now on "reference device") in a controlled desaturation study.

The study protocol follows the requirements of the standard ISO 80601-2-61:2017 "*Medical electrical equipment - Part 2-61: particular requirements for basic safety and essential performance of pulse oximeter equipment*". As such, in this study BrOxy M and the reference device will be tested on healthy volunteers accepting to participate in a controlled desaturation study. Use of both devices will be in accordance with the limitations imposed by and instructions included in the BrOxy M and Reference device User Manual.

Due to the use of a controlled desaturation protocol foreseen by the standard ISO 80601-2-61:2017, this study is classified as an interventional post-market clinical study in accordance with Annex I of EN ISO 14155:2020.

During the execution of the protocol, the following measurements will be acquired in continuous mode:

1) the SpO_2 and HR values from the reference device, and

2) the photoplethysmographic signals with BrOxy M.

As shown in Figure 1, the protocol provides for the maintenance of 4 plateaus at 4 different values of saturation in steps of 5% and, therefore, at 97%, 92%, 87% and 82% SpO_2. The SpO_2 value displayed on the reference device will be used to check the saturation level reached during each plateau level. For each level, at least 30'' will be expected in order to stabilize the SpO_2 value and, subsequently, the plateau will be maintained for 2.5', continuing the parallel acquisition with both the reference device and BrOxy M.

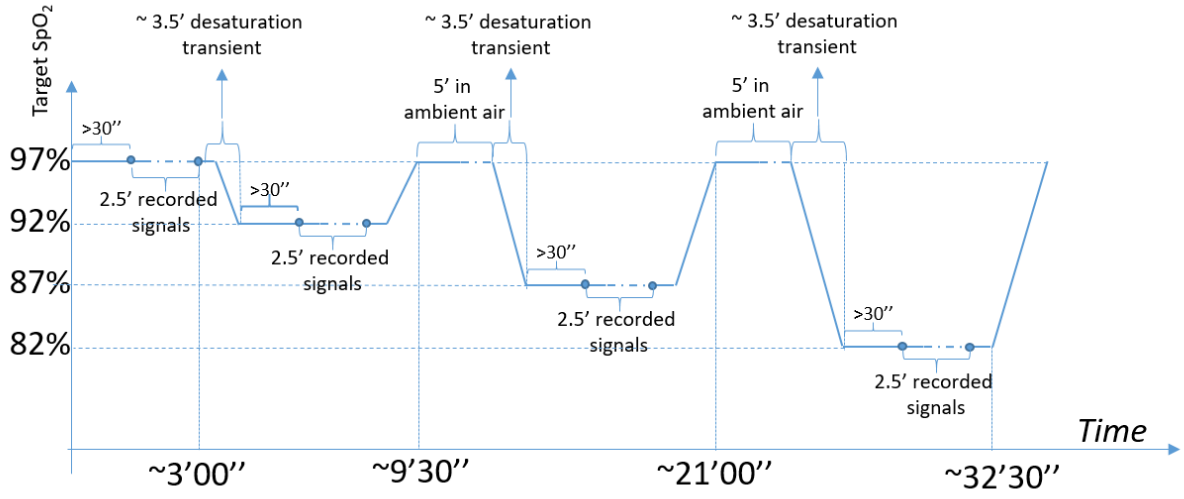


Figure 1: Time distribution of the SpO_2 measurements to be carried out on each individual subject.

Four different saturation plateaus will be used, waiting for the plateau stabilization before starting the 2.5' signal recording from which the series of 8 measurements with at least 20' of pause are extracted by means of a post-processing algorithm.

After data acquisition, up to 8 measurements for SpO_2 and HR will be selected from each plateau as follows:

- Reference device: SpO_2 and HR values will be selected starting from at least 30'' after plateau stabilization, with a time interval between measurements of at least 20''.
- BrOxy M: SpO_2 and HR values generated by BrOxy M will be obtained by processing the photoplethysmographic signals generated by the device with the BrOxy Medical software. Then, SpO_2 and HR values corresponding to the same time points selected for the reference device will be chosen.

As a result, it is expected that from each enrolled subject up to 32 paired measurements of SpO_2 and HR will be obtained from the reference device and BrOxy M. Table 1 below summarises the data collection process.

Plateau no.	**Range SpO_2 (%)**	**Inhaled solution**	**Plateau duration**	**No. of Measurements of SpO_2 and HR extracted from recordings**
I)	95 – 100 (target 97%)	Ambient air (medical air)	2.5‘	8
II)	90 – 94 (target 92%)	O2 15% + N 85%	2.5‘	8
III)	85 – 89 (target 87%)	O2 13% + N 87%	2.5‘	8
IV)	80 – 84 (target 82%)	O2 11% +N 89%	2.5’	8
			Total	**32 measurements**

Table 1: Number of measurements to be performed for each subject, for each target level of SpO_2 and indication of the gaseous solution inhaled during each target level.

After completing the study on 10 subjects, collecting around 320 paired measurements it will be verified that, by dividing the desaturation interval into three equal parts, each part contains around a third of the measurements made. Therefore, considering the minimum target number of 200 measurements (per EN 80601-2-61 standard) and the interval between 98% and 82% of SaO_2 to be divided into three parts, at the end of the protocol on all the subjects recruited, it is expected to have:

- At least 67 measurements between 98% and 93% of SpO_2;
- At least 67 measurements between 92% and 87% of SpO_2;
- At least 67 measurements between 86% and 82% of SpO_2

In the event that this minimum target is not reached to meet the requirements of the EN 80601-2-61 standard, it may be necessary to recruit up to 2 additional subjects.

4 FINANCIAL ASPECTS OF THE STUDY

The study will be financed by Life Meter, the study Sponsor, by means of a signed agreement between Life Meter and the clinical site, in compliance with the applicable regulations and prior to commencement of the study.

5 INVESTIGATIONAL MEDICAL DEVICE

5.1 DESCRIPTION

BrOxy M is a CE-marked medical device consisting of a wearable device (bracelet – Figure 2), a charging and connection base (Figure 3) and medical software to be installed on a Personal Computer (PC).

