Official Title: Validation of the O3 Regional Somatic Tissue Oxygenation Monitor

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A Single-Center, Prospective Study in Healthy Volunteers for Validation of the O3 Regional Somatic Tissue Oxygenation Monitor

Sponsor:	Masimo 52 Discovery Irvine, California 92618, USA
[redacted]	[redacted]
[redacted]	[redacted]
Study Devices:	Masimo Radical-7 Pulse Co-Oximeter Masimo Root Patient Monitoring and Connectivity Platform Masimo O3 Regional Oximeter Masimo O3 Adult rSO_2 Adhesive Sensors Masimo RD SET Adult SpO_2 Sensors
Sponsor Protocol Number:	SCHE0001
Institutional Review Board/ Research Ethics Committee:	METC Brabant PO Box 90151 5000 LC Tilburg info@metcbrabant.nl

Principal Investigator	Title	Signature	Date
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Sponsor	**Title**	**Signature**	**Date**
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1 INTRODUCTION

This document is a clinical investigational plan for a human research study sponsored by Masimo Corporation. The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. By participating in the study, the Investigator agrees to adhere to all stipulations of this protocol, the conditions of the Institutional Review Board (IRB) or Research Ethics Committee approval, federal and local regulatory requirements, 21 CFR 812, ISO-14155, International Conference on Harmonization Good Clinical Practice (ICH GCP) guidance, and Regulation (EU) 2017/745 of European Parliament and of the Council (EU MDR).

1.1 Background and Rationale

For normal cellular function, a continuous supply of oxygen is important. In case of (regional) disturbances of oxygen supply, tissue damage might ensue. Hence, maintenance of sufficient tissue oxygenation is an important target for organ protection in perioperative medicine[1]. In routine perioperative medicine, the focus of hemodynamic monitoring is on macro-circulatory variables such as heart rate and mean arterial blood pressure.[2] Nevertheless, it has been demonstrated before that macro-hemodynamic variables do not necessarily represent actual microcirculatory perfusion[3], so that adequate organ and/or tissue perfusion may be undetected and ultimately, this may lead to adverse postoperative outcome. For example, intraoperative kidney oxygenation during transplantation has been linked to postoperative graft function – and may thus be used as 'target' for perioperative hemodynamic management.[4] In addition, somatic (systemic) oxygenation may be helpful as a guide in resuscitation of critically ill patients with circulatory shock[1,5,6].

In the past decade, technologic advances allow the non-invasive, bed-side measurement of regional oximetry using near-infrared spectroscopy (NIRS). In NIRS, near-infrared light is transmitted through the skin using a non-invasive adhesive probe. The resulting reflected light as obtained by this probe is suggested to resemble regional tissue oxygenation by measuring the ratio of oxyhemoglobin to total hemoglobin in an assumed arterial / venous ratio (typically 30:70 or 25:75, depending on the manufacturer)[7]. Multiple NIRS monitors are currently available on the market. Most frequently, NIRS sensors may be placed on the overlying skin of the tissue(s) of interest, to provide tissue oxygen saturation of that particular region for monitoring oxygenation of that particular region or as an assessment of overall somatic oxygenation[7].

The Masimo O3 regional oximeter (Masimo Corp, Irvine, USA) has been developed recently. This device allows measurement of somatic tissue oxygen saturation (rSO_2) at different sites. Obviously, it is of paramount importance that NIRS readings are reliable during different states of oxygenation, especially during hypoxia. While rSO_2 trending in somatic tissues has been assessed previously[8], and the O3 device has recently received FDA clearance and CE marking for rSO_2 trending in somatic tissues, its absolute accuracy in monitoring rSO_2 in somatic tissues has not been determined yet.

To assess whether somatic rSO_2 values match with microcirculatory oxygenation of the tissue of interest, both the afferent (arterial input) and efferent (venous output) oxygenation needs to be assessed. Hence, in absence of any major circulatory pathology that would influence arterial oxygenation, this is generally derived from a radial artery catheter (SaO_2) and from a venous catheter (SvO_2) as close to the tissue of interest as possible[9].

One study in healthy volunteers examined the absolute and trend accuracy of the O3 oximeter previously. In this study cerebral rSO_2 was measured continuously on the forehead and reference blood samples were taken from the radial artery and internal jugular bulb vein, at baseline and after inducing hypoxic states. This study enrolled 27 subjects and found acceptable absolute and trend accuracy of the rSO_2 measurements when compared with the reference gold standard. [redacted]

[redacted]

A method agreement study was also done in the clinical setting, in which peripheral somatic rSO_2 readings of the O3 device were compared with those measured with another device (EQUANOXTM 7600, Nonin Medical Inc, Plymouth, USA) in cardiac surgical patients. This study suggested that both devices cannot be used interchangeably. A limitation of this study was that the O3 device was not compared to the gold standard (gradient between arterial and venous oxygen saturation).

In this study, controlled hypoxia will be induced in healthy volunteers while Masimo O3 regional oximeter-derived rSO_2 readings of somatic tissue [redacted] will be measured. These readings will be compared with SaO_2 and venous sheath-derived reference SvO_2 values [redacted]

2 STUDY DEVICES

2.1 Study Devices Used

- Masimo Radical-7® Pulse Co-Oximeter®
- Masimo Root® Patient Monitoring and Connectivity Platform
- Masimo O3® Regional Oximeter
- Masimo O3® Adult rSO_2 Adhesive Sensors
- Masimo RD SET™ Adult SpO_2 Sensors

2.2 Device Accountability

2.2.1 Receipt of Study Device

Masimo may ship or hand-carry devices and sensors to the investigative sites. Upon receipt of the study device supplies, an inventory must be performed and the Equipment Shipment Check Form (FRM-2713) and the device accountability log will be completed for each device and signed by the receiver. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study devices in a given shipment will be documented in the study files. The investigator must notify the study sponsor of any damaged or unusable study devices that were supplied to the investigator's site.

