
# Official Title: Spot-Check Noninvasive Hemoglobin (SpHb) Clinical Validation of INVSENSOR00026

Date of Protocol: 18 July 2018

NCT Number: NCT03610269

# Spot-Check Noninvasive Hemoglobin (SpHb) Clinical Validation of INVSENSOR00026

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**Study Device:** INVSENSOR00026 Pulse CO-Oximeter and sensor

**Sponsor Protocol Number:** TORR0003

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| | | | |
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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 IRB approval, federal and local regulatory requirements, 21 CFR 812, ISO-14155 and International Conference on Harmonization Good Clinical Practice (ICH GCP) guidance.

## 1.1 Background and Rationale

Hemoglobin (Hb) is a protein contained in the red blood cells that is responsible for carrying oxygen from the lungs to tissues throughout the body. The concentration of hemoglobin in whole blood is expressed in grams per deciliter (g/dL). Normal Hb range for adult males is 13 to 18 g/dL and 12 to 16 g/dL for adult females. Sufficient hemoglobin level must be maintained to ensure adequate tissue oxygenation; a person with low levels of hemoglobin is referred to as anemic. Some common causes of anemia are:

- Nutritional deficiency - iron, vitamin B12 and folate
- Abnormal hemoglobin structure - sickle cell or thalassemia
- Acute loss of blood due to traumatic injury or surgical procedure
- Chronic diseases - cancer, kidney and liver failure

If left untreated, anemia can cause several complications, such as:

- Fatigue
- Heart problems, such as arrhythmia
- Death in the case of acute blood loss

To assess a patient's anemia status, a physician would typically request a complete blood count (CBC) test to evaluate hemoglobin levels. The National Committee for Clinical Standards (NCCLS) has published an approved gold standard procedure for the manual measurement of hemoglobin using the cyanmethemoglobin method (HiCN). This method breaks down red blood cells to get the hemoglobin into a solution. Then, the free hemoglobin is oxidized to form methemoglobin and combined with cyanide ions, resulting in cyanmethemoglobin. The concentration of hemoglobin is then determined by shining a light through the solution and measuring how much light is absorbed- specifically at a wavelength of 540 nanometers. Manual HiCN has gradually been phased-out of mainstream laboratory use, because it is time and labor intensive and exposes the operator to harmful reagents. Instead, there are automated analyzers based on the same operating principle of HiCN that are more widely used, such as Beckman Coulter and Advia 120, and Sysmex hematology analyzers. However, HiCN still remains that reference method that all automated analyzers are compared against.

These routine CBC tests are invasive, intermittent, increase infection risk to patient, and can expose a clinician to the risk of infection via blood borne pathogens. In addition, multiple blood tests at hospitals have been attributed to patients developing hospital acquired anemia (HAA). A recent study looked at more 17000 myocardial infarction patients across 57 US hospitals and found 20% of the patients had developed HAA over their hospital stay[i].

Recent advancements in pulse oximetry technology have led to the development and FDA-approval of a multi-wavelength Pulse CO-Oximeter, designed for continuous, noninvasive measurement of total hemoglobin concentration (Masimo SpHb™ technology). Thus, noninvasive measurement of total hemoglobin fortify a clinician's toolkit and can potentially offer a great clinical value and provide a simple, portable, safe and accurate method for monitoring patients.

To evaluate the accuracy of hemoglobin measurement, data collection and analysis of blood should be conducted in a controlled manner to reduce variability between noninvasive hemoglobin readings to the hemoglobin value obtained from a reference sample. Small differences in the processing and/or handling of hemoglobin may have dramatic effects in analytical reliability and reproducibility. Therefore, potential bias and confounding factors should be minimized as much as possible by having consistent technique of clinician, posture of the donor, timing of samples, etc.

### 1.2 Investigational Devices

SpHb technology uses a multiwavelength sensor with various light emitting diodes (LEDs) that pass light through the measurement site to a photodiode (detector) as shown in **Figure 1** below. Signal data is obtained by passing various [redacted] wavelengths [redacted] through a capillary bed (for example fingertip) and measuring changes in absorption during the blood pulsatile cycle. The detector receives the light and converts it to an electrical signal which is, in turn, used to predict SpHb.