Figure 2: BrOxy M bracelet

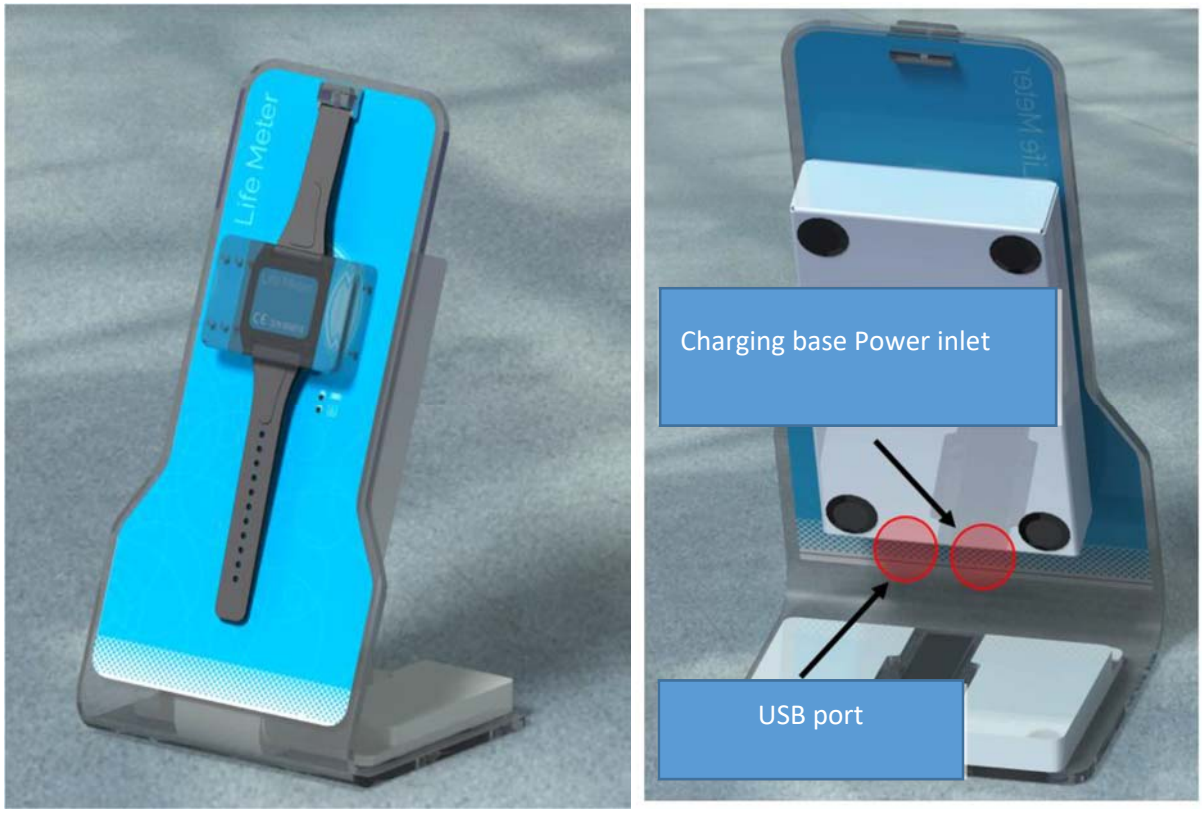


Figure 3: BrOxy M positioned on the one-station charging base (front and back view)

As shown in Figure 4, BrOxy M is equipped with an adjustable strap that allows the device to be adapted to the patient's wrist. The side of the device to be kept in contact with the subject's skin is the one that has the opening of the reflection photoplethysmographic sensor.

The adhesion between the skin of the subject and the device is ensured by a single-use double-sided adhesive film.

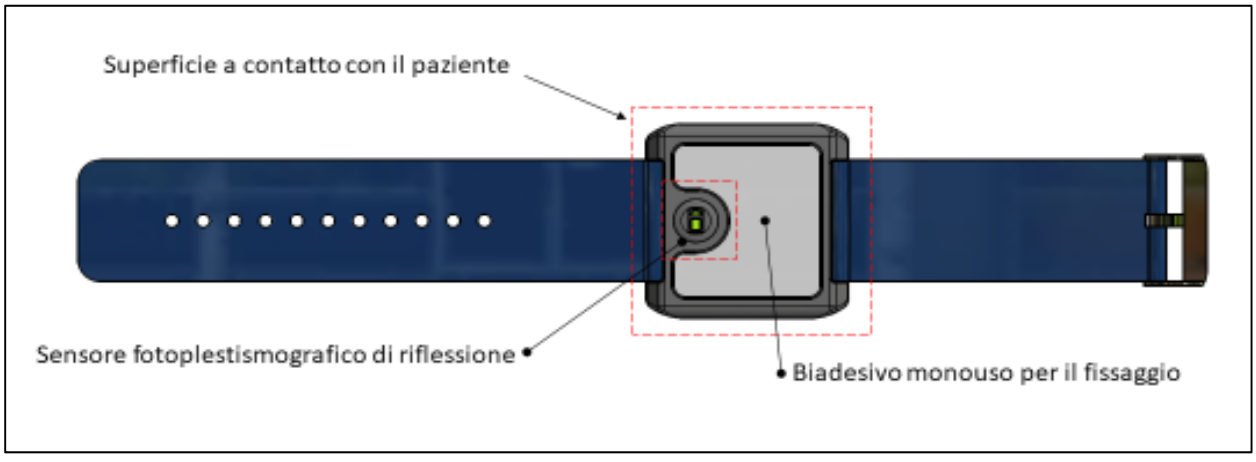


Figure 4: Details of the BrOxy M bracelet (bottom side).

5.2 USE OF BROXY M

The BrOxy M device is a wearable medical device with a size similar to that of a watch (see Figure 2) which, thanks to the sensor placed on the side in contact with the skin of the subject's wrist, is able to acquire photoplethysmographic signals and memorize them. With the use of the proprietary software provided by Life Meter S.r.l., it is possible to download the signals stored in the BrOxy M device, view, archive and export them. Such signals can be used as input for algorithms with diagnostic purposes, provided that they are suitably processed with specifically designed software in order to obtain parameters that describe the physiological state of the patient (such as, for example, heart rate or blood oxygen saturation).

According to the product intended use, registration of photoplethysmographic signals using BrOxy M can occur for up to 24 hours. In this study, signal registration will occur for a maximum of around 35 minutes for each enrolled subject. At the end of the signal registration phase, BrOxy M will be placed on the one-station charging base. Recorded signals will be automatically transferred to the PC in use for the study and archived in an electronic medical record set up for each enrolled subject. The signals recorded with the device will then be processed by a Sponsor delegate with the software for medical use provided by Life Meter S.r.l.

Detailed procedures covering installation of the one-station recharge base, software installation on the PC, use of BrOxy M bracelet, use of proprietary software and other details are fully described in the BrOxy M User Manual.

5.3 BROXY M REGULATORY STATUS AND INTENDED USE

BrOxy M is a CE marked, Class IIa medical device.

According to the User Manual, the BrOxy M intended use is as follows:

- The device is dedicated to recording signals for photoplethysmographic use in the red and infrared frequency, through a wearable module placed on the subject's wrist;
- The device allows the recording and storage of the signals described above, their transfer to the memory of a personal computer and their storage through a software module;
- The software allows to export the recorded signals for further analysis and measurements performed by means of specific algorithms and/or processes possibly developed by the user himself, under his/her responsibility;
- The signals recorded with the device can be used for diagnostic purposes (for example, heart rate calculation, SpO_2 measurement, etc.) as long as they are suitably processed, under the direct responsibility of the user, with appropriate software for medical use provided by Life Meter S.r.l. and/or other compatible software.

The typical contact duration between BrOxy M and the subject’s wrist is 24 hours. BrOxy M can be used by subjects of all ages having wrist diameter between 150 and 200 mm and provided that the skin of the

wrist where the device will be placed is intact and healthy. No other contraindication exists for the use of BrOxy M.

