

Changes in Exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ Breath Delta Value as an Early Indicator of Infection in ICU Patients

Version Date:

August 15, 2016

Protocol Number:

CANARY01

Sponsor

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Name and Description of Investigational Product:

The Isomark Canary™ is a device designed to measure CO₂ concentration and the breath delta value (BDV) in exhaled breath of a patient. The BDV is the difference in the ratio of the two primary naturally occurring isotopic forms of carbon dioxide, ¹³CO₂/¹²CO₂, compared to a standard reference. The BDV may alert the health care practitioner of changes in the patient's metabolic process, onset of systemic infection, and possible need for additional diagnostic tests.

Study Summary

Name and Description of Investigational Device:

The Isomark Canary™ is a device designed to measure the breath delta value (BDV) in exhaled breath samples obtained from a patient. The BDV is a ratio of the two primary naturally occurring isotopic forms of carbon dioxide, $^{13}\text{CO}_2/^{12}\text{CO}_2$. Changes in the BDV can indicate the onset of infection due to the phenomenon known as the acute phase response, which occurs early in the infection process, even before a patient shows typical symptoms of an infection. Breath samples are collected in a disposable, single-use bag. The study is designed to collect data that can demonstrate the effectiveness of the BDV as an indicator of infection as compared to current methods, such as vital signs.

Hypothesis:

BDV is an indicator of infection and provides the earliest possible means for detection of infections.

Specific Aims:

- To determine the positive and negative predictive value of $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV determination as an indicator of infection in adult critically ill subjects.
- To determine baseline variability of exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV in a population of adult critically ill subjects.

General Study Design:

This is a prospective multi-center study using subjects who are not suspected of infection at the time of admission and comparing the Canary's BDV marker to standard infection monitoring methods.

- Open enrollment of subjects, population defined by inclusion/exclusion criteria
- Subject breath sample taken at enrollment and every 4 hours for the length of Intensive Care Unit (ICU) and/or Intermediate Care (IMC) stay until discharge from the ICU/IMC or 28 days (whichever occurs first)
- Subject records monitored/collected for study duration
- Daily research blood samples collected for length of ICU and/or IMC stay and stored for subsequent analysis of c-reactive protein (CRP) and procalcitonin (PCT)

Schedule of Assessments

	Baseline	ICU/IMC Days*	Post ICU/IMC Days	Abx Use Follow-up Days
Eligibility verification	R			
Informed consent by patient or LAR	R			
Demographics, medical history, comorbidity, diagnoses	R			
Pregnancy test	SOC ¹			
Vital signs ²	SOC	SOC x 6 daily ²	CI ³	
CBC with diff	CI	CI	CI	
Standard of Care lab work and cultures	CI	CI	CI	
Blood sample collection, processing for central lab		R ⁴		
Record concomitant medications	R	R	R	
Imaging	CI	CI	CI	
Breath sample collection		R x 6 daily		
Record study specified clinical data		R	R	R
AE review		R	R	R
Assess capacity and re-consent ⁵		R	R	R

Key

CI = if clinically indicated

R = research specific procedure

SOC = standard of care procedure that would occur regardless of study participation

*ICU/IMC Days up to 28 days after Baseline

¹ Pregnancy tests (urine or serum) are performed as part of routine clinical care for women of childbearing potential in this subject population. Results will be used to determine eligibility and be no more than 48 hours old.

² Record Vital signs from medical record that occurred closest to the time of each breath sample collection.

³ Record the first set of vital signs and daily min/max obtained by clinical staff each calendar day.

⁴ Research blood samples will be collected, processed and frozen for batch shipment to a central laboratory.

⁵ Capacity will be assessed daily. If capacity is regained, then encounter for informed consent of the subject will occur.