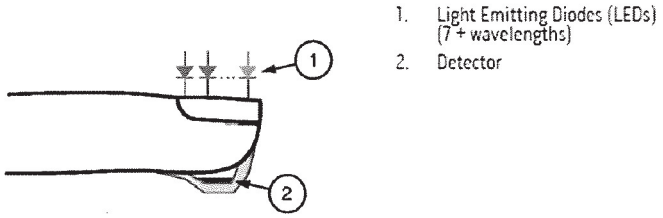


**Figure 1:** SpHb Technology Overview

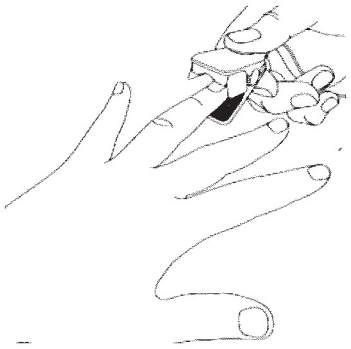

**Figure 2:** SpHb Sensor placement

Devices used for noninvasive hemoglobin evaluation consist of a Masimo Rainbow portable handheld co-oximeter connected to a sensor that measures SpHb, methemoglobin (SpMet), SpO2, pulse rate (PR) and perfusion index (PI). The investigational devices used in this study employ similar material and technology compared to FDA-cleared devices. The investigational devices have undergone risk analysis and safety testing in accordance with applicable safety standards, including electrical safety, current leakage, mechanical safety and biocompatibility testing for patient contacting materials. That risk assessment concluded that the investigational devices do not pose unmitigated risks to operator or subject.

Investigational devices to be used in this study are: INVSENSOR00026 Pulse CO-Oximeter and sensor. The devices used in this study are manufactured per Good Manufacturing Practice (GMP) with traceability of lot or serial numbers and will be labeled as investigational devices, for clinical research only. Final marketed names for the sensor and/or device may change.

The INVSENSOR00026 is a system composed of a handheld Pulse CO-Oximeter and sensor with Rainbow technology similar to already cleared Masimo noninvasive Pulse CO-Oximeters and sensor. The system is intended for spot-check measurements on patients in various care area settings (hospitals, hospital-type facilities, and clinics). The device is a more compact version of already 510(k) cleared Rad-57 and Pronto devices. The Pulse CO-Oximeter device also comes with an upgraded display with touchscreen capabilities. The INVSENSOR00026 Pulse CO-Oximeter and sensor are connected by an accessory patient cable.

The reusable INVSENSOR00026 sensor is intended to measure non-invasive physiological parameters such as SpO2, SpHb, PR, and PI, similar to the existing FDA cleared Masimo rainbow reusable sensors. The sensor has a slimmer and more compact design compared to other Masimo reusable finger sensors. The sensor is intended to be used on patients weighing greater than 3 kg.

## 2 STUDY DEVICE

### 2.1 Description

Investigational Devices:

INVSENSOR00026 Pulse CO-Oximeter and sensor

Laboratory Analysis:

[redacted]

[redacted]

Safety Equipment (FDA-Cleared)
Blood pressure monitoring system
Electrocardiogram (ECG)
Ultrasound system (Bard Site-Rite 8 or equivalent)
Masimo Pulse Oximeters (Radical-7)
Pulse oximeter sensors and cables (Masimo SET)
Masimo Root Patient Monitoring Platform
Medical-grade oxygen tank and mask
Crash cart

The PI, delegated study staff and operator(s) will undergo device training per the Directions for Use (DFU) prior to performing any study-related procedures. All training will be documented.

### 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 of the study device supplies, an inventory must be performed and the Equipment Shipment Check Form ([redacted]) 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 as required per local requirements. 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.

### 2.3 Risk/Benefit

2.3.1 **Benefits:** There will be no direct benefits to the enrolled subjects. Future benefits to subjects might include a reduction in invasive procedures due to the ability to noninvasively measure blood parameters.

2.3.2 **Pulse oximeter risks:** The INVSENSOR00026 Pulse CO-Oximeter used during this study are modifications of FDA-cleared devices, are similar in material and technology, and pose no additional risk to the subject.