In this study, BrOxy M will be used on healthy volunteers accepting to participate in a controlled desaturation study, in full accordance with the limitations imposed by and instructions included in the BrOxy M User Manual. The purpose of this study is to confirm the performance of BrOxy M in comparison with a reference, CE marked, pulse oximeter equipment. The study will follow the procedures described in the standard ISO 80601-2-61:2017 "*Medical electrical equipment - Part 2-61: particular requirements for basic safety and essential performance of pulse oximeter equipment*".

6 REFERENCE MEDICAL DEVICE

The device used as the reference for measuring SpO_2 and heart rate is the Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N (Covidien LLC, USA). It is a CE marked device normally used in a hospital environment for monitoring patients while they are in bed. The Nellcor pulseoxymeter will require the use of the Nellcor™ Adult XL SpO_2 Sensor mod. MAXALI (sterile, single-use only).

In addition to being a precise device for measuring HR and SpO_2, it allows the storage of the displayed measurements and the downloading of data on a PC memory via USB. These features make the Nellcor™ mod. PM1000N suitable to be employed as reference device in this clinical trial.

The main features of the Nellcor mod. PM1000N are as follows:

- Precision on adult subjects, even with low perfusion:
  - Error +/- 2 digits, on the 70-100% range of SpO_2;
  - Error +/- 3 bpm, on the 20-250 bpm range of HR;
  - The measurements used as a reference were carried out on healthy, voluntary subjects, by means of the use of a CO-oximeter with analysis of arterial blood samples.

- Data and measurement recording:
  - Up to 48 hours of recording
  - Sampling: 1 measure per second
  - USB connection to download recorded data (SpO_2 and heart rate recorded values) by means of specific software;
  - Downloaded data shows time indication (hour, minute and second) and operating status, indicating signal quality such as motion, alarms, loss of pulse, etc.

- Alarms:
  - Acoustic and visual feedback to report critical conditions;
  - Possibility to customize the alarm levels (for threshold value and minimum duration of the event);

- Product compliance:
  - IEC 60601-1:2005

- IEC 60601-1-2: 2007
- IEC 80601-2-61:2001

The rationale for the selection of the reference device is that it meets the requirements specified in the standard ISO 80601-2-61:2017, section EE.3. In particular, the Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N has been calibrated against:

1) a Co-Oximeter for measuring SpO_2
and
2) an ECG for measuring cardiac frequency.

7 OBJECTIVES AND ENDPOINTS

7.1 OBJECTIVES

The **primary objective** of the study is to confirm the performance of BrOxy M in comparison with a reference, CE marked, pulse oximeter equipment in a controlled desaturation study.

The **secondary objectives** of the study are:

- To assess BrOxy M performance in subjects with different skin pigmentation (Note: this is a tentative objective, since few subjects are planned to be enrolled and there is no guarantee that they will have different skin pigmentation)
- to confirm BrOxy M safety

7.2 ENDPOINTS

The **primary endpoint** of the study will be a comparison of the accuracy of SpO_2 and HR measurements obtained with BrOxy M with that obtained with the reference pulse oximeter in paired observations over a range between 80% and 100% SpO_2.

For each subject, four different saturation plateaus will be set up (i.e. at 97%, 92%, 87% and 82% SpO_2), waiting 30'' before starting to keep the SpO_2 level on each plateau for 2.5’ in order to obtain 8 measurements in post-processing. These measurements will be at least 20'' away from each other, for a total of 32 measurements for each subject, as summarized in Table 1.

The study will follow the procedures described in the standard ISO 80601-2-61:2017 “*Medical electrical equipment - Part 2-61: particular requirements for basic safety and essential performance of pulse oximeter equipment*”.

As **secondary endpoints,** the following will be verified:

- whether any relation exists between the accuracy of BrOxy M for both SpO_2 and HR and subject skin pigmentations as measured with the Fitzpatrick scale.

- that no adverse events related to the use of BrOxy M, besides a temporary slight skin irritation of the wrist, will occur.

8 RISK BENEFIT ASSESSMENT

8.1 BENEFITS

Enrolled subjects will not receive any direct benefit from participating in the study besides the possibility to have some clinical parameters assessed at screening, e.g. ECG, hemoglobin, etc.

8.2 RISKS

Each subject enrolled in the study will be exposed to experimentally generated, controlled hypoxia, starting from 97% O_2 and reaching 83% O_2 as the lowest level. During the desaturation protocol, which is expected to last a maximum of 35 minutes for each subject, SpO_2 and cardiac frequency will be measured continuously using BrOxy M and the reference device.

The following considerations were made about the potential risks that subjects enrolled in this study will be exposed to:

- Risks associated to the use of BrOxy M and the reference device are negligible, i.e. for BrOxy M a slight irritation of the skin where the bracelet is placed can sometimes be expected. For the reference device subjects may exhibit allergic reactions to the adhesive tape. For this reason subjects with known allergy to adhesive tapes should be excluded from the study
- The absence of pre-existing cardiovascular or pulmonary disease that may expose subjects to increased risks when participating in the study will be carefully checked at screening (see exclusion criteria in Section 10.2)

It is expected that, due to the use of a controlled desaturation protocol foreseen by the standard ISO 80601-2-61:2017, this study is classified as an interventional post-market clinical study in accordance with Annex I of EN ISO 14155:2020.

- Values may be below 90% for a maximum continuous period of 3 minutes. The Principal Investigator will be equipped with a chronograph with a timer and alarm function to avoid exposure of the subjects to longer periods of hypoxia.
- The lowest SpO_2 that subjects will be exposed to is 82% for maximum 3 minutes (30'' for stabilization and 2.5' for signal recording). This situation is similar to the one that a subject would have by staying at an altitude of around 3,500-4,000 meters for 3 minutes. Data in literature indicate that there are no risks for healthy subjects for permanence time below 30 minutes at altitudes up to 5,500 m (32).
- The full test will be performed only once on each subject.
- The study will be performed in an outpatient pneumology department, where medical assistance can be promptly provided if necessary.

- The experience of research laboratories performing desaturation studies involving up to 10,000 subjects exposed to arterial saturation levels as low as 45% for brief periods, indicates that brief hypoxia levels much higher than those in this study (i.e. SaO_2 50%-70% for 10-30 minutes) is well tolerated in healthy individuals with normal cardiopulmonary function (33).

In light of the above considerations the Sponsor believes that all potential risks related to study participation have been minimized. As a result, residual risks are minimal and can be considered acceptable.

9 STUDY SITES

The study will be conducted in a single Center in Italy. The list of Centers is maintained separately.

10 STUDY SUBJECTS

The study population will include adult healthy volunteers who will accept to participate in this oxygen desaturation study in a controlled clinical environment. At least 11 healthy subjects will be enrolled. Data collected on the first subject will not be used for accuracy measurements but will serve to finalize the test set up phase and ensure adequate recording procedures are used by site staff. On each of the remaining 10 subjects, 32 paired measurements will be performed in order to obtain a maximum of 320 "valid" measurements (see Section 17.2) which guarantees a statistically significant accuracy study (see point EE2.3.4 of the ISO 80601-2-61: 2017 standard).

To take into account invalid data and potential drop out samples up to 2 additional subjects may need to be enrolled. For this reason sample size for this study will be between a minimum of 11 and a maximum of 13 healthy volunteers.

