Document: Study Protocol

Official Title: Characterization of Preterm Neonatal Skin by Diffuse Reflectance Spectroscopy Pilot Study

NCT05127004

Document Date: 06JUN2023

Study Title: Characterization of Preterm Neonatal Skin by Diffuse Reflectance Spectroscopy Pilot Study

Principal Investigator:
Rebecca P. Sartini, DNP, RNC-NIC
University of Arkansas for Medical Sciences
4301 W. Markham Street, Slot 526
Little Rock, AR 72205
Telephone: 501-526-1580
Email: RPSartini@uams.edu

Sub-Investigator (s):
Kyle P. Quinn, PhD
University of Arkansas
700 W. Research Center Blvd.
Fayetteville, AR 72701
Telephone: 479-575-5364
Email: KPQuinn@uark.edu

Michael Blair, BS
University of Arkansas
700 W. Research Center Blvd.
Fayetteville, AR 72701
Telephone: 479-575-5364
Email: MJBlair@uark.edu

Rebecca K. Smith, MSN, RNC-NIC
University of Arkansas for Medical Sciences
4301 W. Markham Street, Slot 526
Little Rock, AR 72205
Email: SmithRebeccaK@uams.edu

Jack Murphy
University of Arkansas
700 W. Research Center Blvd.
Fayetteville, AR 72701
Email: JWMurphy@uark.edu

Medical Monitor:
Dr. R. Whit Hall
Neonatal Intensive Care Unit
4301 W. Markham Street, Slot 526
Little Rock, AR 72205
HallRichardW@uams.edu

Study location:
University of Arkansas for Medical Sciences
Neonatal Intensive Care Unit
4301 W. Markham Street, Slot 526
Little Rock, AR 72205

Sponsor: University of Arkansas for Medical Sciences

NCT Number: NCT05127044

Table of Contents

Abbreviations

ADE	Adverse Device Effect
AE	Adverse Event
APR	Annual Progress Report
CFR	Code of Federal Regulations
DRS	Diffuse Reflectance Spectroscopy
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Council on Harmonization
IRB	Institutional Review Board
LAR	Legally Authorized Representative
ms	Millisecond
NICU	Neonatal Intensive Care Unit
ORRA	Office of Research Regulatory Affairs
PI	Principal Investigator
SAE	Serious Adverse Event
UADE	Unanticipated Adverse Device Effect
UAF	University of Arkansas Fayetteville
UAMS	University of Arkansas for Medical Sciences
UPIRTSO	Unanticipated Problem Involving Risks to Subjects or Others

Background and Rationale

Preterm neonates are plagued with an abundance of hardships, many of which are related to the incomplete development of their epidermis. The epidermal layer of the skin develops slowly and is still incomplete in the third trimester. For preterm neonates, this layer may not be fully developed at birth [1]. With an underdeveloped epidermis, the preterm infant can experience the following challenges: trans-epidermal water loss, trouble regulating temperature, and increased skin irritation or wounding from adhesives, prolonged contact with tubing, and frequent blood draws. Unfortunately, there are no quantitative methods of characterizing the development of the epidermis in preterm neonates. Furthermore, the means of assessing the development of a wound achieved by visual inspection can be highly subjective, depending on the provider performing the assessment. With the trend of a larger number of preterm infants surviving and at lower gestational ages, there is an urgent need for a quantitative method of characterizing the condition of preterm neonatal skin. This can increase the quality of care and decrease the risk of wound development. Previous work with optical spectroscopy has proven effective in the transdermal characterization of hemoglobin count, oxygen saturation levels, tissue scattering properties, and bilirubin levels [2,3]. However, no study or device has integrated this optical information to evaluate skin maturity and its susceptibility to injury in preterm neonates. Our long-term goal is to develop a non-invasive, quantitative means of characterizing the optical properties of neonatal skin that will be capable of evaluating skin status, guiding care and minimizing injury.

Objectives

The objective of this pilot study is to characterize the response of chromophores in preterm neonatal skin using diffuse reflectance spectroscopy (DRS) with the aim of understanding how the optical properties change with gestational age, race, ethnicity, and sex. This characterization will be used to develop novel methodologies to quantitatively analyze aspects of the stability of the infant.

Primary Objectives

1. Compare bilirubin concentrations derived from the DRS system with that collected from the Philip's BiliChek System using a linear regression and a Pearson’s correlation coefficient assessment.