2.3.3 **Sensor risks:** As with all optical sensors, the INVSENSOR00026 sensor investigational device has the risk of thermal burn. The design includes safeguards, and this risk is believed to be low. All patient-contact materials, including the adhesive used in the design of the Masimo Rainbow sensors, have been subjected to biocompatibility tests per ISO 10993-1 and results demonstrate that the materials are non-toxic, non-irritating, and non-sensitizing. The sensors have been subjected to performance, mechanical, and electrical testing and results demonstrate that the sensors meet the requirements for safety and effectiveness for the intended use of the product. The modifications to the sensor are specifically designed so as not to introduce any new risks to the subject, and have been developed pursuant to the design control regulations.

2.3.4 **Arterial, and Capillary Blood Draw Risks:** Discomfort is generally associated with needle puncture. The most common complications associated with blood draws and capillary sticks are hematomas or bruising. There is also a possible risk of infection, tendon or tissue damage, damage to the blood vessel and surrounding nerves, and/or loss of feeling in hand and/or arm. Other anticipated adverse events that may occur, include but are not limited to: Vasovagal syncope (passing out/fainting), lightheadedness, feeling flush/ warm, feeling nauseated, throwing up, sudden drop in blood pressure/ sudden increase in blood pressure, sudden drop in heart rate/ sudden increase in heart rate, tingling sensation of face, arms and/or legs, sweating, and/or mouth dryness. These anticipated adverse events are expected to be temporary.

2.3.5 **Lidocaine (injection) Risks:** Administration of lidocaine injection 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 site, 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; swelling of the face, lips, tongue or throat.

2.3.6 **Topical Anesthetic (such as Pain Ease):** Topical anesthetic which is used to prevent pain by cooling the skin. Although unlikely, the anticipated adverse events that may occur, include but are not limited to: changes in skin color (i.e. flushing or redness of the skin), delayed wound healing, rash, itching and swelling. These adverse events are expected to be temporary.

# 3 STUDY OBJECTIVES

The primary objective of this clinical investigation is to evaluate the accuracy of using Masimo's INVSENSOR00026 Pulse CO-Oximeter and Sensor to measure hemoglobin, as compared to hemoglobin measurements obtained from various lab analyzer(s) and point of care device(s).

# 4 STUDY DESIGN

## 4.1 General Design

This is a multi-center, prospective, nonrandomized study design. Subjects will be tested in a clinical setting. For each sample point, the SpHb value from the Masimo device will be compared to the hemoglobin value from an arterial blood draw measured by one or more reference laboratory methods. [redacted]

### 4.2 Study Endpoint

Accuracy will be reported as the root mean squared error ($A_{RMS}$) of the SpHb-tHb parameter. Per ISO 80601 Part 2-61, accuracy of existing pulse oximetry instruments will be reported using $A_{RMS}$ standard calculations which are also applicable to SpHb testing.

$$Bias = \frac{1}{n}\sum_{i=1}^{n}(SpHb - tHb)$$

$$A_{RMS} = \sqrt{\frac{\sum_{i=1}^{n}(SpHb - tHb)^2}{n}}$$

### 4.3 Clinical Sites

Study subjects will be recruited from the following sites. Additional sites will be added through the IRB but will not require an amendment to the protocol.

| Site | Site Name and PI | Site Description | Age (years) |
|---|---|---|---|
| 1 | Torrance Clinical Research<br>[redacted]<br>Lomita, CA | Family Practice tied to research unit | ≥18 |

### 4.4 Population Base

Subjects will be healthy and non-healthy subjects older than 18 years of age. Subjects will be recruited in diversified demographics (age, gender, ethnicity, skin tone, comorbidities, etc.).

### 4.5 Number of Subjects

There will be up to 300 subjects enrolled into this study. The anticipated duration of subject participation in this study will be approximately 1-2 hours in the clinic and up to 72 hours from the time subject leaves the clinic, if subject receives an arterial blood draw. The study enrollment period is expected to be approximately 3 months.