10.1 INCLUSION CRITERIA

1. Healthy subjects of both sexes with an age ≥ 18 and ≤ 50.
2. ASA classification 1
3. Positive Allen's test
4. Intact and healthy skin on the selected wrist
5. Wrist circumference between 150 mm e 200 mm.
6. Normal ECG obtained at screening or within 2 months prior to screening
7. Ability to understand and execute the required study procedures and provide an informed consent to the study.

10.2 EXCLUSION CRITERIA

1. Presence of at least one of the following altered hemoglobin parameters at screening:

a. αHb ≤ 10 gr/dl
b. COHb ≥ 3%
c. MetHb ≥ 2%

2. For pre-menopausal female subjects only: positive pregnancy test performed at screening on urines or capillary blood
3. Presence of any cardiovascular pathology in medical history
4. Any episodes of respiratory infection during the 30 days prior to screening
5. Any prior experience of dyspnea
6. Hospitalization during the 2 months prior to screening, for any reason.
7. Chronic drug intake known to interfere with gaseous exchanges or induce changes in cardiac frequency
8. Presence of any medical condition not allowing the subject to perform the required test
9. Known allergy to adhesive tapes
10. Participation in an interventional study with a medicinal product or a medical device in the 30 days prior to screening.

10.3 POINT OF ENROLLMENT

Point of enrollment is defined as the point where the subject, after having signed the informed consent form, is confirmed to fulfill all criteria for eligibility.

Following the point of enrollment, each subject will undergo study procedures and measurements.

10.4 WITHDRAWAL/DISCONTINUATION FROM THE STUDY

Subjects may choose not to participate in the study or to withdraw from the study at any time by informing the Investigator. Participation in the clinical investigation is entirely voluntary.

Subjects' participation in the study will be terminated early by the Investigator in the following situations:

- Study is terminated by the investigator/Sponsor
- For other reasons, e.g. repeated protocol violations or inability to meet protocol requirements, at Investigator's discretion

Sponsor should be informed of all subjects whose participation in the study is terminated early.

Subjects who are withdrawn from the study due to adverse events (AE), on the basis of Investigator's judgement, will be followed until medical condition returns to baseline, or is considered stable, and will be recorded in the Case Report Form (CRF).

All subjects enrolled in the clinical investigation (including those withdrawn early from the clinical investigation) shall be accounted for and documented.

10.5 PROCEDURE FOR SUBJECT REPLACEMENT

Should there be circumstances not allowing to proceed with the experimental protocol, for whatever reason, related to subject's reaction or to the poor quality of the photoplethysmographic signals recorded and verified in real time (see Section 11), the following steps shall be followed:

- The actual interruption of the clinical protocol for that subject will be registered on the subject's CRF, noting the reasons;
- Any "valid" measurement collected before study interruption will be kept (see Section 17 for a definition of "valid measurement");

Please note that, based on minimum data requirements for the study, it is possible that up to 2 additional subjects may need to be enrolled in order to reach the established number of target measurements (see Section 3).

10.6 EXPECTED STUDY DURATION

Expected duration for each subject is approximately two weeks from screening up to completion of test session. The test session is expected to last approximately 50 minutes (including subject and equipment preparation, measurement session and final subject status verification).

Total duration of the study is expected to be about 3 weeks from first subject enrolled until last subject completed.

11 STUDY PROCEDURES

A schematic representation of the procedures performed in the study is shown below in Table 2:

Procedures	**Screening Visit**	**Visit 1 - Test session (Within 2 weeks from Screening)**
Informed Consent	X	
Medical history	X	
ECG	X*	
Physical exam (including wrist skin evaluation)	X	
Skin Pigmentation assessment (Fitzpatrick scale)	X	
Wrist circumference measurement	X	
ASA classification	X	
Vital signs	X	X§
Allen test	X	

Procedures	Screening Visit	Visit 1 - Test session (Within 2 weeks from Screening)
Concomitant medications	X	
Blood test for Hb parameters	X	
Pregnancy test (urinary or capillary blood)	X**	
Inclusion/exclusion criteria assessment	X	
BrOxy M recording		X
Reference device recording		X
Adverse Events		X

Table 2: Study flowchart

§ 5 minutes after end of test session
* ECG required at screening unless a 12-lead ECG has been performed in the last 2 months prior to screening
**pregnancy test only for pre-menopausal women.

The following sections include a detailed description of study procedures at each visit.

11.1 SCREENING VISIT (FROM DAY -14 TO DAY 0)

11.1.1 Informed consent

Informed consent shall be obtained in writing from each subject. The subject will sign and personally date the informed consent form attesting that the information was accurately explained and that informed consent was freely given. The original signed informed consent form will be kept in the site file and a copy will be given to the patient.

The process of obtaining the informed consent shall be documented before any procedure specific to the study is applied to the subject.

11.1.2 Eligibility assessment

Subjects will be assessed for eligibility and all inclusion/exclusion criteria will be verified.

Demographic data and medical history of past or present cardiovascular or pulmonary illnesses as well as information on medication currently being taken to address current illnesses will be obtained by the investigator.

Patients will be permitted to receive concomitant therapy as medically required. A record of all concomitant prescription medications will be maintained. Subjects undergoing drug treatments known to interfere with gaseous exchanges or cardiac frequency will be excluded.

A physical examination will be performed which will include the examination of general appearance and body systems as indicated by patient symptoms, with particular attention to the wrist skin. The subject

will be asked about any known reaction/allergy to any of the device components. Subjects will be assessed for ASA classification and Allen's test.

Systolic/diastolic blood pressure will be recorded after 5 minutes of rest. Wrist circumference will be measured. Skin pigmentation will be measured using the Fitzpatrick scale (see Appendix A).

A 12 lead ECG will be performed (unless the subject had an ECG tracing done in the last 2 months) and blood sample taken to assess hemoglobin parameters (αHb; COHb and MetHb).

For pre-menopausal females a urine or capillary blood pregnancy test will be obtained.

All measurements will be recorded in the CRF.

Once all screening tests/assessments are completed the subject will be determined to be eligible or excluded.

11.2 VISIT 1 – TEST SESSION (DAY 1)

The study involves, starting from medical air (O2 concentration 20.98%), the subsequent use of cylinders with an oxygen concentration of 15%, 13% and 11%, rest nitrogen. We will use a Hans Rudolph one-way valve connected to the subject with a nose piece to eliminate nasal ventilation and with a disposable mouthpiece and filter. The valve on the inhalation side will be connected, with a two-way tap, to a Douglas bag supplied by the gas cylinder with a concentration selected according to the target level of SpO_2 to be obtained (see Table 1).

From each session of measurements on each subject, the photoplethysmographic signals in the red and infrared frequency will be collected from the subject's wrist, while the comparison measurements will be carried out using the reference oximeter.

As shown in Figure 1, during the execution of the protocol, four different saturation plateaus will be used (in steps of 5% and, therefore, at 97%, 92%, 87% and 82%), waiting 30'' before starting to keep the SpO_2 level on each plateau for 2.5' in order to obtain 8 measurements in post-processing. These measurements will be at least 20'' away from each other, for a total of 32 measurements for each subject as summarized in Table 1 (on page 17 and replicated below).