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List of abbreviations

Abbreviation	Meaning
‰	parts per mil (i.e. parts per thousand)
AE	adverse event
BDV	breath delta value
BPC	best practices control
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulation
CRP	C-reactive protein
CT	computerized tomography
EAC	endpoint assessment – adjudication committee
EDC	electronic data capture
FDA	Food and Drug Administration
HAI	healthcare-associated infections
HIPAA	Health Insurance Portability and Accountability Act
ICU	intensive care unit
IDE	investigational device exemption
IgG	immunoglobulin G
IgM	immunoglobulin M
IMC	Intermediate Care Unit
IRB	institutional review board
ISS	injury severity score
LAR	legally authorized representative
LOCF	last observation carried forward
LPS	lipopolysaccharide
MAP	mean arterial pressure
MEWS	modified early warning system
MRI	magnetic resonance imaging
MOF	multiple organ failure
PCT	procalcitonin
PHI	protected health information
PI	principal investigator
RT	respiratory therapist
SAE	serious adverse event
SD	standard deviation
SEM	standard error of the mean
SIRS	systemic inflammatory response syndrome
UADE	unanticipated adverse device event
UO	urine output rate (mL/hr-kg)
WBC	white blood cell count

Introduction

This study is designed to determine if Isomark's Canary infection monitor can provide an early indicator of the onset of infection.

Healthcare-associated infections (HAIs) are the most common complication of hospital care, resulting in 1.7 million infections and 99,000 deaths each year. HAIs extend the average length of hospital stay by five times, costing the U.S. healthcare system more than \$35 billion each year (1). Serious HAIs that lead to extended hospital stays, and ultimately increased cost and risk of mortality, include blood stream infections, catheter-associated urinary tract infections, surgical site infections, and ventilator-associated pneumonia. These four infections account for more than 80 percent of all HAIs (2). Ventilator associated pneumonia, a common complication in mechanically ventilated patients, increases length of stay in the ICU by an average of six days, increases treatment costs by \$10,000 to \$40,000 per patient (3) and may result in a mortality rate greater than 30 percent (4).

Critically ill patients are at risk for infectious complications. Early detection of infections may lead to earlier treatment and reduce progression to systemic infectious processes like sepsis. Earlier diagnosis of sepsis and treatment as part of a resuscitation bundle has been shown to decrease sepsis related death rate in the critically ill (2). Localized infections that progress to sepsis and its more severe forms, severe sepsis and septic shock, have an unpredictable onset and progression. Sepsis is a leading cause of death in ICUs with mortality rates as high as 30% at some hospitals. Annually in the U.S., 1.4 million sepsis cases involve hospitalization, 750,000 cases of severe sepsis or septic shock, and ~260,000 cases of sepsis related death have been reported in recent years (1). Therefore, infection detection methods aimed at earlier diagnosis may result in earlier initiation of treatment and perhaps mitigate progression to sepsis and associated sequelae. However, the early detection of infection is a significant clinical challenge with diagnosis subject to clinician's clinical judgment and interpretation of overt infectious symptomology and laboratory profile. The healthcare system needs a better way to easily detect infections early and with confidence.

Carbon-12 and carbon-13 are naturally abundant isotopes in expired breath carbon dioxide, can be measured accurately and have been demonstrated in proof-of-concept studies to be reliable indicators of the onset of severe infection. A change in the ratio of carbon-13 to carbon-12 (i.e. $^{13}\text{CO}_2/^{12}\text{CO}_2$) in exhaled breath is known as the breath delta value (BDV). The ratio of $^{13}\text{CO}_2/^{12}\text{CO}_2$ is expected to decrease, and produce a negative BDV trend, during severe stress such as infection. In animal models of sepsis, the BDV preceded other physiologic changes associated with infection (5, 6). In these preclinical studies, BDV was shown to be a possible early indicator of the onset of infection that precedes current, less direct indicators, such as changes in vital signs, mental status or white blood cell count.

Isomark, LLC (Madison, WI) has developed an investigational device, the Canary™ infection monitor which is intended to determine the BDV in critically ill patients. The Canary will be used in this study to determine the BDV of breath samples collected from patients who agree to participate as research subjects.