Secondary Objectives

1. Compare bilirubin concentrations from the DRS to gestational age, race, ethnicity, and sex using a linear regression and a Pearson’s correlation coefficient assessment.
2. Compare hemoglobin concentrations from the DRS to gestational age, race, ethnicity, and sex using a linear regression and a Pearson’s correlation coefficient assessment.
3. Compare melanin concentrations from the DRS to gestational age, race, ethnicity, and sex using a linear regression and a Pearson’s correlation coefficient assessment.

Study Population

Subject’s gestational age, race, and ethnicity are expected to affect scattering properties and melanin absorption. Our analysis of DRS spectra will account for these parameters, which can be validated by extraction of bilirubin levels that match the BiliChek system measurements. Up to 80 subjects will be consented for a total of 44 completed subjects while inpatient in the University of Arkansas for Medical Sciences (UAMS) Neonatal Intensive Care Unit (NICU), noting current average daily census of 60 infants are cared for daily. A recruitment flyer will be posted at nurse’s stations to advertise the study to families of potential subjects and provide information about how parents/legally authorized representative (LARs) can speak to a study team member about

enrolling their infants. Potential subjects will be identified in the UAMS NICU and maternal floor.

Recruitment of subjects will be performed by the Principal Investigator (PI) and delegated UAMS study staff who have routine access to patient schedules of NICU admissions by census review. Parents/LARs will be approached by or directed to the PI and/or delegated UAMS study staff for study details.

To the extent possible, the 44 completed subjects should be evenly distributed between males and females. Recruitment will pause for males or females when either begins to exceed 50% of the total population. Additionally, to the extent possible, the 44 completed subjects should be distributed across races and ethnicities so that no particular race or ethnicity is overrepresented. Recruitment will pause for a particular race or ethnicity if that race and/or ethnicity reaches 50% of the total intended completers (i.e., 22 of the planned 44 complete subjects).

Eligibility Criteria

Inclusion Criteria

- Infants, ≤ 37 weeks gestation at birth
- Current care provided by the UAMS NICU
- Parents/LARs must be able to provide written consent

Exclusion Criteria

- Entering palliative care
- Known diagnosis of liver disease
- On isolation precautions
- Approaching end-of-life
- On minimal stimulation protocol
- Any other condition, that in the opinion of the investigator, might interfere with the safe conduct of the study or place the subject at increased risk

Study Design and Procedures

This study is a cross-sectional pilot study to characterize the response of neonatal premature skin to DRS when excited with a white light source, and provide a baseline of how the collected spectra change with the maturation of the epidermal layer in the weeks to term gestation after birth by using a correlation analysis. It will be conducted in the UAMS NICU. A device employing related technology, the Philip's BiliChek System, is currently used to assess the level of bilirubin in infants with proven accuracy. As such, BiliChek readings will be taken as an evidenced-based practice reference along with DRS spectra. Accordingly, DRS spectra and BiliChek readings will be performed on consented study subjects admitted to the NICU. The pilot data will be used to evaluate whether DRS spectra can be used to extract optical information (scattering coefficient) related to skin maturity as well as hemoglobin, melanin, and bilirubin concentrations (mg/mL). DRS spectra will be compared to a lookup table created from published absorbance spectra (collected with a spectrophotometer) for bilirubin, hemoglobin, and melanin, and converted into relative concentrations for each chromophore [,5,6,7]. The DRS-derived bilirubin concentrations and BiliChek-derived bilirubin measurements will be compared with the primary criteria for success being a high, positive correlation ($r \geq 0.85$, Pearson's correlation coefficient).

The following coded data will also be collected only once during a 24-hour window before and/or after DRS readings as routine standard of care to investigate correlations with the DRS spectra: blood oxygen saturation, weight, race, ethnicity, corrected age, gestational age, sex, most recent

complete blood count (if available in the 24-hour window,), most recent chemistry levels , lipids, liver profile, and blood gas (all if available). No laboratory tests will be performed solely for study purposes. Data will only be collected if values are available as part of regular medical care.

Procedure

A prospective subject's parent or LAR will be provided with a copy of the informed consent form (ICF)/HIPAA Authorization, including details of the study in English. Non-English speaking parents/LARs will not be approached for the study. Study-specific questions will be directed to the PI.