Plateau no.	**Range SpO_2 (%)**	**Inhaled solution**	**Plateau duration**	**N. of Measurements of SpO_2 and HR extracted from recordings**
I)	95 – 100 (target 97%)	Ambient air (medical air)	2.5'	8
II)	90 – 94 (target 92%)	O2 15% + N 85%	2.5'	8
III)	85 – 89 (target 87%)	O2 13% + N 87%	2.5'	8
IV)	80 – 84 (target 82%)	O2 11% +N 89%	2.5'	8
			Total	**32 measurements**

Table 1: Number of measurements to be performed for each subject, for each target level of SpO_2 and indication of the gaseous solution inhaled during each target level.

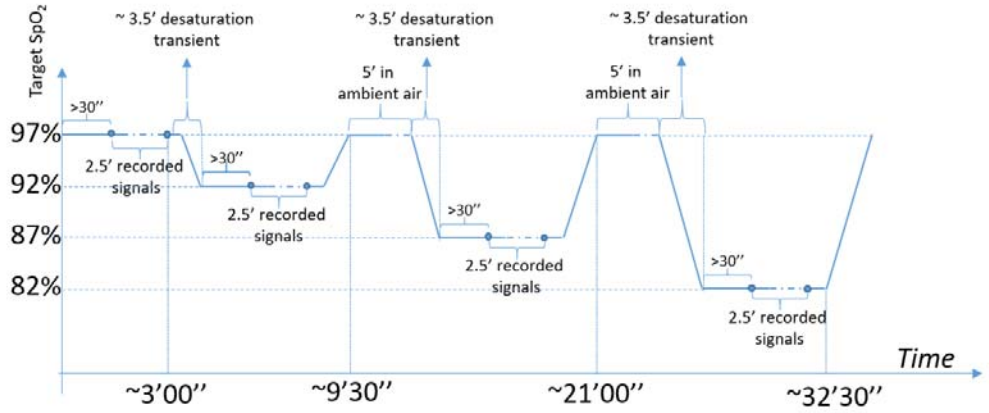


Figure 1: Time distribution of the SpO_2 measurements to be carried out on each individual subject. Four different saturation plateaus will be used, waiting the plateau stabilization before starting the 2.5' signal recording from which the series of 8 measurements with at least 20' of pause are extracted by means of a post-processing algorithm.

Before starting the measurement protocol, as shown in Figure 1, in order to prepare the above instrumentation, it is necessary to carry out the following operations:

1. Start the PC;
2. Start the reference pulse oximeter device for monitoring the SpO_2 and HR on the subject's left index:
    - Verify that the registration of SpO_2 and HR measurements is effective;
    - Verify that the safety alarms are active and correctly set;
    - Verify that the clock of the reference device is consistent with the time shown on the PC clock used to manage BrOxy M.
3. Connect the medical power supply of BrOxy charging base to the power outlet;
4. Connect the medical power supply to the charging base;
5. Connect the charging base to the PC via the USB cable;
6. Place the BrOxy M wearable device on the charging base;
7. Start the "BrOxy software" by double clicking on the icon on the desktop and log in with your account.

Subject preparation (see figure 5):

- The subject will be invited to sit in in a comfortable position and breathe normally
- The subject will be asked to move as little as possible during the test and to notify the Investigator immediately in case of any adverse event suffered during the test

- On the BrOxy software GUI, follow the procedure for inserting a new medical record (see User Manual, par. 14.3.1), entering only requested personal data according to the following scheme:
  - Last name: enter the patient code used for the registration;
  - Name: enter again the patient code
  - Date of birth: enter the date of birth of the patient;
  - Weight, height, etc.: leave the fields blank.

- Carry out the procedure for the assignment of a device (see User Manual, par. 14.4.1), following the steps provided for by the software.

- The subject is made to wear nose clip, the respiratory mask with filter correctly connected to the circuit for the administration of the gaseous solution with controlled oxygen concentration (starting from standard conditions).

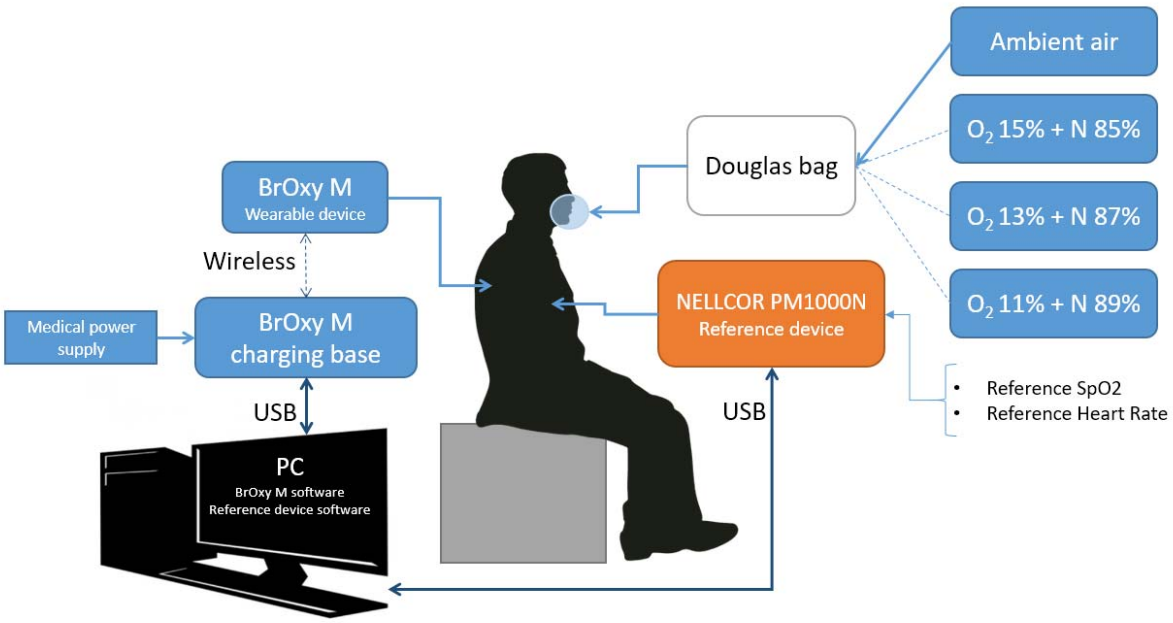


Figure 5: Experimental setup to be used during the measurements.

The test protocol on the single subject will then be started. Detailed instructions will be provided to the Investigators in writing and during training sessions performed prior to study start.

After 5 minutes following test completion blood pressure will be measured and if no abnormalities are recorded, the subject will officially leave the study.

In case of abnormalities the subject will be followed-up until resolution and return to a “normal state”.

11.3 ADVERSE EVENTS ASSESSMENT

In the study, all Adverse Events (AEs) will be recorded by Investigator(s), whether the event is considered related to the investigational medical device/procedures or not.

All AEs, regardless of relatedness or outcome, will be reported to Sponsor via the Adverse Event Form of the CRF.