### 4.6 Inclusion Criteria

- 18 years of age or older
- Subject has given written informed consent to participate in the study

### 4.7 Exclusion Criteria

- (*) Pregnant or positive human chorionic gonadotropin (hCG) test
- (*) Any severe coagulopathy, chronic bleeding disorders (i.e., hemophilia) or recent thrombolysis
- (*) Hemoglobinopathies and synthesis disorders (i.e., sickle cell, thalassemias, etc.)
- (*) Subjects who are currently taking anticoagulant medication
- (*) Subjects with allergies to lidocaine, Pain Ease, latex, adhesives, or plastic
- (*) Subjects with skin abnormalities at the planned application sites that may interfere with sensor application, per directions-for-use (DFU) or trans-illumination of the site, such as psoriasis, eczema, angioma, burns, scar tissue, substantial skin breakdown, nail polish, acrylic nails, infections, abnormalities, etc.
- (*) Subjects unlikely to be able to refrain from excessive motion during data collection. Excessive motion includes postural changes, making hand gestures, involuntary muscular movements, etc.

- (*) Subjects with elevated blood pressure, skin or wrist abnormalities that may interfere with an arterial blood draw as determined by investigator or research medical staff.
- (*) Subjects who intend on participating in heavy lifting, repetitive movement of their wrist (including riding a motorcycle) or exercise (working out, riding a bike, etc.), or any activity that will put additional stress on the wrist within 24 hours following a study involving an arterial blood draw
- Subjects deemed not suitable for the study at the discretion of the investigator or research medical staff

Note: (*) May be self-reported by subject

# 5 STUDY PROCEDURES

## 5.1 Subject Recruitment and Screening

Subjects may be recruited for the study from the physician's clinical practice or by advertisement. Information to be disseminated to subjects and any advertisements will be approved by the IRB. Once a potential subject sees the recruitment material (i.e. advertisement) they can contact the clinical site to schedule an appointment. Potential subjects may also be identified by review of the site's appointment schedule for subjects that may be eligible for the study. Potential subjects may be contacted by phone by study staff using the IRB-approved phone recruitment script.

Subjects will be screened to determine their potential eligibility and interest in a study. Subjects' potential eligibility can be evaluated during their routine intake at the clinic when health history and other information are gathered for their pre-existing appointment with the clinic, or during the recruitment phone call. All subjects screened will be documented on the Screening and Enrollment log.

Subjects are considered enrolled in the study once the subject has signed and dated the informed consent. Subjects must also meet all of the inclusion criteria and none of the exclusion criteria. If the subject signs the Informed Consent form and is identified later as failing to meet all eligibility criteria, the subject will be classified as a screen failure. Upon determination that the subject does not qualify for enrollment, the reason for the subject's screen failure status will be documented on a Screening and Enrollment Log, in order to minimize bias. In case a subject leaves the study prematurely, another volunteer may be recruited.

## 5.2 Informed Consent

The Investigator(s) and/or staff delegated for this task are responsible for conducting the informed consent process and for obtaining written informed consent, including HIPAA and the California Experimental Subject's Bill of Rights, 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 approval of the informed consent form. Subjects must provide written informed consent prior to being enrolled in the study, in accordance with applicable federal and state regulations.

5.2.1 Following identification of a potential eligible subject as defined by the inclusion and exclusion criteria, the subject will be approached by the study staff, who will thoroughly explain the purpose, procedures of the study in respect to subject's involvement and responsibilities, potential risks & benefits, subject's rights & privacy of the data collected, and that participation is voluntary.

5.2.2 If the subject expresses interest in participating in the study, they will be asked to read the written informed consent approved by the local IRB and Sponsor.

5.2.3 Subject will be given adequate time to ask questions regarding the study and to discuss with family and friends before making a decision. 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.

5.2.4 The Investigator shall retain a copy of the signed informed consent document in each subject's record and provide a copy to the subject.

The Investigator is also responsible for ensuring any new information related to the study will be provided to the subject. Subject may need to be re-consented to continue participation as required by IRB.

### 5.3 Study Procedure

Operators conducting tests shall be health care professionals. [redacted]

5.3.1 Verification of Eligibility and Subject Demographics

5.3.1..1 All subjects will be asked to complete a Health Assessment Questionnaire that will be reviewed and discussed with a medical staff. If the subject does not meet all eligibility criteria, they will be notified and removed from the study.