The consent process will occur prior to any research procedures being carried out; although, medical records may be reviewed prior to consent solely to assess eligibility for the study. Eligibility confirmation and study procedures must be conducted within 7 days of obtaining consent. If study procedures are not completed in the 7-day timeframe, subjects must be re-consented and eligibility re-confirmed.

Study procedures include 1 study episode in which DRS and BiliChek measurements will be collected on intact skin at 4 anatomical sites per subject. Readings (i.e., when the probe makes contact with the skin) will only be performed on intact skin while broken or excoriated skin will not be tested. Qualified medical personnel will conduct a visual observation to assess for intact skin per standard of care. The 5 BiliChek readings will be performed first at each of the 4 anatomical locations on the subject (chest, upper arm, outer thigh, and forehead) to produce a single BiliChek measurement at each anatomical location, followed by 5-10 DRS readings (each of which produces a DRS average measurement) at each of the 4 anatomical locations for a total of 4 BiliChek measurements and a total of 20-40 DRS measurements. The Bilichek measurements will be recorded on the subjects electronic medical record following established NICU protocol, to include documentation in the nurses note. The testing locations were chosen to provide a wide range of data without having to move the subject from the position during the handling time with a nurse. Prior to beginning testing, each engineer will review the Bilichek measurement process steps and will duplicate the same practice for DRS readings with the PI or Sub-I to confirm aseptic techniques are followed. Spectra will be initiated within 10 minutes (+ 1 minute) of the completion of the final BiliChek reading. The cumulative light exposure that occurs over those 5-10 readings at each given anatomical location will not exceed 1 minute since the time to collect each individual reading is 0.1-0.2 seconds. Approximately 15 seconds (± 5 seconds) is needed between each anatomical location to allow for repositioning of the DRS probe and following unit guidelines for device use and cleaning. After all the data acquisition has been collected for the subject, the DRS probe and the BiliChek device will be wiped down with germicidal disposable cloths, as per unit cleaning protocol.

The spectrometer is a portable device and will be placed in the NICU room with the subject. The entire procedure is not expected to take more than 15 minutes for both the BiliChek and DRS readings. Delivery of the excitation light to the subject is through a fiber-optic probe and only triggered once it is placed against the subject's skin. No adhesive or transmitting medium will be used. The light source and subsequent data collection are initiated by a foot pedal. As a precautionary measure, subjects will wear the same eye protection used in the NICU during blue light therapy. Since the light intensity of the DRS is low enough and does not pose any danger, the researchers and personnel in the room will not be required to wear eye protection. The expected total time exposed to the DRS light is less than 10 seconds.

Study staff will conduct a visual inspection of the subject's skin at each anatomical site every 2

hours (± 15 minutes) for 24 hours for possible adverse skin changes after the DRS readings. The visual inspection will be recorded using the Visual Inspection Tool, developed for this study to document any changes in skin. Qualified medical personnel will conduct the visual observation to assess intact skin per standard of care for any change using the unit-based Neonatal Skin Condition Score [8], to grade dryness, erythema, and breakdown. All skin observations or adverse events, regardless of relatedness to device, will be documented in the subject's electronic medical record, as well as on the study Adverse Event Log.

Investigational Device

The DRS system is the device utilized in this study, and when not in use at the study site is located at the University of Arkansas Engineering Research Center, 700 W. Research Center Blvd., Fayetteville , AR, 72701, in room 3407, an authorized-personnel-only biomedical engineering research laboratory. The device introduces excitation light energy through a fiber-optic probe, then collects and analyzes scattered light energy using DRS intended to characterize the response of chromophores in preterm neonatal skin. It will be used to evaluate whether DRS spectra can be used to extract optical information on skin maturity, as well as hemoglobin, melanin, and bilirubin concentrations. The following devices used collectively are the main components of the DRS system:

- Flame Spectrometer FLAME-T-VIS-NIR-ES (Ocean Insight, Inc, Orlando, FL, USA)
- HL-2000 Light Source (Ocean Insight, Inc, Winter Park, FL, USA)
- Custom Reflectance Probe (Fibertech Optica, Inc., Kitchener, ONT, CA)

Risks and Benefits

The risks involved with the DRS procedure are expected to be no greater than what might be encountered in daily clinical care or during routine physical examinations. The DRS system has never been utilized on neonates and may involve unforeseeable risks to the subject such as a soft warming sensation of skin where the probe is placed and discomfort from laying down for the duration of the procedure. During the procedure, there is minimal risk that the light from the probe may be harmful to the subjects. Subjects will wear the same eye protection used in the NICU during blue light therapy.