2.2.2 Use of Study Device

Use of devices and sensors will be documented on Case Report Forms (CRF) for each subject. Any unused devices must be returned to the Sponsor at the end of the study or before product expiration date.

2.2.3 Return or Destruction of Study Device

At the completion of the study, there will be a final reconciliation of study devices. This reconciliation will be logged on the device accountability log. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study devices. Devices destroyed on site will only be upon written instruction from the Sponsor and will be documented in the study files.

2.2.4 Device Deficiency

Device deficiency is defined as inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling. (ISO 14155:2011 3.15). All Masimo device related deficiencies should be reported to the Sponsor and must be recorded in the CRF in a timely manner. This excludes computer issues. These should be reported to the IRB/ethics committee as required per local

requirements. The sponsor shall also report to the Member State any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate. When a Masimo device deficiency is observed, every effort should be made to return the device and its packaging to the Sponsor in a timely manner.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to validate Masimo O3 regional oximeter-derived somatic rSO_2 readings in healthy volunteers under controlled hypoxia, by comparison of these readings with SaO_2 / SvO_2 blood reference values as the gold standard.

[redacted]

4 STUDY DESIGN

4.1 General Design

This study is a prospective, single-center healthy volunteer validation study. [redacted] If the volunteer decides to participate in this study, informed consent is explained and a copy is handed over or sent by email and a date is set for the screening visit, and for the hypoxia procedure study session, which will be at least 24 hours after obtaining informed consent. A screening blood and urine test will be performed as well as physical examination and ECG recording. All inclusion criteria and exclusion criteria needs to be confirmed before a volunteer is eligible for the study and can be assigned a study number. For women, a negative pregnancy test must be confirmed before the onset of study session.

To derive a representative population sample size, volunteer inclusion will be stratified to skin pigmentation, and at least 4 subjects should have a dark skin pigmentation. Skin pigmentation will be assessed using the Massey Scale[10].

The names and study numbers of those who sign a consent form will be recorded in an enrollment form stored in a password protected electronic file. Demographic data (ASA physical status classification, age, gender, weight, height, skin pigmentation, co-morbidity, and regular medication history) will be recorded in a case report form (CRF) kept separate from any subject identifiable data. After IRB/ethics committee approval for the study and written informed consent of each volunteer, the volunteers will undergo the study session. The study will take place in a controlled environment at [redacted] UMCG, under supervision of at least one qualified anesthesiologist. [redacted]

We aim to include 25 subjects in the study, and withdrawn subjects will be replaced by another volunteer. Therefore, several extra subjects are recruited to account for withdrawn subjects and screen-failures. Subjects who did not complete the study procedures due to errors of technical nature and who did not meet any of the stopping criteria during the procedure, can be asked to repeat the study procedures. [redacted]

4.2 Study Endpoints

4.2.1 Primary Endpoint

The primary endpoint for this study is to assess regional oximeter-derived somatic rSO_2 with Masimo O3 sensors and to compare this to invasively determined somatic oxygenation values (SaO_2 / SvO_2 [redacted]) during normoxia and

controlled hypoxia [redacted]

4.3 Clinical Site

University Medical Center Groningen (UMCG)
[redacted]

4.4 Population Base

Subjects will be healthy men and/or women between 18 and 45 years of age (inclusive) consisting of members of the community at large. At least 4 subjects should have a dark skin pigmentation, in order to have a representative sample size.

4.5 Number of Subjects

A total of 25 subjects may be enrolled at the Clinical Site. Withdrawn subjects will be replaced by another volunteer. Therefore, several extra subjects are recruited to account for withdrawn subjects and screen-failures. Subjects who did not complete the study procedures due to errors of technical nature and who did not meet any of the stopping criteria during the procedure can be asked to repeat the study procedures. If the subject is willing to discuss repeating the study session, a person who is independent from the study will discuss this with the subject to ensure they do not feel obligated to do so and there is no serious error. If, after this discussion, they still want to return for a second study session, informed consent will be obtained a second time and a second study session will be scheduled. If a subject were to repeat the study procedures, the subject would receive financial compensation for performing the study procedures a second time.

4.6 Inclusion Criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Age between 18 and 45 years, inclusive
- Willing and able to provide written informed consent
- Healthy subjects

4.7 Exclusion Criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Pregnant women
- Presence of any cardiovascular or pulmonary disease
- Exposure to high altitude(s) (>2000 m) within 30 days prior to the study
- Known allergy to intravenous contrast medium or heparin
- Subject has skin abnormalities affecting the digits such as psoriasis, eczema, angioma, scar tissue, burn, fungal infection, substantial skin breakdown, nail polish or acrylic nails that would prevent monitoring of SpO_2 levels during the study
- Patients deemed not suitable for the study at the discretion of the Investigator

5 STUDY PROCEDURES

5.1 Subject Recruitment and Screening

Volunteers will be recruited by the UMCG research group. [redacted]

The complete study will take place at [redacted] UMCG that will be equipped with all required study and safety equipment. All volunteers will be selected and screened for eligibility in the study by a certified physician in the [redacted] of the UMCG. All volunteers will fast for solid foods for at least 6 hours before the start of the study. The volunteer is allowed to drink clear fluids until 2 hours before the start of the study. No premedication will be administered. All participants will be encouraged to restrain from alcohol on the day prior to the study.

5.2 Informed Consent

The study staff delegated for this task are responsible for conducting the consent process and for obtaining consent, prior to each subject's inclusion into the study. The Investigator shall not allow subjects to participate in the study or consent any subjects prior to receiving IRB/ethics committee approval of the consent process. Informed consent may be obtained directly or via mail.