The reporting period for adverse events is from the day the patient performs screening examinations until study exit. Pre-existing conditions (present before the signature of the informed consent) are considered "concurrent medical conditions" and should NOT be recorded as AEs. However, if the subject experiences a worsening or complication of such a concurrent condition, the worsening or complication should be recorded as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g. "worsening of…."). Worsening of pre-existing conditions will be classified as SAE, if matching criteria listed in Section 15.

Investigators will record AE severity and causality. All AEs should be followed to resolution.

The Investigator is responsible for maintaining adequate source documentation relating to AE diagnosis, and treatment. Procedures for investigation and treatment of AEs should be according to site standard of care and Investigator judgment.

Refer to Section 15 for further details.

11.4 ACTIVITIES PERFORMED BY THE SPONSOR REPRESENTATIVE

Activities performed by the Sponsor representative will include:

- training of site staff prior to study start about the desaturation protocol and use of BrOxy M (see Section 13).
- downloading data from the BrOxy M software and the reference software recordings, and data processing (see Section 17).
- monitoring activities (see Section 14)

12 STUDY SUPPLIES/MATERIAL

The following materials will be provided by the Sponsor:

- 3 BrOxy M wearable devices (bracelets)
- One-station charging base (mod. "BrOxy M x1 charging base")
- a cable for USB connection between BrOxy M and PC
- a medical AC / DC converter power supply
- BrOxy M User and installation Manual
- BrOxy M double-sided tape
- 1 Chronograph with timer function
- A PC for the management of measurement acquisitions with BrOxy M software and reference device software installed
- Paper case report forms and study specific binder for archiving of study documents
- Gaseous solutions with 15, 13 and 11% oxygen (rest nitrogen) + medical air

- One way air "HANS RUDOLPH" valve
- 4 Douglas bags (50, 100 or 150 liters)
- Tubing from Gaseous solutions to Douglas bag
- Tubing from Douglas bag to one way air valve
- Disposable mouth filter (Neumofilt Ergo- CE 0051)

All study products will be stored in a safe place and according to package labeling directions, with access limited to study personnel. BrOxy M will be used in accordance with the Instructions for Use (IFU) included in the package and in the study operations binder.

The following materials will be provided by the study site:

- A medical marker or pen to trace references on the subject's skin;
- A flexible tape measure for measuring the circumference of the subject's wrist;
- Reference pulse oximeter.

The equipment provided by the site will be maintained and calibrated according to the site's standard procedures.

12.1 STUDY SUPPLIES/MATERIAL ACCOUNTABILITY

Sponsor delivery of BrOxy M devices and related accessories will be performed in accordance with the site local procedures.

Access to these products should be restricted to study site authorized staff. Use of BrOxy M and the accessories provided by the Sponsor outside of the clinical protocol is strictly prohibited.

The Principal Investigator or Authorized Delegate must keep records documenting the receipt, use, return or disposal of BrOxy M in an appropriate manner and/or in the CRF, as described in the monitoring plan.

12.2 PROCEDURE FOR THE RETURN OF SUSPECTED DEFECTIVE DEVICES

In the event that Investigators and/or Sponsor's representatives suspect the existence of possible defects in the BrOxy M devices or a damaged accessory, such devices shall be returned to Sponsor for further investigation. Investigator or delegate should contact Sponsor for instructions on returning the product.

13 SITE STAFF TRAINING

The study protocol involves procedures which are specific to this study and are not routinely performed during patient care. For this reason, Sponsor expert personnel will provide training to site staff as follows:

- Use of reference device (Nellcor mod.PM1000N) and BrOy M will be reviewed

- The desaturation procedure and all associated materials used in the study will be described
- Test simulations will be carried out to create the ventilation circuit (Douglas bag filled with a mixture of O_2 and N_2 at a known concentration), the connection system to the inspiratory route of the one-way valve, patient connection with filter and fitting to the valve and the bag supply system during the test.
- The procedures for replacing the Douglas bag with a mixture of the inspired gas at different O_2 concentration will also be verified.
- Correct data transmission from BrOxy M and the reference device to the PC will be verified
- Instrument synchronization will be checked
- Before starting the study protocol, tests will be carried out to verify compliance with all the procedures planned and the delivery times of the gas mixtures as required by the Study protocol.

Overall, site staff training, preparation of materials and test simulations, including at least 2 complete tests, will take approximately 4-5 days.

All training will be documented.

In order to ensure that site staff is fully familiar with study procedures, data collected on the first subject will serve to finalize the test set up phase and ensure adequate recording procedures are used by site staff

14 MONITORING AND QUALITY ASSURANCE

Monitoring activities will be performed to ensure that the Investigator and his/her study team conduct the clinical investigation in accordance with the signed Study Agreement, the Study Protocol, any conditions imposed by the Ethics Committee, the Declaration of Helsinki, applicable Good Clinical Practices, and any other applicable regulations, to ensure adequate protection of the rights and safety of subjects and the quality and integrity of the resulting data. The monitor(s) will be qualified by training and experience and possesses the appropriate skills necessary to properly monitor the study.

Due to the ongoing Covid-19 pandemic, monitoring visits may be performed on site or remotely, provided that all necessary systems required to protect data confidentiality are adopted.

In addition to regular monitoring visits, the Sponsor (or designees) may conduct audits at the study site.

15 SAFETY REPORTING

15.1 DEFINITIONS

Adverse Event (AE) (ISO 14155:2020)

Untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device and whether anticipated or unanticipated

Note 1: This definition includes events related to the investigational medical device or the comparator.
Note 2: This definition includes events related to the procedures involved.
Note 3: For users or other persons, this definition is restricted to events related to the use of investigational medical devices

Serious Adverse Event (SAE) (ISO 14155:2020)

Adverse event that led to any of the following:
a) death,
b) serious deterioration in the health of the subject, users, or other persons as defined by one or more of the following:
1) a life-threatening illness or injury, or
2) a permanent impairment of a body structure or a body function including chronic diseases, or
3) in-patient or prolonged hospitalization, or
4) medical or surgical intervention to prevent life-threatening illness or injury, or permanent impairment to a body structure or a body function,
c) foetal distress, foetal death, a congenital abnormality, or birth defect including physical or mental impairment
Note 1: Planned hospitalization for a pre-existing condition, or a procedure required by the clinical investigation plan, without serious deterioration in health, is not considered a serious adverse event.

Device deficiency (ISO 14155:2020)

Inadequacy of a medical device with respect to its identity, quality, durability, reliability, usability, safety or performance
Note 1: Device deficiencies include malfunctions, use errors, and inadequacy in the information supplied by the manufacturer including labelling.
Note 2: This definition includes device deficiencies related to the investigational medical device or the comparator.

15.2 ANTICIPATED ADVERSE EVENTS

AEs that have been identified as risks through the risk management process and therefore can be considered as “anticipated” for the purposes of this study are:

- temporary slight skin irritation of the wrist related to the use of BrOXy M
- temporary transitory non critical increase of the heart rate and blood pressure
- mild hyperventilation

It is expected that the above symptoms will be resolved in few minutes (<5 minutes) after the interruption/completion of the test session.