As with all clinical research, there exists the potential risk to the study's loss of confidentiality. Measures to protect the confidentiality of study subjects and data will be implemented as described in the Data Handling and Recordkeeping section below.

There will be no direct benefits to the study subjects; however, knowledge gained from the study could potentially benefit the care of NICU patients in the future. These indirect benefits are improvements in assessment techniques for preterm skin, assist with early diagnosis of preterm skin breakdown, and advancements in treatments for preterm skin breakdown.

To ensure this minimal risk protocol maintains the same level of risk/benefit throughout the study, the PI will monitor the risk/benefit ratio status. This will be accomplished through review of adverse events and ensuring it is appropriate to continue the study as outlined in this protocol.

Data Safety Monitoring Plan

The PI has overall responsibility for assuring safety, conducting the study, gathering study data, overseeing the data safety plan, and complying with reporting requirements with assistance from the sub-investigators and study staff, under the guidance of the Institutional Review Board (IRB) and the study Sponsor (UAMS). Safety will be monitored by assessment of AEs (serious and non-

serious) throughout the study. The PI, or designated sub-investigator, will assess all AEs for seriousness, relatedness (attribution), and expectedness. (See Adverse Events section below).
The Medical Monitor will serve as a resource to the clinical investigators and the Sponsor for advice about management of AEs but may not be involved in other aspects of the study. The Medical Monitor will independently review all Grade 3 or higher AEs as well as all serious adverse events (SAEs) related to the investigational device submitted by the study team in real time to ensure good clinical practice and to identify safety concerns quickly. Grade 3 AEs are severe or medically significant (but not immediately life-threatening) events, hospitalization, or prolongation of hospitalization. The Medical Monitor's review will include AEs and SAEs with the PI's assessments of attribution and severity as well as any quality assurance issues that have emerged. These reviews will examine whether risks of participation remain acceptable under the present protocol, modifications are needed, or the study needs to be halted.

The Medical Monitor may choose to halt the study temporarily if serious concerns arise regarding subject safety. If the study is prematurely terminated or suspended, the PI will promptly inform study subjects, the IRB, and Sponsor and will provide the reason(s) for the termination or suspension. Study subjects will be contacted, as applicable, and be informed of changes to study visit schedule. If only suspended the study may resume once all concerns have been addressed, and satisfy the Sponsor, IRB, and/or the US Food and Drug Administration (FDA).

Adverse Events (AEs) and Serious Adverse Events (SAEs)

The term AE encompasses all adverse events, which are classified as either serious or non-serious. Following consent, safety will be measured by assessment of AEs through the duration of the study (i.e., study period). AE data collection and reporting, which are required as part of every study, are done to ensure the safety of subjects enrolled in the studies and those who will enroll in future protocols.

Definitions

Adverse Event (AE)

An AE is any untoward, unintended, unfavorable, or undesirable medical occurrence, symptom, sign (including an abnormal laboratory finding), illness/disease, or experience that develops or worsens in severity during the course of the study, regardless of relatedness to the investigational device. This includes any new medical problem, or exacerbation of an existing problem, whether or not it is considered device-related.

Each AE is a unique representation of a specific event used for medical documentation and scientific analysis.

Serious Adverse Event (SAE)

SAEs are a subset of AEs. An AE is considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- Death - (i.e., the AE actually causes or leads to death)
- Life-threatening experience - (i.e., the AE, in the investigator's opinion, places a subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions)
- Congenital anomaly/birth defect in subject's offspring

- Important medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, but may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. [Examples include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.]

Unanticipated Adverse Device Effects (UADEs)

A UADE is any serious adverse effect on health or safety, or any life-threatening problem, or death caused by, or associated with, a device; if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan, or application (including supplementary application); or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects [21 CFR 812.3(s)].

Adverse Device Effects (ADEs)

ADEs include any event that is a result of a use error or intentional misuse as well as any AE resulting from insufficiencies or inadequacies in the instructions for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational device.