The study staff will thoroughly explain the purpose and procedures of the study, the subject's involvement and responsibilities, the potential risks and benefits of participation, the subject's rights and privacy of the data collected, and that participation is voluntary. Subjects will be given 7 days to ask questions regarding the study. Once all questions have been answered and the informed consent signed, the subject will be enrolled in the study. If the subject refuses to participate, they will not be enrolled in the study. The subject will be assigned a non-identifying subject number for the data collected in this study, and the names and study numbers of those who sign consent forms will be recorded in an enrollment form stored in a password protected electronic file. The Investigator shall retain a copy of the signed informed consent document in each subject's record, and the subject shall be provided with a copy of the signed informed consent document.

Once a volunteer decides to participate in the study and informed consent is obtained, screening can be started. The hypoxia procedure will be at least 24 hours after obtaining informed consent. Prior to the onset of study sessions, all subjects will perform screening blood- and urine tests as well as an ECG. Women will also undergo a pregnancy test before their study session.

5.3 Treatment of Subjects

Each subject will undergo the following interventions in a standardized sequence:

- Stepped Hypoxia Plateau Sequence Protocol by changing inspiratory oxygen fraction (FiO_2):
    - Normoxia: room air (FiO_2 0.21) (baseline)
    - Hypoxia [redacted]
- After measurements have been completed, the subject will be allowed to breathe room air.

[redacted] oxygen saturation (SaO_2 or SvO_2, respectively) will be measured by drawing blood samples [redacted]

5.4 **Study Procedures**

- O3 rSO_2 sensors will be applied before each study session.
- All subjects will have their medical history done at the time of the screening period. Female subjects will have a pregnancy test prior to being admitted to the study.
- Upon passing the screening tests, each subject will read and sign an Informed Consent to Act as a Human Research Subject form prior to being enrolled in the study.

[redacted]

- A (20G) radial artery catheter will be inserted in the radial artery of the non-dominant hand. It will be inserted under local anesthesia (Lidocaine), only after the Allen Test is negative. The arterial catheter will be used for SaO_2 measurements and for continuous monitoring of arterial blood pressure.
- The venous sheath and radial artery will be continuously flushed with NaCl 0.9% by a pressure infusion bag (per institutional policy). [redacted]
- The Masimo O3 regional oximeter rSO_2 sensor will be placed on the skin [redacted]
  The rSO_2 sensors may be placed on the forehead as well.
- The face-mask will be applied to the subject and it will be connected to the breathing circuit. Note, the subject will still breathe room air at this moment.
- Hypoxia Plateau Sequence Protocol by changing inspiratory oxygen fraction (FiO_2):

  After all catheters and sensors have been placed, the following intervention(s) will be applied during the stepped hypoxia plateau sequence:

  - *Baseline* blood samples will be obtained [redacted]
  - [redacted] *Hypoxia* breathing mixtures of O_2 / N_2 in air via a tight-fitting facemask. [redacted]

[redacted]

- After measurements have been completed, the subject will be allowed to breathe room air.
- A study event flowchart of the stepped hypoxia plateau sequence is given below (figure 1).

[redacted]

[redacted]

- Recovery Period:

[redacted]

  - The volunteer should remain in bed for one hour, and will be monitored using the remaining basic monitoring (see above) for another hour.
  - The volunteer will be allowed to go home after the medical personnel determine that it is safe to do so. The volunteer should not drive a car by themselves, until 1 day after the study procedure. The volunteer is asked to refrain from strenuous exercise the first 48 hours after the study procedure.

[redacted]

- All blood samples will be immediately analyzed using satellite-lab blood gas analysis. [redacted]

Early Termination Conditions: At any point in the study, if the subject feels uncomfortable, the subject will be given 100% oxygen and the study interrupted/stopped. For the safety and welfare of the subjects, the study will be prematurely terminated if any of the following conditions are met: a rise or fall in arterial blood pressure of more than 30% from baseline, a rise in heart rate to 130 BPM or greater, dysrhythmia, complaints of feeling faint, altered mental status, clinical

suspicion of an allergic reaction, or if the subject requests the study to stop for any reason. In the unlikely event that a drug-related allergic reaction is suspected, the study will be stopped directly and if required, the infusion of the drug will be halted. All routinely available drugs and monitoring equipment is directly available for treatment of an allergic reaction. The attending researcher-anesthesiologist is experienced in the management of allergic reaction.

5.5 Early Withdrawal of Subjects

Subjects can withdraw from the study at any time for any reason if they wish to do so without any consequences or loss of benefits to which they are otherwise entitled. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

Any data collected until the time of subject withdrawal may be included in the final data analysis. Information on the subject's withdrawal should be documented on the CRF and the Screening and Enrollment Log, and should include clear documentation of the reason for withdrawal. Subjects that withdraw from the study may be replaced by another volunteer.

6 STATISTICAL CONSIDERATIONS

6.1 Sample Size Calculation

The current study will be a repeated measures study with one group. The study is focused at assessing the agreement of SaO_2/SvO_2 readings with O3-derived rsO_2 readings during a stepped hypoxia plateau sequence protocol. A high level of correlation among repeated measures (0.9) is assumed.

[redacted]

The calculation(s) of the required sample size comply with formal requirements in the U.S, by the FDA, which require for NIRS validation studies the inclusion of at least 12 subjects[9].

6.2 Statistical Plan

All analyses will be performed for pooled data from all sensors, as well as broken down per individual sensor. As the aim of the study is to investigate agreement of Masimo O3 regional oximeter-derived rSO_2 readings with the invasive blood gas derived gold standard(s), conventional method comparison statistics (i.e. bias, precision and repeatability) will be performed.