5.3.1..2 Females will be asked to take a urine pregnancy test. If the subject declines the pregnancy test or if the pregnancy test is positive, the subject will be notified and removed from the study.

5.3.1..3 An Allen's test will be performed to determine adequate blood flow to the hand. Results will be noted on the CRF. If the Allen's test is negative, the subject will be notified and removed from the study.

5.3.1..4 Subject's demographic information (including, but not limited to, age, weight, height, race, ethnicity, [redacted], and medical history) will be recorded on CRF.

5.3.2 Set-up for Noninvasive Readings and Blood Draws

5.3.2..1 The subject's vital signs will be monitored with FDA-cleared monitors, such as pulse oximetry, ECG, and blood pressure monitor throughout the procedure. Data captured from these safety monitors may be manually recorded by medical staff or electronically recorded. Study procedures will be immediately discontinued if the subject shows signs of discomfort or distress, indicates his/her desire to discontinue with study participation, or upon discretion of the clinical or research medical staff.

5.3.2..2 Subjects will be offered a snack (e.g. granola bar) and/or beverage (e.g. water, juice) prior to having any measurements collected or blood drawn.

5.3.2..3 Subjects will be [redacted] during noninvasive readings and blood draws.

5.3.2..4 [redacted].

5.3.2..5 [redacted]

5.3.2..6 [redacted]

5.3.3 Noninvasive Readings

5.3.3..1 Sensor should be placed on the appropriate finger(s), following manufacturer's Directions for Use and study procedure manual, and then connected to Pulse CO-Oximeter to perform noninvasive measurement.

5.3.3..2 Noninvasive measurements will be performed by following instructions included in the study procedure manual.

5.3.3..3 At the end of measurement, study data will be recorded on the CRF.

5.3.3..4 [redacted].

5.3.4 Blood Draws

Individuals who perform the capillary and/or arterial blood draw(s) will be qualified health professionals. Arterial blood draws will be performed by registered nurses, certified registered nurse anesthetists, or doctors/anesthesiologists. Capillary and/or arterial sampling and handling procedures will follow accepted blood collection practices.

5.3.4..1 Subjects will have, at most, two attempts per capillary and/or arterial puncture.

5.3.4..2 Blood samples will be collected [redacted] Not all blood sampling sites may be done for each subject.

5.3.4..3 Arterial samples should be obtained [redacted]. Approximately [redacted] of blood may be collected. Blood samples will be collected into vacuum sealed tubes containing di-potassium ethylenediaminetetraacetic acid (K2 EDTA).

5.3.4..4 A capillary sample should be obtained [redacted]. Approximately one or two drops of blood may be collected from each sampling. [redacted] results will be recorded on the CRF.

5.3.4..5 For arterial blood draws, lidocaine injection and/or topical anesthetic may be used to numb the site where the needle is inserted into the radial artery. Subjects will be given the option to receive or decline local anesthetics. An ultrasound may be used to visualize blood vessels and guide the placement of the needle for the blood draw. After the blood sample has been collected, firm, constant pressure should be maintained on the puncture site for at least 5 minutes to ensure that the bleeding has stopped.

5.3.4..6 Total blood withdrawal will be approximately [redacted] total of blood, allowing for sample collection/or handling issues.

5.3.5 Blood Analysis

5.3.5..1 Blood tube(s) will be identified with a unique barcode [redacted] for processing and additional analysis.

5.3.6 Study Completion

Study completion is defined as when the subject has completed the noninvasive device readings and blood draw(s).

5.3.6..1 The sensors will be removed and the skin inspected for signs of redness or irritation.

5.3.6..2 Subjects will be offered a snack (e.g. granola bar) and/or beverage (e.g. water, juice).

5.3.6..3 Subjects will be allowed to leave after medical personnel determine it is safe to do so. Subjects will be encouraged to remain in the study area until they feel fit to leave.

5.3.6..4 Vital signs (including heart rate and blood pressure) will be recorded for subject safety monitoring before subject exits the study.

5.3.6..5 Subjects will be given post-care instructions for arterial blood draws.