Classification of an AE

Severity of Event

AEs will be graded using:

- **Mild** – Events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

To avoid confusion, as the terms "serious" and "severe" are not synonymous, the following clarification is given: The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate or severe myocardial infarction); the event itself; however, may be of relatively minor medical significance (such as a severe headache). This is not the same as "serious", which is based on subject/event outcome or action usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations [ICH-E2A(II)(B)].

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Intervention

All AEs must have their relationship to study intervention or study participation assessed by the clinician who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

Attribution categories are as follows:

- **Definite** - The AE is clearly related to study treatment. The AE onset occurs in a plausible time relationship to study treatment and other contributing factors (e.g. concurrent disease or concomitant medications/treatments) can be ruled out.
- **Probable** - The AE is likely related to study treatment. The AE onset occurs in a plausible time relationship to study treatment and the influence of other contributing factors (e.g. concurrent disease or concomitant medications/treatments) is unlikely.
- **Possible** - The AE may be related to study treatment. The AE onset occurs in a plausible time relationship to study treatment; though, other factors (e.g. concurrent disease or concomitant medications/treatments) may have contributed to it.
- **Unlikely** - The AE is doubtfully related to study treatment. The AE onset does not occur in a plausible time relationship to study treatment, and other contributing factors (e.g. concurrent disease or concomitant medications/treatments) are likely.
- **Unrelated** - The AE is clearly NOT related to study treatment. There is not a causal relationship between the AE and the study treatment.

Expectedness

Expected AEs are those that are known to occur for the study intervention being studied. Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study intervention. The investigator or delegated clinician will be responsible for determining whether an AE is expected or unexpected.

Unexpected AEs are those not listed in the device manual, protocol, or not previously identified. This includes AEs for which the specificity, nature, intensity, severity, incidence, or frequency is not consistent with the description in the device manual or protocol or is not consistent with the risk information previously described for the study intervention.

Pre-Existing Conditions

Any medical condition, laboratory abnormality or physical finding that is present prior to initiation of the study intervention will be considered as baseline (i.e. pre-existing condition) and not reported as an AE. Instead, it should be reported as part of the subject's medical history. This includes all relevant historical medical conditions (as determined and documented by Investigator / Clinician) that are known/diagnosed prior to the start of the first study activity involving the investigational device. If the study subject's condition deteriorates or exacerbates at any time during the study period, it will be recorded as an AE.

A pre-existing medical condition should be re-assessed throughout the study period and reported as an AE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors (e.g., "more frequent headaches").

Time Period and Frequency for Event Assessment and Follow-Up

Study Period

The study period will be from time of consent through 24 hours after completion of all DRS Spectra.

Follow-Up of AEs

The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that study participation is not the cause. If an AE does not return to baseline, the resolution date is recorded as "ongoing". All AEs, which completely resolve and then recur, should be recorded as a new AE, regardless of relatedness.

Subjects will be monitored every 2 hours (± 15 minutes) for 24 hours while admitted in the UAMS NICU following completion of the procedure (completion of all DRS Spectra) to evaluate for any potential AEs related to the procedure or the device. This contact will be documented in the subject record. All AEs discovered during this contact will be assessed, documented and reported according to the investigational plan as outlined in this section.

Documenting and Recording of AEs and SAEs

All AEs occurring during the study period whether volunteered by the subject; discovered by study personnel during questioning; or detected through physical examination, observation of clinical symptoms, laboratory, pathological, radiological, or surgical findings, or other appropriate means must be recorded and reported appropriately, regardless of relationship. AEs are to be reported in a routine fashion at scheduled times during the study.

To ensure consistency of AE recording, information to be collected will include: description of event, duration (i.e., start and end dates), clinician assessment of severity (grade) [Mild, Moderate, Severe], relatedness (relationship to the investigational device or study procedures, to be assessed only by those with the training and authority to make a diagnosis), expectedness and actions taken. All AEs will be followed to adequate resolution/stabilization of the event.

All AEs will be documented in the subject record (captured in source documents, including but not limited to the electronic medical record) and recorded on the on the appropriate case report form. All corroborative information related to the AEs will be filed with the source documents.

At each study visit, the investigator, or a designee, will inquire about the occurrence of AEs/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Diagnosis versus Signs and Symptoms

If known that a sign or symptom is one component of a diagnosis or syndrome, the diagnosis or syndrome should be reported as the AE (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

Hospitalizations for Medical or Surgical Procedures

If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, document the event responsible for the procedure, not the procedure itself, as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations or prolonged hospitalization required to allow efficacy measurement for the study, for scheduled therapy of the target disease, or for diagnostic or elective surgical procedures for pre-existing conditions do not require reporting.