Primary Study Parameter(s): The data from this study will be analyzed using a mixed effects analysis with subject as random effects terms. By adding an additional factor identifying replicate data points, the repeatability of cerebral and somatic O3 rSO_2 values (ΔrSO_2(O3) – ΔrSO_2(Gold Standard)) will be estimated and used to correct Limits of Agreement for repeated measures on the same subject. Bland Altman Plots will be created showing the calculated Limits of Agreement. These will be evaluated to check whether the variance of rSO_2 from the investigated devices and the invasive gold standard, is constant over the range of hypoxia studied. [redacted]

Other Study Parameter(s): To evaluate the evolution of continuous hemodynamic variables (stroke volume, arterial blood pressure) during the study protocol, the Mann-Whitney test will be used. Categorical variables (gender, skin pigmentation as assessed by the Massey scale) will be analyzed with the Fisher's Exact test.

7 BENEFITS / RISK ANALYSIS

Benefits: There are no benefits to enrolled subjects. Information gained from this research study may help develop new technologies that may be beneficial for future patients.

Non-Invasive Measurement Risks: The risk from non-invasive devices is minimal since the measurements are non-invasive and derived with optical technology.

Sensor Risks: As with all optical sensors, the investigational device has the theoretical risk of thermal burn. The design includes safeguards, and this risk is believed to be very low. Pressure damage may occur to the tissue if the sensor is placed too tightly. Sensors will be attached with adhesive, and those on the forehead may be secured by a supplemental headband. This risk is believed to be low. Optical exposure is minimized by procedure and low power. This risk is believed to be low.

Lidocaine Risks: Administration of lidocaine injection, as a local anesthetic to facilitate placement [redacted] may be discomforting and can feel like a slight pinch along with warm/burning sensation. Other anticipated adverse events that may occur include but are not limited to: flushing or redness of the skin, itching skin, small red or purple spots on the skin, unusually warm skin, bruising, bleeding at the application sites, and swelling. These adverse events are expected to be temporary. Although not common, it is also possible to have an allergic reaction to injectable lidocaine. Subjects should not take part in this study if they are allergic to lidocaine injection or other types of numbing medicine, or if they have a heart rhythm disorder such as Wolff-Parkinson-White Syndrome or Stokes-Adams Syndrome. Subjects are instructed to tell the study staff right away if they experience hives; difficulty breathing; or swelling of the face, lips, tongue, or throat.

[redacted] **Venous Catheter Placement Risks:** Placement of a venous catheter over a sheath will be performed by a certified and qualified interventional radiologist. Placement of a venous catheter via a [redacted] venous sheath is, when placed using ultrasound-guidance, regarded safe[18]. Typical – but rare – complications may include local hematoma and hemorrhage. The catheter will be a [redacted] for which the correctness of its positioning will be verified by [redacted] x-ray. The (carcinogenic) risk of a single x-ray is negligible.

Radial Artery Line Placement Risks: Placement of the arterial line will be done by a certified and qualified anesthesiologist. These cannulas are used routinely in standard perioperative anesthetic care. Arterial catheters have been found to be relatively safe with a low incidence of serious complications[13]. Typical complications include temporary radial artery occlusion and hematoma. (Very) rare complications (less than 1% of procedures) include localized catheter site infection, hemorrhage, sepsis, permanent ischemic damage and pseudo-aneurysm formation. All these risks are very rare in volunteer populations, especially when used for short-term monitoring [17].

Stepped Hypoxia Plateau Sequence Protocol Risks: The risks of the brief exposures to hypoxia include feeling short of breath, headache, dysrhythmia, and dizziness. Brief loss of consciousness may occur, but is not expected at the levels of oxygen targeted for these tests given the magnitude of comparable studies, in which none of the events mentioned previously, occurred.[8,9,14-16] The methods comply with FDA and ISO standards [9,11].

Mitigating Risks: This study employs methods that are similar to standardized protocols as set by the FDA and ISO standards for testing pulse oximetry devices under controlled hypoxia[11]. For this study, only capacitated healthy adult volunteers will be included. During the study, the volunteers will be observed closely by at least one certified anesthesiologist, and at least one qualified nurse. Placement of the arterial line will be done by a certified and qualified anesthesiologist. These cannulas are used routinely in standard perioperative anesthetic care.

In a similar healthy volunteer study, we have previously performed controlled hypoxia procedure(s) without any unwanted sequelae[14]. During the study, the volunteer will remain under the care of the research team which includes at least a certified nurse or doctor after the study is concluded. If, after 2 hours in the recovery phase of the study, the volunteer meets the UMCG standard day care discharge criteria he/she can be discharged home. If the volunteer does not meet the discharge criteria he/she will be observed until deemed fit for discharge.

8 SAFETY AND ADVERSE EVENTS

8.1 Definitions

The definitions for adverse event, adverse device effect, serious adverse event, serious adverse device effect, and unanticipated adverse device effect are provided below (ISO 14155:2011, 21 CFR 812.3(s)).

- Adverse Event (AE): an adverse event is any untoward medical occurrence in a subject which need not be related to the device under investigation.
- Adverse Device Effect (ADE): an adverse device effect is any untoward or unintended response to a medical device which may result from insufficiencies in the instructions for use or deployment of the device, or from use error.
- Serious Adverse Event (SAE): a serious adverse event is an adverse event that results in death, inpatient hospitalization, severe or permanent disability, a life threatening illness or injury, fetal distress, fetal death, a congenital abnormality, a birth defect, or medical or surgical intervention to prevent permanent impairment to body or structure.
- Serious Adverse Device Effect (SADE): a serious adverse device effect is an adverse device effect that results in death, inpatient hospitalization, severe or permanent disability or is life threatening.
- Unanticipated Adverse Device Effect (UADE): any serious adverse effect on health or safety or any life threatening problem or death cause by or associated with, a device, if the effect, problem, or death was not previously identified in nature, severity or degree of incidence in the investigational plan, or application (including a supplementary plan or application) or any other unanticipated serious problem associated with a device that related to the rights, safety or welfare of subjects.

Refer to the Risk/Benefit section for details on anticipated adverse device effects and potential risks associated with the study procedures.