5.3.6..6 Subjects who received an arterial stick will be instructed to contact the principal investigator in the event of any potential adverse event up to 72 hours after being discharged from the clinic. AE(s) will be recorded on the CRF and/or Adverse Event Report Form.

## 5.4 Early Withdrawal of Subjects

5.4.1 Withdrawal of Individual 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. Subjects may also be withdrawn from the study at any time prior to expected

completion at the discretion of the Principal Investigator or research medical staff for safety concerns, failure to adhere to protocol requirements, malfunction that prevents accurate collection of data, sampling errors, inability to collect noninvasive device and/or hemoglobin readings, administrative reasons, etc.

Any data collected until the time of subject withdrawal may be included in the final data analysis. Information on the subject's withdrawal should either be documented in the medical record or on the CRF and should include clear documentation of the reason for withdrawal to the Sponsor.

No follow-up is required for subjects who did not receive an arterial stick and are withdrawn from the study, as there are no long term effects anticipated from participating in this study. Subjects who received an arterial stick will be instructed on wound care and to contact the principal investigator in the event of any potential adverse event up to 72 hours after being discharged from the clinic. AE(s) will be recorded on the CRF and Adverse Event Report Form.

Subjects that withdraw from the study may be replaced by another volunteer.

# 6 STATISTICAL PLAN

[redacted]

## 6.1 Sample Size Determination

### 6.1.1 Earlier Studies

Earlier studies of Pulse CO-Oximeters using similar technology were used to provide estimates of the required variances used in the sample size calculation. In the earlier study, the variability of both the test (SpHb) and the reference method ([redacted]) method were calculated. [redacted]

### 6.1.2 Deming Regression

Since the reference method has a measureable variation, Deming regression is indicated. (Pasing Bablok regression is an alternate regression method for data with variation in the reference method, however, it is not necessary to run both procedures.) [redacted]

### 6.1.3 Effect Size

It is desired to determine if the slope of the Test on Reference regression is significantly different than [redacted]. All regression lines will pass through the mean point. Therefore, the slope and intercept terms are not independent and it is only necessary to check if the slope is significantly different than [redacted]. The levels of significance were set at [redacted]

For a slope range between [redacted], and a mean data point of [redacted] the intercept term will be between -[redacted]. Additional input constants used in the calculations are shown in Table 1 for both the Deming Slope and Deming Intercept.

Additional input constants used in the calculations are shown in Table 1 for both the Deming Slope and Deming Intercept.

6.1.4 Monte Carlo Simulation

The data from the earlier study contained data from [redacted]. This data was resampled by Subject (with replacement) to simulate data sets with differing numbers of Subjects.

A Deming regression was calculated for each of [redacted] iterations. The Slope and Intercept from each regression were stored and the statistics required to meet the effect size requirements were calculated for each level of subjects. These simulations indicated that the Slope effect size of [redacted]. Similarly, [redacted] would be required to meet the Intercept requirement. The initial sample size, assuming the measurements are all independent, is set to [redacted]

The initial sample size, assuming the measurements are all independent, is therefore set to [redacted]

6.1.5 Intraclass Correlation Coefficient

Since this protocol will be run in several clinical sites, an evaluation of the Intraclass Correlation[iii] was performed. Based on the Site to site variation seen in the earlier study, the Between Site variance component was [redacted]. The Across Site variance was calculated as [redacted]. The ICC is found by:

$$ICC = \frac{AcrossSiteVariance}{AcrossSite{=}Variance{+}BetweenSiteVariance} = [redacted]$$

This gives a Sample Size Inflation factor of:

[redacted]

Therefore, this study will use a minimum of [redacted] total subjects. Additional subjects may be required to fill in demographic bins or account for subject drop out of [redacted]

In order to collect data across multiple subject health conditions, [redacted] sites will be utilized. The number of subjects per site will be determined by patient availability and patient demographics at each site. This will ensure that other study endpoints related to the distribution of observed hemoglobin values are also met.

Final statistical analysis will be performed on a population set who have enrolled and completed the study procedure as defined per the protocol.