AE and SAE Reporting

The PI is responsible for ensuring that all AEs/SAEs/UADEs/ADEs observed or reported during the study, regardless of relationship to the investigational device, are collected and reported to the sponsor and the IRB in accordance with 21 CFR 812. A summary of AEs will be included in the annual IRB Continuing Review and the IDE Annual Progress Report (APR).

Certain AEs must be reported in an expedited fashion to allow for timely monitoring of subject safety and care. Although an event may be considered 'serious' based on previous criteria and should be reported to sponsor immediately, not all SAEs/ UADEs/ ADEs meet expedited reporting criteria.

An investigator must immediately (at least within 72 hours of awareness) report to the sponsor any SAEs/UADEs/ADEs (see definitions above), whether or not considered device related, including those listed in the protocol or device manual and must include an assessment of whether there is a reasonable possibility that the device caused the event. SAEs/ UADEs/ ADEs should be reported using the MedWatch Form FDA 3500A.

Any event occurring within 24 hours after the study procedure that is possibly, probably, or definitely attributable to the investigational device must be reported according to the instructions above.

AEs and SAEs at the End of the Study Period

Any SAE occurring up to 30 days after the study period and considered possibly, probably, or definitely attributable to the investigational device or study participation must be recorded and reported immediately to the sponsor. SAEs that are still ongoing at the end of the study period must be followed for up to 30 days to determine the final outcome.

Reporting of UADEs

In the case of an UADE, an investigator should submit to the sponsor and 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. In addition, sponsor will conduct an evaluation of a UADE and report the results of such evaluation to Food and Drug Administration (FDA) and IRB within 10 working days after the sponsor first receives notice of the potential UADE. Thereafter, the sponsor will submit such additional reports concerning the effect as FDA requests.

Reporting of ADEs

Investigators should immediately notify the sponsor of any adverse device effect within 24 hours of first learning of the event.

Reporting to the Sponsor

The sponsor will be promptly notified of all potential SAEs/UADEs by the investigator for evaluation. Sponsor will report these evaluations to FDA, as necessary, in accordance with 21 CFR 812.

SAEs that do not meet the requirements for expedited reporting to FDA will be reported to the IRB at Continuing Review and on the IDE APR.

All deaths that occur during the study period will be reported to the sponsor as soon as possible, preferably within 24 hours, but no later than 48 hours of learning of the subject's death, regardless to relatedness to the device or the study. A death due to a terminal condition of the research

subject would be considered anticipated and not related to the research, but would still be reported as an SAE.

Reporting to the FDA and IRB

All three (3) of the definitions contained in the requirement must be met for expedited reporting to FDA:

1. Serious,
2. Unexpected, and
3. Possibly, Probably, or Definitely related to use of the device

Sponsor will review all SAEs and report serious, possibly/probably/definitely related events to FDA within 15 calendar days of initial receipt or after determining that the information qualifies for reporting under 21 CFR 812.

In cases of unexpected possibly/probably/definitely related events that are fatal or life-threatening, sponsor will report to FDA as soon as possible, but no later than 7 calendar days after initial receipt of the information.

Investigator will report possibly/probably/definitely related events immediately to the IRB. Any life-threatening event, including those resulting in death, occurring while the subject is actively participating in the study will be reported by investigator immediately to the IRB.

The sponsor will report deaths to FDA in accordance with 21 CFR 812. THE CAUSE OF DEATH AND THE INVESTIGATOR'S DISCUSSION REGARDING WHETHER OR NOT THE DEATH WAS DEVICE-RELATED SHOULD BE DESCRIBED IN A WRITTEN REPORT.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOs)

AEs meeting the criteria of Unanticipated Problem Involving Risks to Subjects or Others (UPIRTSO) will need to be reported to the IRB within the required 10-day allotment of being notified of the event. UPIRTSO requires that an unanticipated problem meet the following qualifications: a) unanticipated or unexpected; b) related to the research; and c) involves new or increased risk to the subject(s) (including physical, psychological, economic, or social harm). Examples of other UPIRTSOs include theft of a computer containing subject information or incarceration of a subject (if not approved for research on prisoners). UPIRTSO may or may not result in actual harm.