8.2 Temporary Halt for Reasons of Subject Safety

The sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited IRB/ethics committee without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited IRB/ethics committee. The investigator will take care that all subjects are kept informed.

8.3 Anticipated Adverse Events

8.3.1 Device-Related Adverse Events

- Sensors may cause thermal burn, pressure damage, mild allergic reaction, dicomfort, redness, or skin irritation.

8.3.2 Procedure-Related Adverse Events

- Refer to the Benefits/Risk Analysis Section.

8.4 Adverse Event Reporting

The PI must follow the guidelines below and promptly report adverse events to the Sponsor through the CRF and Adverse Event Report Form, and must follow their IRB/ethics committee policy for safety event reporting:

- All Adverse Events, both Anticipated and Unanticipated and serious and non-serious, for the duration of the subject's enrollment in the study until subject completion of the study, shall be reported to the Sponsor and/or IRB/ethics committee.
- SAEs must be reported to the Sponsor within 48 hours. The sponsor shall report without delay any serious adverse event that has a causal relationship with the investigational device, the comparator or the investigation procedure or where such causal relationship is reasonably possible. The sponsor shall also report any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate. All other Adverse Events should be reported to the Sponsor within 5 business days. The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited IRB/ethics committee that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. SAEs will be reported to the Member State within 48 hours of awareness by the Sponsor. New information in relation to an already reported event will also be reported to the Member State within 48 hours.
- All Unanticipated Adverse Device Effects (UADEs), for the duration of the subject's enrollment in the study until subject completion of the study, shall be reported to the Sponsor and/or IRB/ethics committee. The investigator shall submit to the sponsor and to the reviewing IRB a report of any UADE occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect. The Sponsor will evaluate the UADE to determine if it presents an unreasonable risk to study subjects. If the Sponsor determines there is an unreasonable risk to subjects, the Sponsor will notify the FDA, IRB, and investigator within 10 working days after the Sponsor first received the notice of the UADE.

8.5 Adverse Event Follow-Up

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of the study within the Netherlands. Any new findings in relation to SAEs or device deficiencies that may have led to SAEs shall be reported without delay.

8.6 Annual Safety Report

In addition to the expedited reporting of UADEs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited IRB/ethics committee, competent authority, and competent authorities of the concerned Member States. This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation

8.7 Deviations from the study protocol

Deviations from the protocol must receive both Sponsor and the investigator's IRB/ethics committee approval before they are initiated, with the exception that 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 or the IRB/ethics committee. Any protocol deviations initiated without Sponsor and the investigator's IRB/ethics committee approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be documented and reported to the Sponsor and to the investigator's IRB/ethics committee as soon as a possible, but no later than 5 working days after the occurrence of the protocol deviation. All deviations must be recorded on the CRF and on the Protocol

Deviation Form. If protocol deviations continue to occur frequently at a study site, a corrective and preventive action (CAPA) may be opened by the Sponsor.

8.8 Withdrawal of IRB/ethics committee approval

An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB/REB as soon as a possible, but no later than 5 working days of the IRB/ethics committee notification of withdrawal of approval.

9 VULNERABLE POPULATIONS

9.1 Definition

Vulnerable populations are defined as disadvantaged sub-segment of the community requiring utmost care, special considerations and protections in research. This study may recruit subjects from the following: economically disadvantaged or unemployed, and educationally disadvantaged.

9.2 Protection of vulnerable subjects

- Reasonable compensation may be provided for economically disadvantaged subjects to eliminate possibility of undue influence due to financial incentive.
- Educationally disadvantaged subjects will be provided ample time to ask questions and comprehend information.
- Medical care will be provided to these subjects after the clinical investigation has been completed if they are injured as a direct result of participating in this research study. The cost of treatment for any research related injury will be covered by Masimo.

9.3 Responsible Parties

The IRB/ethics committee will review research with vulnerable populations and evaluate consent, level of risk, coercion, and the reason for choosing this particular subject population. The IRB/ethics committee will be responsible for determining what practices will include continuing review for compliance while monitoring these studies.

The Investigator holds the ultimate responsibility for protecting the rights, safety, and welfare of research subjects by ensuring that all regulations and proper documentation of consent is handled in a compliant and timely manner.

10 DATA MANAGEMENT

10.1 Confidentiality of Records

Information about the patients will be kept confidential. Study data that will be released to Masimo and other regulatory authorities will be de-identified.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records potentially include: questionnaires, memoranda, recorded data from automated instruments, and copies or transcriptions certified after verification as being accurate and complete. For this study, the case report forms may also be used as source worksheets. The investigator will permit trial-related monitoring, audits, IRB/ethics committee review and regulatory inspection(s), providing direct access to source data/documents.

10.3 Case Report Forms

The Site shall capture study data in the CRFs for each subject enrolled. The CRFs will be completed and initial and dated by the PI or delegated personnel. This also applies to those subjects who fail to complete the study. If a subject withdraws

from the study, the reason must be noted on the CRF. Case report forms are to be completed on an ongoing basis. CRF entries and corrections will only be performed by study site staff, authorized by the investigator. Entries and corrections to the CRF will be made following Good Documentation Practices.

The CRF may contain the following information, including but not limited to: inclusion/exclusion criteria, whether patient consent obtained before start of study, demographic information, patient medical history, comorbidities, prescription medication records, concomitant medications, patient alcohol consumption, over-the-counter medication information, relevant urine drug testing results, device readings, and occurrence of any adverse event, protocol deviation, and device deficiencies, etc. The CRF may also contain questions regarding the subject's experience with the sensor, such as ease of use, comfort, etc. The CRFs will be signed by the PI to attest that the data are complete and accurate and forward a copy to Masimo.

CRF entries will be verified by study monitor and any errors or inconsistencies will be queried to the site on an ongoing basis. Any changes will be made directly on the CRFs and re-verified. Query resolution will be assessed and confirmed by study monitor during site visit.