## 6.2 Statistical Methods

6.2.1 Accuracy will be reported as the $A_{RMS}$ using the following equation:

$$Bias = \frac{1}{n}\sum_{i=1}^{n}(SpHb - tHb)$$

$$\text{Precision} = \sqrt{\frac{\sum_{i=1}^{n}(\,(SpHb - tHb) - Bias)^2}{n}}$$

$$A_{RMS} = \sqrt{\frac{\sum_{i=1}^{n}(SpHb - tHb)^2}{n}}$$

### 6.3 Additional Analyses, as applicable

6.3.1 Bland-Altman bias plot

6.3.1..1 Bland Altman plots will be provided by site, by subject, and for all data combined.

6.3.1..2 A correction for repeated measures on the same subject and multiple subjects at each site will be applied during the analysisi.

6.3.1..3 The limits of agreement will be calculated using the corrected variance components.

6.3.2 Regression Analysis

6.3.2..1 Data will be randomly resampled to provide a uniform distribution of data points across the measurement range.

6.3.2..2 To account for variation in the reference tHb values, Deming linear regression analysis will be calculated.

6.3.2..3 Estimates of standard deviation in both the SpHb and tHb data (required by Deming regression) will be calculated from earlier studies [redacted]

6.3.2..4 Estimates and 95% confidence intervals for both Y-intercept and Slope will be calculated for a Deming regression across all [redacted] sites and within each site individually.

6.3.3 Coefficient of Variation (CV)

CV will be defined as (100*MSE)/Mean tHb value where MSE = variance of measured values to the line of identity.

### 6.4 Criteria for excluding data from quantitative analysis

[redacted]

6.4.3 Other reasons, to be fully described in the final test report.

### 6.5 Handling of missing data

A mixed-effects analysis will be used to assess the impact of missing data on the analysis of the accuracy endpoint.

## 7 SAFETY AND ADVERSE EVENTS

### 7.1 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.

### 7.2 Anticipated Adverse Events

- Sensor may cause slight, temporary redness, which should fade away shortly after sensor removal. Sensor may cause thermal burn; however, the design includes safeguards and this risk is believed to be minimal.
- Capillary sticks may cause swelling, bruising, momentary discomfort at site of blood draw, and in some rare cases lightheadedness, nausea, or fainting.
- Arterial draws may cause momentary discomfort and pain at site of blood draw, arteriospasm, hematoma, infection, nerve damage, fainting or a vasovagal response.

Refer to the Risk/Benefit section for details on anticipated adverse events.

### 7.3 Adverse Event Reporting

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

- All Adverse Events, both Anticipated and Unanticipated and serious and non-serious, after the first Masimo sensor is placed and up to discharge. For subjects who receive an arterial stick, all adverse events will be reported up to 72 hours after being discharged from the clinic, as well.
- All Unanticipated Adverse Device Effects, after the first Masimo sensor is placed and up to discharge. For subjects who receive an arterial stick, all adverse events will be reported up to 72 hours after being discharged from the clinic, as well.
- Serious Adverse Events and Unanticipated Adverse Device Effects must be reported to the Sponsor within 48 hours. All other Adverse Events should be reported to the Sponsor within 5 business days.

### 7.4 Deviations from the study protocol

Deviations from the protocol must receive both Sponsor and the investigator's IRB 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. Any protocol deviations initiated without Sponsor and the investigator's IRB 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 as soon as a possible, but no later than 5 working days 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.

### 7.5 Withdrawal of IRB approval

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

## 8 VULNERABLE POPULATIONS

### 8.1 Definition

8.1.1 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, educationally disadvantaged, and employees/colleagues/students of the Principal Investigator and/or study staff.

### 8.2 Protection of vulnerable subjects

- Reasonable compensation will 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.
- If subjects are employees, colleagues, or students of the Investigator and/or study staff, the IRB will add non-coercion language to the informed consent form and Investigator will explain that participation does not affect subject's employment, benefits, or position at the company and cannot be the grounds for termination. Likewise, if employees refuse to participate or withdraw from the study, their employment, benefits, and position at the company will in no way be affected.
- 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.

### 8.3 Responsible Parties

- The IRB will review research with vulnerable populations and evaluate consent, level of risk, coercion, and the reason for choosing this particular subject population. The EC/IRB 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.