Refer to Section 8.2 RISKS for further details.

15.3 REPORTING OF ADVERSE EVENTS AND DEVICE DEFICIENCIES

All Adverse Events (AEs) shall be recorded by Investigator(s) on the CRF, whether the event is considered related to the investigational medical device/procedures or not.

Any Serious Adverse Event (SAEs) and device deficiency has to be recorded in the CRF and reported by Investigator to Study Sponsor within 24 hours of having become aware of the event. The Investigator shall also comply with any applicable Ethics Committee requirements for reporting of SAEs.

Study Sponsor has to evaluate any SAE / device deficiency reported from clinical site.

15.4 REPORTING OF INCIDENTS

If an incident is identified or a field safety corrective action is implemented, it shall be reported to the competent authorities concerned according to the applicable regulation for post-market vigilance and per Study Sponsor internal vigilance procedures.

If foreseen by local regulation, any SAE / device deficiency fulfilling the definition of “Incident” shall be reported by the Investigator to the national Competent Authority, according to the procedure in place at the time of occurrence of event.

16 STATISTICAL ANALYSIS

16.1 OVERVIEW

Statistical analyses will be performed with SPSS, Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL), by following the requirements of the standard ISO 80601-2-61:2017 (“*Medical electrical equipment - Part 2-61: particular requirements for basic safety and essential performance of pulse oximeter equipment,* Procedure for non-invasive laboratory testing on healthy adult volunteers).

The *significance level,* that is the probability of rejecting the null hypothesis when it is true, will be set to 0.05 (two-sided, corresponding 5% risk of concluding that a difference exists when there is no actual difference).

16.2 ANALYSIS SETS

Analyses will regard 320 pair measurements (80 in the ambient air, and 80 for each of the four defined target levels, that will be obtained from 10 healthy subjects).

16.3 EFFICACY ANALYSIS

16.3.1 Baseline characteristics

Frequency analyses will be used to produce descriptive statistics of the sample as regards demographic, anthropogenic, clinical, or other characteristics (gender, age, race, weight, height, smoking habits, etc.)

16.3.2 Primary endpoint

The **primary endpoint** of the study regards the accuracy of SpO_2 and HR measurements of BrOxy M. The *accuracy* of the experimental BrOxy M will be evaluated by comparing SpO_2 readings of the BrOxy M to the values obtained with the secondary standard pulse oximeter equipment (Nellcor™ Bedside Respiratory Patient Monitoring System model PM1000N, Covidien LLC, USA), that is traceable to co-oximeter SaO_2 values.

The following routines will be used:

- The paired t test, to evaluate the differences between experimental and standard values: the paired sample *t*-test procedure determines whether the mean difference between two sets of observations is zero. It regards *pairs* of observations.
- Correlation analysis, to quantify the degree to which experimental and standard values are related: it is a bivariate analysis that measures the strength of the association between two variables. The degree of association is measured by a correlation coefficient (r) on a scale that varies from + 1 through 0 to – 1. We expect a positive correlation: r>0.70 indicate a strong correlation, and r=1 a perfect correlation.
- Concordance correlation coefficient (CCC), to evaluate whether the experimental value is interchangeable with the standard; this statistic quantifies the agreement between a new measurement and a gold standard measurement. Also CCC varies from -1 to 1, with perfect agreement at 1.
- Bland -Altman (B&A) plot, to evaluate the agreement between experimental and standard values; the B&A plot analysis is a simple way to evaluate a bias between the mean differences, and to estimate an agreement interval, within which 95% of the differences of the second method, compared to the first one, fall. Data can be analyzed both as unit differences plot and as percentage differences. The Bland-Altman chart is a scatterplot with the difference of the two measurements for each sample on the vertical axis and the average of the two measurements on the horizontal axis. Three horizontal reference lines are superimposed on the scatterplot - one line at the average difference between the measurements, along with lines to mark the upper and lower control limits of plus and minus 1.96*the standard deviation of the measurement differences. Bland and Altman also discuss the option of using confidence interval bounds, based on the standard error of the mean, for the upper and lower reference lines. If the two methods are comparable, then differences should be small, with the mean of the differences close to 0, and show no systematic variation with the mean of the two measurements. 'Small' would be an amount that would be clinically insignificant for the factor being measured.

- Linear regression analysis: to give further information about any potential proportional bias about the mean differences and averages. It is a simple test for a linear trend, with the mean difference as dependent variable and the average difference as independent. The aim is to evaluate if the mean difference can be predicted from the average. The resulting coefficients indicate whether one can accept the null hypothesis that there is no proportional bias.

16.3.3 Secondary endpoint

To verify whether any relation exists between the accuracy of BrOxy M for both SpO_2 and HR and subject skin pigmentations, and that no adverse events related to the use of BrOxy M will occur, the following routines will be used:

- Sensitive analyses by group of skin pigmentation: above reported analyses can be repeated after stratifying by group.
- ANOVA (analysis of variance): this test allows a comparison of two or more groups.
- Frequency analyses describing whether adverse effects exist.
- Bivariate and multivariate logistic regression analyses evaluating which risk factors may be associated with adverse effects, if these exist.

17 DATA MANAGEMENT

17.1 DATA COLLECTION

This study will enroll healthy volunteers. For this reason, study data will not be collected in hospital medical records but rather as follows:

1. Subject data, including the date when the Informed Consent is signed, subject demographics, screening test results (printouts of laboratory results signed by the Principal Investigator), patient eligibility, adverse events and any other measurements will be collected on paper CRFs, which will be signed by the Principal Investigator after completion.
2. Measurements obtained with the BrOxy M: the photoplethysmographic signals recorded during the measurements are stored on a magnetic support (hard disk of the PC used for the BrOxy M software). The files will contain:
    a. The recorded values of the red and infrared sensors integrated in BrOxy M, acquired with a constant frequency over time and stored continuously until the measurement is stopped;
    b. The time when the registration is started;
    c. The values of the accelerometric sensor integrated in BrOxy M, recorded in parallel to the photoplethysmographic signals;
3. Measurements obtained with the reference device Nellcor™ mod. PM1000N. The files will contain:
    a. The time when each sample is recorded;

b. SpO_2 measurements
c. HR measurements

17.2 DEFINITION OF VALID MEASUREMENT

A "valid measurement" is defined as a measurement obtained under the following conditions:

1. Signal compliant with quality control for both the reference and BrOxy M
2. No movement above predefined threshold for both the reference and BrOxy M

The quality control of the signal is carried out automatically by the HR and SpO_2 calculation algorithm and is based on the following conditions:

1. Starting from the signals, through appropriate band-pass filters, the following components are calculated as follows:
   a. RED_DC = continuous part of the red signal;
   b. RED_AC = alternating part of the red signal;
   c. IR_DC = continuous part of the infrared signal;
   d. IR_AC = alternating part of the infrared signal
2. Average values of RED_DC and IR_DC have to be in the expected range, determined empirically;
3. If the previous point occurs, peak-peak amplitude values of RED_AC and IR_AC have to be in the empirically determined acceptability range;
4. If the previous condition is verified, the correlation coefficient calculated between IR_AC and IR_AC has to be higher than the empirically determined threshold;