10.4 Data Transfer and Storage

Training on CRF completion will be provided to study personnel prior to data collection. Original CRFs, if paper-based, will be stored in a secure location at site. Paper CRFs will be scanned and sent to sponsor. Only authorized sponsor personnel will have access to study data, and will move it to a secure and backed-up drive at Masimo. CRFs will be checked for accuracy and completeness of data. If there are inconsistent or missing data points, a data query list will be generated and submitted to the PI or designee, who shall both follow GDP practices for data correction by striking through the old entry, adding in new entry with initial and date, and resend to Masimo the corrected CRF. Once all queries have been resolved, Masimo engineers are notified that data is ready for analysis. To ensure data integrity, Masimo engineers will only have read access to study data, therefore are unable to unintentionally tamper with the original data files.

10.5 Record Retention

All study information, including but not limited to study correspondence, study logs, device accountability records, consent forms, subject records, and copies of CRFs should be maintained in the Investigator site files.

Study records shall be retained during the study and for a period of at least 20 years after the clinical investigation has ended or at least 20 years after the last device has been placed on the market.

The Sponsor is responsible for verifying study data, retaining records, analyzing data, and authoring study reports.

11 MONITORING PLAN

As the Sponsor of this clinical investigation, Masimo Corporation is required by 21 CFR, Part 812, of the Food and Drug Administration regulations to monitor and oversee the progress of the investigation. The monitor(s) assigned by Masimo Corporation to this task will trained on departmental SOPs on conduct and monitoring of Sponsored studies.

In accordance with good clinical practices guidelines, there will be at least three scheduled monitoring visits to ensure overall regulatory compliance of the study:

- An initiation visit, prior to any subject enrollment to confirm site readiness, and to document training on the study protocol and procedures, and use of equipment.
- At least one periodic monitoring visit during initial enrollment, preferably when enrollment has reached approximately 10-15% of subjects, and then every 2 months thereafter.
- A final close out visit after the last patient has finished the study.

Depending on the quality of the data and/or changes to factors affecting patient safety, additional monitoring visits may be necessary according at the sponsor's discretion.

The Investigator shall provide the monitor access to all necessary records to ensure the integrity of the data (21 CFR 812, EU MDR). The monitor will verify source documents and records to entries in the CRFs and other GCP-related documents (IRB/ethics committee approvals, correspondences, and ICFs) provided that subject confidentiality is maintained, in agreement with local privacy regulations.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the CIP and the completeness, consistency and accuracy of the data being entered on them. During each visit, the monitor will verify presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs/SADEs and protocol deviations/violations, and check CRF against site records.

After each visit, the monitor will provide a monitoring follow-up letter to the investigator. The follow-up will detail findings and open action items observed during the visit. It is the responsibility of the Principal Investigator and Study Coordinator(s) to respond to the findings of the monitoring letter, and complete any open action items as soon as possible. Any open action items not completed within the time allowed may be sufficient grounds for study site suspension or termination; it will be up to the sponsor to determine whether any incomplete action items are sufficient grounds for suspension or termination. See Section 12.5 for details on suspension and termination.

12 ADMINISTRATIVE ASPECTS

12.1 Protection of Human Subjects

In accordance with EU MDR and 21 CFR 50, written consent must be obtained from each subject prior to any study procedures in accordance with applicable federal, state, and study site regulations. The Investigator must keep a copy of the signed consent form in each subject's record and provide a copy to the subject as well. The Investigator shall not allow a subject to participate in a study or sign consent prior to IRB/ethics committee approval. Oral consents may be used in the study with approval by IRB/ethics committee.

Prior to the start of data collection or subject enrollment, the Investigator must provide documentation of IRB/ethics committee approval of the study protocol and a copy of the approved informed consent form (EU MDR, 21 CFR 50).

12.2 Institutional Review Boards/Research Ethics Committee

The Sponsor and/or Investigator must submit the protocol to the appropriate IRB/ethics committee and obtain a copy of the written and dated approval letter. The approval letter should state the name of the documents reviewed, date of review, date of approval, and reference the study name (protocol title, study number, and version).

The informed consent used by the Investigator must be reviewed and approved by the Sponsor prior to submission to the IRB/ethics committee. The Investigator cannot enroll subjects until a copy of the approved informed consent is obtained from the IRB/ethics committee.

Any amendments to the protocol or informed consent should be submitted to the IRB/REB for review and approval (21 CFR 56, EU MDR). The IRB/ethics committee should be notified of any changes that may affect conduct of the study or pose safety risks to the subjects.

12.3 Confidentiality

All data collected will be kept confidential and de-identified. It can only be accessed by researchers and will be used for research purposes only.

12.4 Protocol Amendments

Any changes made to the clinical investigational plan/study protocol will be documented by way of an amendment. Before submitting protocol amendment to the IRB/ethics committee for approval, the protocol amendment must be agreed upon and signed by both the Investigator and the Sponsor. The Investigator shall not make any changes to the protocol without Sponsor approval and documented approval from the IRB/ethics committee. Both PI and Sponsor will retain the IRB/ethics committee approval letter and approved protocol as confirmation that the protocol amendment was approved.

A 'substantial amendment' is defined as an amendment to the terms of the IRB/ethics committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial
- the scientific value of the trial
- the conduct or management of the trial, or
- the quality or safety of any intervention used in the trial

All substantial amendments will be notified to the IRB/ethics committee and to the competent authority.

Non-substantial amendments will not be notified to the accredited IRB/ethics committee and the competent authority, but will be recorded and filed by the sponsor.

12.5 Suspension or Termination of Study Site

The Sponsor can suspend or prematurely terminate the PI's and study site's participation in the study, particularly if Sponsor finds serious non-compliance by the PI or site, and if such non-compliance was not resolved in a timely manner. The Sponsor will document the decision to suspend or terminate the investigation in writing. A suspended study site cannot enroll new subjects.