Clinical Site Monitoring

Clinical site monitoring will be conducted by the UAMS Office of Research Regulatory Affairs (ORRA) to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable from source documents, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), ICH GCP, and applicable regulatory requirements.

UAMS ORRA Monitoring Specialists will conduct periodic, comprehensive monitoring (either on-site or remote) as determined by a protocol-specific monitoring plan, which will be provided to the Investigator by the ORRA Monitoring Unit.

Deviations and Violations

Protocol Deviation: any unintentional change, divergence, or departure from the study design or procedures defined in the protocol. Protocol deviations will be tracked and compiled in a Protocol Deviation Log. Deviations that potentially cause concern for the subject's health, safety, or rights will be reported to the Sponsor as soon as possible for guidance on reporting.
Protocol Violation: a change to, or non-compliance with, the IRB-approved procedures without prior Sponsor and IRB approval (excluding changes made to eliminate apparent immediate hazard to subjects). A violation may affect health, safety, or rights of a subject. Any violation will be reported immediately to the Sponsor for guidance on reporting.

If the protocol deviation/protocol violation does not represent a significant alteration in the approved protocol and/or affect the safety or welfare of the subject, it will be reported to the UAMS IRB at the time of Continuing Review. If the protocol deviation/violation represents a significant alteration in the approved protocol and/or if it affects the safety or welfare of the subject, it must be reported to the Sponsor and UAMS IRB immediately.

Study Discontinuation and Withdrawal

Subjects have the right to withdraw from the study at any time for any reason. The study will be stopped in the event of a sentinel clinical outcome to one or more subjects. A sentinel event is a subject safety event that results in death, permanent harm, or severe temporary harm. Sentinel events are debilitating to both subjects and health care providers involved in the event.

Being a pilot study, all collected data will be analyzed and no subjects will be considered withdrawn from the study due to incomplete data collection. Subjects who have at least 5 readings at each anatomical location with both the BiliChek and DRS devices are considered completers of the study. Incomplete subjects will not be replaced; however, enrollment will continue until data for 44 completed subjects has been collected.

Data Handling and Recordkeeping

The PI will carefully monitor study procedures to protect the safety of research subjects, the quality of the data, and the integrity of the study. The engineers from University of Arkansas Fayetteville (UAF) will be responsible for data recording. Specifically, the optical instrument is connected to a password-protected laptop that controls the acquisition and storage of spectral data. All data files will be duplicated after the measurements are taken for each subject, and one copy will be zip archived. A CRC-32 checksum value will be generated for a subject's archived folder, and stored with the data in a password-protected, multi-factor-authenticated Box folder. The other copy of the data stored on the laptop will be electronically transferred to password-protected, multi-factor-authenticated UAF servers for processing and analysis using light-tissue interaction models with the program Matlab (version 2022b). The data analysis will be completed within 90 days from acquisition. All study subject materials will be coded by assigning a unique identifying number and will not include additional identifiers. The study demographics, bilirubin concentrations, and clinical lab results will be extracted from the subject's electronic medical record and/or the UAMS Power BI Reporting System NICU Reports. This information will be downloaded in an Excel spreadsheet for analysis on the password-protected device. All data gathered for the analysis and the results of the analysis will be kept in each subject's folder on the secure UAF servers. The key to the subject's coded file, as well as completed paper consent forms, will be kept in a locked file in the PI's office.

Readings for the study will be taken at the UAMS NICU; however, the device and a maintenance log will subsequently be taken to UAF for data transfer to UAF servers and device storage. The

UAF will cede IRB approval to the UAMS IRB, though approved documents will be filed with the UAF IRB. Data gathered during the study will be kept on a password-protected device provided by UAF, which will be stored at the Engineering Research Center located at 700 Research Center Blvd, Fayetteville, AR and analyzed in the Biomedical Engineering department at UAF. The device will be stored in a secure location (Room #: 3407). A second copy of the data will be stored in a password-protected Box folder.

t the conclusion of the study, the data will be permanently de-identified. The data will be retained for a minimum of seven years after the study and final reporting are completed. It will thereafter be destroyed per all applicable UAMS institutional policies and federal regulations.