## 9 DATA MANAGEMENT

### 9.1 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.

### 9.2 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 include: questionnaires, hospital records, clinical and office charts, laboratory notes, 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 review and regulatory inspection(s), providing direct access to source data/documents.

### 9.3 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 will include the following information, including but not limited to: inclusion/exclusion criteria, whether patient consent obtained before start of study, demographic information, device readings, and if occurrence of any adverse event, protocol deviation, and device deficiencies, etc. The CRFs will be signed by the PI to attest that the data is 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 paper CRFs and re-verified. Query resolution will be assessed and confirmed by study monitor during site visit. The monitor or study manager will collect original completed and signed CRFs at the end of the study. A copy of the completed and signed CRFs will remain on site

### 9.4 Data Transfer and Storage

9.4.1 Training on CRF completion will be provided to study personnel prior to data collection.

9.4.2 Original CRFs will be stored in a secure location at site. Original CRFs will be scanned and sent to sponsor.

9.4.3 Only authorized sponsor personnel will have access to study data, and will move it to a secure and backed-up drive at Masimo.

9.4.4 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.

### 9.5 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 minimum of two years after date of study closure or date when records are not required to support 510(k) clearance. The Institution's own retention policies and regulations may apply in addition to the minimal requirement.

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

## 10 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 be a direct employee from the Clinical Research department 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 monitoring visit during enrollment, or within 4-6 weeks of a round of data collection.
- 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). The monitor will verify source documents and records to entries in the CRFs and other GCP-related documents (IRB approvals, IRB correspondences, and ICFs) provided that subject confidentiality is maintained in agreement with HIPAA 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 also verify presence of informed consent, adherence to the inclusion/exclusion criteria, and documentation of SAEs/SADEs and protocol deviations/violations, and check CRF against medical records and real-time data stored within the INVSENSOR00026 Pulse CO-Oximeter.

After each visit, the monitor will provide a monitoring follow-up letter to the investigator within 4 weeks of visit completion. 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 but no later than 60 days of receiving the monitoring follow-up letter. 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 11.5 for details on suspension and termination.

# 11 ADMINISTRATIVE ASPECTS

## 11.1 Protection of Human Subjects

Per 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 approval.

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

## 11.2 Institutional Review Boards

The Sponsor and/or Investigator must submit the protocol to the appropriate IRB 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. The Investigator cannot enroll subjects until a copy of the approved informed consent is obtained from the IRB.

Any amendments to the protocol or informed consent should be submitted to the IRB for review and approval per 21 CFR 56. The IRB should be notified of any changes that may affect conduct of the study or pose safety risks to the subjects.

## 11.3 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.

### 11.4 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 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. Both PI and Sponsor will retain the IRB approval letter and approved protocol as confirmation that the protocol amendment was approved.

### 11.5 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 determine that the study site's compliance to GCP and federal regulations to be inadequate at any point during the study, and Sponsor move to suspend or terminate the study site, the Sponsor will provide notification in writing to the principal investigator and IRB 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.

### 11.6 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 IRB approval if the device is non-significant risk.

## 12 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 approval of the study.
- Ensure all subjects are consented prior to enrollment, per FDA Code of Federal Regulations titled 21 CFR 50.
- Conduct the clinical investigation in accordance with the protocol, all applicable laws and federal regulations, and conditions or restrictions implemented by the governing IRB.
- 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.

## 13 REVISION HISTORY:

| Version Number | Version Date | Summary of Revisions Made: |
|---|---|---|
| 1.0 | July 11, 2018 | Original version |
| 2.0 | July 18, 2018 | Administrative changes |

[i] Bland and Altman. Agreement between methods of measurement with multiple observations per individual. Journal of Biopharmaceutical Statistics (2007) vol. 17 pp. 571-582

[ii] Salisbury AC, Amin AP, Reid KJ, et al. Hospital-acquired anemia and in-hospital mortality in patients with acute myocardial infarction. Am Heart J. 2011; 162(2):300-309.e3.

[iii] Wears Robert L, Advanced Statistics: Statistical Methods for Analyzing Cluster and Cluster-randomized Data, Academic Emergency Medicine, 2002; 9:330-341