If the Sponsor determines that the study site's compliance to GCP, federal and EU MDR regulations to be inadequate at any point during the study, and Sponsor may move to suspend or terminate the study site, the Sponsor will provide notification in writing to the principal investigator, IRB/ethics committee and Member State as necessary. The study site is eligible for reinstatement upon correction of any findings and any open action items prior to the suspension, and provides a written guarantee that the same non-compliance will not reoccur in the future. Site can only resume patient enrollment upon receiving written notification of reinstatement from the Sponsor and/or IRB/ethics committee.

12.6 Termination of Clinical Investigation/Study due to UADE

The clinical investigation may be terminated if Sponsor determines that an unanticipated adverse device effect presents an unreasonable risk to the subjects. Termination shall occur not later than 5 working days after the Sponsor makes this determination, and not later than 15 working days after the Sponsor first received notice of the effect.

The Sponsor may resume the terminated clinical investigation with prior ethics committee approval if the device is non-significant risk.

12.7 Annual Progress Report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited IRB/ethics committee once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

12.8 Temporary Halt and (premature) End of Study Report

End of Study

The end of the study is defined as the last patient's last visit. The sponsor will notify the IRB/ethics committee and the Member State of the end of the clinical investigation within 15 days of the end of the study. Within one year after the end of the study, the investigator/sponsor will submit a final study report to the IRB/ethics committee and Member State with the results of the study, including any publications/abstracts of the study.

Premature Termination of Study

The sponsor will notify the IRB/ethics committee within 15 days of the study ending prematurely, including the reason of such an action. If the premature termination is for safety grounds, the sponsor will inform the Member State within 24 hours. The sponsor will notify the Member State within two working days and no later than four calendar days after premature termination of the study. Within three months of a premature termination, the sponsor will submit a clinical investigation report to the IRB/ethics committee and Member State.

Temporary Halt of Study

The sponsor will notify the IRB/ethics committee and Member State within 15 days of a temporary halt in the study, including the reason of such an action. If the temporary halt is for safety grounds, the sponsor will inform the Member State within 24 hours. Within three months of a temporary halt in the study, the sponsor will submit a clinical investigation report to the IRB/ethics committee and Member State.

13 AGREEMENT BETWEEN INVESTIGATOR AND SPONSOR REGARDING RESPONSIBILITIES FOR GOOD CLINICAL PRACTICE

International Conference of Harmonization (ICH) E6 Good Clinical Practice guidance is an international ethical and scientific quality standard for designing, conducting, recording, and reporting trials that involve the participation of human subjects.

It specifies general requirements intended to:

- Protect the rights, safety and well-being of human subjects,
- Ensure the scientific conduct of the clinical investigation and the credibility of the clinical investigation results,
- Assist Sponsors, monitors, Investigators, ethics committees, regulatory authorities and other bodies involved in the conformity assessment of medical devices.

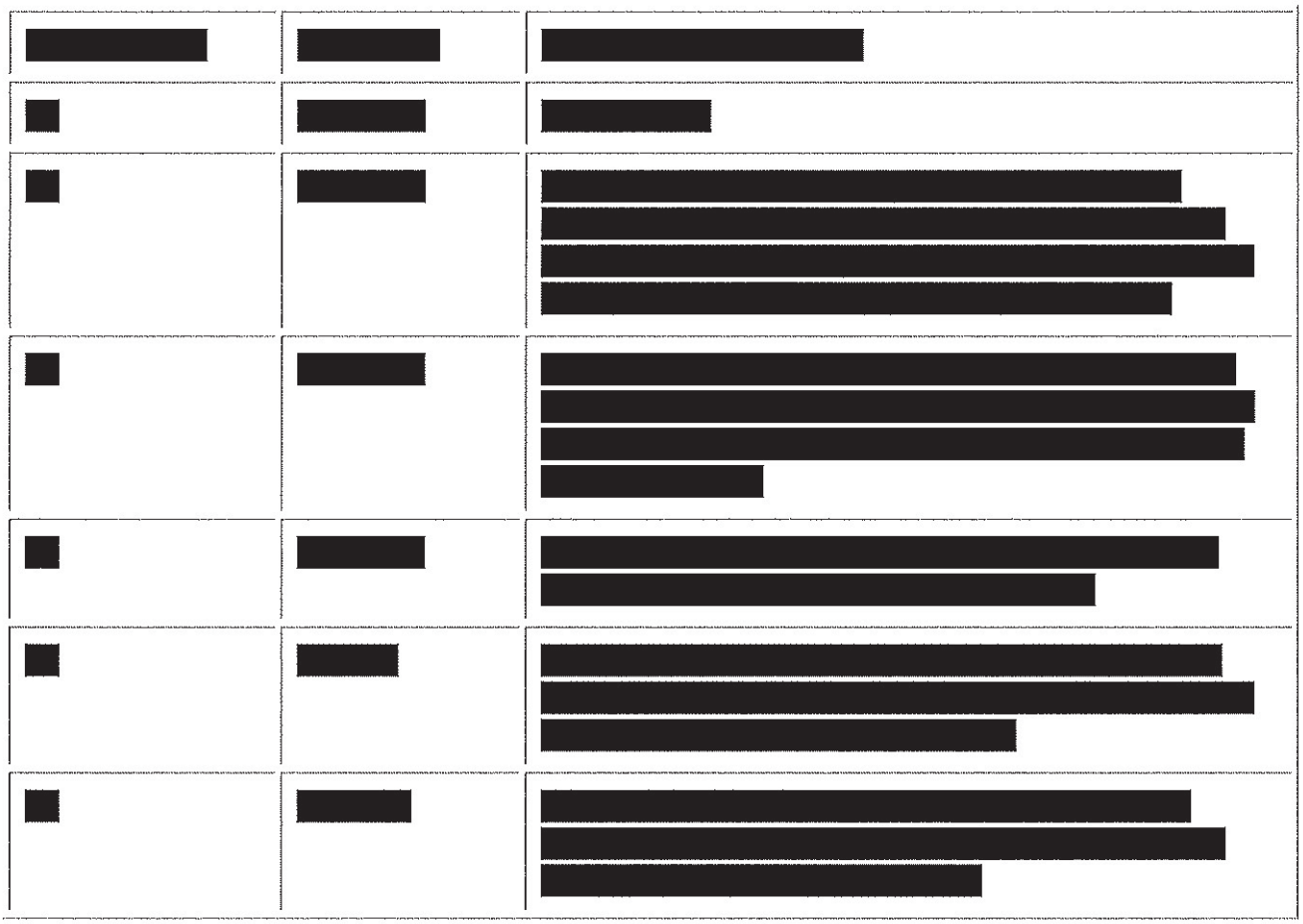
The Principal Investigator of the clinical investigation shall:

- Obtain and maintain IRB/ethics committee approval of the study.
- Ensure all subjects are consented prior to enrollment, per FDA Code of Federal Regulations titled 21 CFR 50 and EU MDR.
- Conduct the clinical investigation in accordance with the protocol, all applicable laws and federal regulations, and conditions or restrictions implemented by the governing IRB/ethics committee.
- Ensure only appropriately trained personnel will be involved in clinical investigation.
- Maintain study records mentioned in the CIP.
- Maintain logs for study team delegation, site visit/monitoring, equipment disposition, study team training, subject recruitment and enrollment.
- Evaluate all adverse events and adverse device effects and determining whether the study is safe to continue.
- Allow the Sponsor to conduct periodic monitoring of study activities to ensure GCP compliance.
- Not promote device prior to clearance by FDA for commercial distribution, except for academic purposes and scientific presentations.

The Sponsor shall ensure existence and record of all necessary compliance documents, and will conduct monitoring visits to ensure appropriate conduct of the study. Additionally, the Sponsor will finance all aspects of this study as indicated in the

fully executed contract.

14 REVISION HISTORY:



15 REFERENCES

1. Vos JJ, Ellermann SF, Scheeren TWL. Journal of clinical monitoring and computing 2017/2018 end of year summary: Monitoring-and provocation-of the microcirculation and tissue oxygenation. J Clin Monit Comput 2019; 33: 201-209.

2. van Lier F, Wesdorp, F. H. I. M., Liem VGB et al. Association between postoperative mean arterial blood pressure and myocardial injury after noncardiac surgery. Br J Anaesth 2018; 120: 77-83.

3. Meddeb B, Hajjej Z, Gharsallah H, Trabelsi B, Labbene I, Ferjani M. Relationship between macrocirculation and microcirculation monitored by microdialysis during septic shock. ICMx 2015; 3: 1-2.

4. Scheeren TW, Martin K, Maruschke M, Hakenberg OW. Prognostic value of intraoperative renal tissue oxygenation measurement on early renal transplant function. Transpl Int 2011; 24: 687-696.

5. Mesquida J, Espinal C, Gruartmoner G et al. Prognostic implications of tissue oxygen saturation in human septic shock. Intensive Care Med 2012; 38: 592-597.

6. Mesquida J, Masip J, Gili G, Artigas A, Baigorri F. Thenar oxygen saturation measured by near infrared spectroscopy as a noninvasive predictor of low central venous oxygen saturation in septic patients. Intensive Care Med 2009; 35: 1106-1109.

7. Scheeren TW, Schober P, Schwarte LA. Monitoring tissue oxygenation by near infrared spectroscopy (NIRS): Background

and current applications. J Clin Monit Comput 2012; 26: 297-287.

8. Redford D, Paidy S, Kashif F. Absolute and trend accuracy of a new regional oximeter in healthy volunteers during controlled hypoxia. Anesth Analg 2014; 119: 1315-1319.

9. Benni PB, MacLeod D, Ikeda K, Lin HM. A validation method for near-infrared spectroscopy based tissue oximeters for cerebral and somatic tissue oxygen saturation measurements. J Clin Monit Comput 2018; 32: 269-284.

10. Ferraris A, Jacquet-Lagreze M, Fellahi JL. Four-wavelength near-infrared peripheral oximetry in cardiac surgery patients: A comparison between EQUANOX and O3. J Clin Monit Comput 2018; 32: 253-259.

11. Massey D, Martin JA. The New immigrant survey (NIS) skin color scale: Office of population research. . Princeton University, Princeton, NJ 2003.

12. International Organization for Standardization, (ISO). ISO 80601-2-61:2011: Medical electrical equipment — part 2-61: Particular requirements for basic safety and essential performance of pulse oximeter equipment. ; 2019.

13. Vos JJ, Willems CH, van Amsterdam K et al. Oxygen reserve index: Validation of a new variable. Anesth Analg 2019; 129: 409-415.

14. MacLeod DB, Ikeda K, Vacchiano C, Lobbestael A, Wahr JA, Shaw AD. Development and validation of a cerebral oximeter capable of absolute accuracy. J Cardiothorac Vasc Anesth 2012; 26: 1007-1014.

15. Schober A, Feiner JR, Bickler PE, Rollins MD. Effects of changes in arterial carbon dioxide and oxygen partial pressures on cerebral oximeter performance. 2018; 128: 97-108.

16. Apfelbaum JL, Silverstein JH, Chung FF et al. Practice guidelines for postanesthetic care: An updated report by the american society of anesthesiologists task force on postanesthetic care. Anesthesiology 2013; 118: 291-307.

17. Cousins TR, O'Donnell JM. Arterial cannulation: A critical review. AANA J 2004; 72: 267-271.

18. Saugel B, Scheeren TWL, Teboul JL. Ultrasound-guided central venous catheter placement: A structured review and recommendations for clinical practice. Crit Care 2017; 21: 225-y.