Data Analysis

Previous work has shown that the BiliChek results were very highly correlated to total serum bilirubin levels (r = 0.88) [9]. An *a priori* power analysis was conducted to determine the number of subjects needed to detect a similar strong correlation between the DRS and BiliChek data (α = 0.05, power = 0.8, r_0 = 0.7, r_1 = 0,85; G*Power3.1). In this study, n = 44 completed subjects (≤ 37 weeks gestation at birth) will be recruited with the goal of maximizing biological variability (gestational age, sex, race/ethnicity) and validating our DRS system during the fewest visits possible. Subjects who have at least 1 measurement with both the BiliChek and DRS systems at a single anatomical location are considered completers of the study. Measured DRS spectra will be analyzed using a lookup table created from published absorbance spectra for each chromophore (e.g. hemoglobin, melanin, bilirubin) and the relative concentrations of the chromophores will be extracted [4]. The primary criteria for success will be a high, positive correlation (r ≥ 0.85, Pearson's correlation coefficient) between the extracted DRS bilirubin concentration (dependent variable) and BiliChek-derived concentration (independent variable). The concentrations will be compared to blood oxygen saturation, weight, complete blood count, chemistry levels, lipids, liver profile, and blood gas, as available, by linear regression. In summary, the spectral and concentration data elements will be compared using linear regression and assessed using Pearson's correlation coefficient.

Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and UAMS research policies and procedures. This protocol/ICF and any amendments will be submitted and approved by the UAMS IRB to conduct the study. All protocol and ICF amendments must be submitted to ORRA prior to IRB review.

The informed consent process will occur prior to any testing. The informed consent of each subject, using IRB-approved consent materials, will be obtained from the parent/LAR before the subject begins any study procedures. The parent/LAR of the subjects for this study will be provided the ICF on paper or electronically via email describing this study in language understandable to the study population. Consent materials will provide sufficient information for the parent/LAR to make an informed decision about their infant's participation in this study. The person obtaining consent will thoroughly explain to the parent/LAR important information about the study, including study requirements, study risks, and benefits. The consent process will take place on the maternity floor prior to the mother's discharge from the hospital or the patient's room in the NICU.

Privacy will be maintained throughout the consent process and questions regarding participation will be answered by the study doctor or staff. No coercion or undue influence will be used in the consent process. The ICF must be signed by the parent/LAR and the person obtaining the consent. The parent/LAR will receive a paper copy of the signed ICF and the informed consent process will be documented in the research record for each subject. A copy of the signed ICF will be placed in the subject's medical record.

The PI and study staff do not have or hold any financial conflict of interest or potential future financial interests in the investigational device involved in this study.

Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a subject. Furthermore, the study

will be registered and maintained in the ClinicalTrials.gov database, and results of this study will be submitted to ClinicalTrials.gov within one year of the study's completion date.

References

1. Afsar FS, Physiological skin conditions of preterm and term neonates. Clin Exp Dermatol, 2010. 35(4): p. 346-50.
2. Zonios G, Bykowski J, and Kollias N, Skin melanin, hemoglobin, and light scattering properties can be quantitatively assessed in vivo using diffuse reflectance spectroscopy. J Invest Dermatol, 2001. 117(6): p. 1452-7.
3. Subhash N, et al., Oral cancer detection using diffuse reflectance spectral ratio R540/R575 of oxygenated hemoglobin bands. J Biomed Opt, 2006. 11(1): p. 014018.
4. Rajaram N, Nguyen TH, and Tunnell JW, Lookup table-based inverse model for determining optical properties of turbid media. J Biomed Opt, 2008. 13(5): p. 050501.
5. Prahl, S. (2005). Bilirubin. Retrieved May 27, 2022, from https://omlc.org/spectra/PhotochemCAD/html/119.html
6. Prahl, S. (1999). *Optical Absorption of Hemoglobin*. Optical absorption of hemoglobin. Retrieved May 27, 2022, from https://omlc.org/spectra/hemoglobin/
7. Jacques, S. (2018). *Melanin*. Optical absorption of Melanin. Retrieved May 27, 2022, from https://omlc.org/spectra/melanin/
8. Lund CH, Osborne, JW, Validity and Reliability of the Neonatal Skin Condition Score. Journal of Obstetric, Gynecologic & Neonatal Nursing, 2004. 33(3): 320-7. doi: 10.1177/0884217504265174.
9. Qualter YM, et al., Transcutaneous bilirubin--comparing the accuracy of BiliChek® and JM 103® in a regional postnatal unit. J Matern Fetal Neonatal Med, 2011. 24(2): p. 267-70.