The algorithm used to recognize the time windows to be excluded for motion artifacts is organized with the following steps:

1. Control of the amplitude of photoplethysmographic signals: the amplitude of the alternating component of red and infrared signals (obtained by filtering signals with an anti-causal high pass filter must not exceed a threshold determined empirically;
2. Control of the correlation between photopletismographic signals and accelerometric signals: the module of accelerometric signals recorded on three orthogonal axes of space (A_x,A_y and A_z) is calculated as $|A_{XYZ}[t]| = \sqrt{A_x[t]^2 + A_y[t]^2 + A_z[t]^2}$and, subsequently, it occurs that:
   a. The correlation between and $|A_{XYZ}[t]|$ plethythygraphic signal recorded in the red frequency is less than a certain threshold derived empirically;
   b. The correlation between $|A_{XYZ}[t]|$ and plethythygraphic signal recorded in the infrared frequency is less than a certain threshold derived empirically;
3. If the conditions in point 1 and 2 occur, the time window from which to derive heart rate and SpO_2 data is excluded from the collection of data deemed useful in the context of the study.

At this point, once all the above conditions are verified, the values of SpO_2 and HR are calculated for the signal windows considered. These values are considered acceptable if the following conditions occur at the same time:

- Heart rate values between 40 and 280 bpm
- SpO_2 values between 80 and 100 %

17.3 DATA PROCESSING

Subject's data from the paper CRF will be entered manually on an electronic clinical database.

Measurements obtained with the reference device and with BrOxy M will be accessed by the Sponsor Data Manager, and processed as follows. After data acquisition from the devices, first 8 valid paired assessments of SpO_2 and HR will be selected from each plateau as follows:

- Reference device: SpO_2 and HR valid values will be selected starting from at least 30'' after plateau stabilization, with a time interval between measurements of at least 20''.
- BrOxy M: SpO_2 and HR values generated by BrOxy M will be obtained by processing the photoplethysmographic signals generated by the device with the BrOxy Medical software. Then, SpO_2 and HR values corresponding to the same time points selected for the reference pulse oximeter will be chosen.

As a result, it is expected that up to 32 paired (and synchronized) measurements of SpO_2 and HR will be obtained from each enrolled subject, from the reference device and BrOxy M. Once paired measurements for SpO_2 and cardiac frequency have been obtained at the preselected time points (0, 20'', 40'', etc.) the list of paired observations will be merged with subject's data in the clinical database.

18 PROTOCOL AMENDMENTS

All amendments to the protocol must be reviewed and approved by the reference Ethics Committee(s) prior to implementation, except where necessary to eliminate apparent immediate hazards to human subjects.

For non-substantial changes (e.g. minor logistical or administrative changes) not affecting the rights, safety and well-being of human subjects or not related to the clinical investigation objectives or endpoints, a simple notification to the ECs will be performed.

19 PROTOCOL DEVIATIONS

19.1 DEFINITIONS

Protocol deviation(s):

Instance(s) of failure to follow, intentionally or unintentionally, the requirements of the Clinical Investigation Plan.

- Major deviation: CIP deviations that may have affected the rights, safety or well-being of the subject or validity of study data
- Minor deviation: CIP deviations that have NOT have affected the rights, safety or well-being of the subject or the validity of study data.

19.2 DEVIATION MANAGEMENT

Except in emergency situations, implementation of any CIP change that affects patient safety, investigational scope, or the scientific quality of the study will not be permitted until all of the following conditions have been met:

a. The Investigator and the Sponsor have approved the protocol amendment, and

b. The responsible EC has reviewed and approved the protocol change.

Under emergency circumstances, deviations from the CIP to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Sponsor and the EC. Such deviations shall be documented and reported to the Sponsor and the EC as soon as possible.

19.3 RECORDING AND NOTIFICATION REQUIREMENTS

Investigators are required to maintain accurate, complete and current records, including documentation of any deviations from the Protocol, including the date of and reason for the deviation. All major deviations must be reported immediately to Life Meter and/or designee and documented appropriately.

Deviations shall be reported regardless of whether medically justifiable, pre-approved by the Sponsor, or taken to protect the patient in an emergency.

Deviations from the CIP performed in emergency circumstances to protect the rights, safety and well-being of human subjects shall be documented and reported to the Sponsor and, if required, to the EC as soon as possible.

19.4 CORRECTIVE AND PREVENTIVE ACTIONS

Protocol deviations are only allowed in case of medical and clinical urgent need. The investigator should consult with the Sponsor if feasible.

The Sponsor will review records of deviations and will classify them as major or minor, consider the need for corrective and preventive action and further external reporting to regulatory authorities and Ethics Committee according to applicable local regulation.

20 STUDY SUSPENSION OR PREMATURE TERMINATION (IF APPLICABLE)

The study will end when all of the following apply:

- The planned sample size has been achieved

- The last patient has completed the last visit

The Sponsor may decide to suspend or prematurely terminate the study site for any reason, including site non-compliance with the Protocol.

If the site is suspended or prematurely terminated, the Sponsor or designee shall promptly inform the clinical Investigator(s) of the termination or suspension and the reason(s) for this. The Sponsor or designee shall then promptly inform the Ethics Committees.

If the study is terminated on grounds of safety, the Ethics Committees will be notified in writing of the termination of the study, with a clear explanation of reasons and details of follow-up measures, if any. Notification will be performed without unjustified delay, and within timelines foreseen by local regulation.

21 PUBLICATION POLICY

All data generated from this study are the property of Life Meter and shall be held in strict confidence along with all information furnished by Life Meter.

Independent analysis and/or publication of these data by the Investigator or any member of his/her staff are not permitted without prior written consent of Life Meter.

Written permission to the Investigator will be contingent on the review by Life Meter of the statistical analysis and manuscript and will provide for nondisclosure of Life Meter confidential or proprietary information.

In all cases, the parties agree to submit all manuscripts or abstracts to all other parties 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

22 RECORDS AND RETENTION

22.1 INVESTIGATOR RECORDS

The Investigator must retain all records pertaining to this study, including:

- All site-specific correspondence that pertains to the investigation
- Records of receipt, use, or disposition for all study devices
- Patient records, including but not limited to:
    - Signed and dated Informed Consent Form for each enrolled subject
    - Observations of Adverse Device Effects, whether anticipated or unanticipated; and
    - All Case Report Forms which include a record of exposure of each subject to the study device and study procedures.

- A copy of the Protocol, with documentation showing the date of and reason for each deviation from the Protocol
- A copy of signed study Agreements
- EC approval documentation and correspondence

Records must be retained at clinical site in accordance with local law and regulations for a minimum period of 10 years (or longer if local law requires) after the date on which the study is terminated or completed.

The Investigator should take measures to prevent accidental or early destruction of the study related materials and must obtain permission from the Sponsor or designee in writing before destroying or transferring control of any study records.

22.2 SPONSOR RECORDS

All records and reports related to this investigation are subject to inspection and must be retained together in an organized, retrievable manner for 10 years after the date the last device has been placed on the market.

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Appendix A

Fitzpatrick scale