The objectives of this study are:

1. To measure variation of BDV with time in adult critically ill ICU patients who agree to participate as research subjects;
2. To determine the magnitude of change of BDV in subjects who are subsequently diagnosed with severe infection and/or sepsis;
3. To define variation of BDV in adult critically ill ICU subjects who do not develop severe infection.

In this multi-center study of 100 subjects, breath samples will be collected six times per day and research blood samples will be collected once per day. Analysis results of these research samples will be compared with data from subjects' medical records.

1.0 Background and significance

The response to a wide variety of insults has been termed systemic inflammatory response syndrome (SIRS). SIRS is nonspecific and develops following a severe physiologic stress or insult, such as infection, trauma, surgery, pancreatitis, or burn. At least two SIRS criteria coupled with a known or suspected infection is termed “sepsis.” In a prospective survey of adult patients admitted to a tertiary care center, 68% of admissions met SIRS criteria, and 26% developed sepsis (SIRS due to an infection) (7). Hospital death associated with severe sepsis ranges from 18% to 28% in adults (8). Sepsis remains the leading cause of death in adult surgical ICU patients(9).

Clinical data are often unreliable in distinguishing between patients with sepsis (defined as SIRS with a known or suspected infection) and those with SIRS secondary to a cause other than infection. Trauma is frequently associated with SIRS (10), and, in the US, 5.7% of patients admitted after vehicular trauma develop an infection (9). Followed by evidence based management, early detection of infection after trauma could decrease complications and death due to sepsis (11). Overdiagnosis of infection and associated unnecessary use of antibiotic drugs is believed to promote antibiotic resistance and complications related to antibiotic exposure, such as morbidity associated with hospital acquired *C. difficile colitis*.

Analyses of some biomarkers is available and some may be useful for prompt diagnosis of infections, including systemic infections like sepsis. However, they are insufficient for monitoring infection progress to more severe forms like sepsis. WBC and absolute neutrophil count are inexpensive laboratory tests, but lack adequate sensitivity and specificity to detect serious infections (12). C-reactive protein (CRP) is an inexpensive test, but does not detect severe infections early (13). Procalcitonin (PCT) may be more specific than CRP in response to bacterial infections(14, 15), but is an expensive test, not commonly available in US clinical laboratories, and specificity is confounded in postoperative, burn, trauma, pancreatitis, vasculitis, and other non-infectious conditions that cause SIRS (13).

The $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV has been shown to be an early indicator of catabolic infectious states in several animal models of bacterial sepsis (6). In animal studies, $^{13}\text{CO}_2/^{12}\text{CO}_2$ begins to decrease immediately after administration of lipopolysaccharide (LPS), and the change in $^{13}\text{CO}_2/^{12}\text{CO}_2$ precedes other physiological changes associated with infection, such as hypotension, gut hypoperfusion, or alterations in blood pH, pCO_2 or pO_2 (6). No animal studies have assessed $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV as an indicator of infection in non-infectious systemic inflammatory conditions, such as surgery, severe trauma, severe burn injury, or pancreatitis.

The objective of this pilot study is to determine the positive and negative predictive values of exhaled breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV as an early diagnostic marker of infection in adult critically ill adult subjects. If BDV accurately indicates onset of infection in subjects subsequently diagnosed with infections, and discriminates onset of infection and non-infectious causes of deranged vital signs in subjects, then BDV would be an effective monitor of infection, could prompt early diagnosis, early treatment, and improve outcomes of critically ill patients.

1.1 Prior studies

Human studies

In a proof-of-principle study at the University of Wisconsin Hospital and Clinics (IRB #2011-0045), 17 mechanically ventilated pediatric subjects and 15 mechanically ventilated adult subjects were enrolled (12). Figure 1 shows pediatric BDV at enrollment for SIRS vs no SIRS cohorts (Panel A) and categories of inflammation risk factors (Panel B). Adult data are similar (Figure 2). Clinical data collected to date correlate well with the hypothesized breath delta value paradigm illustrated below (Figure 3).

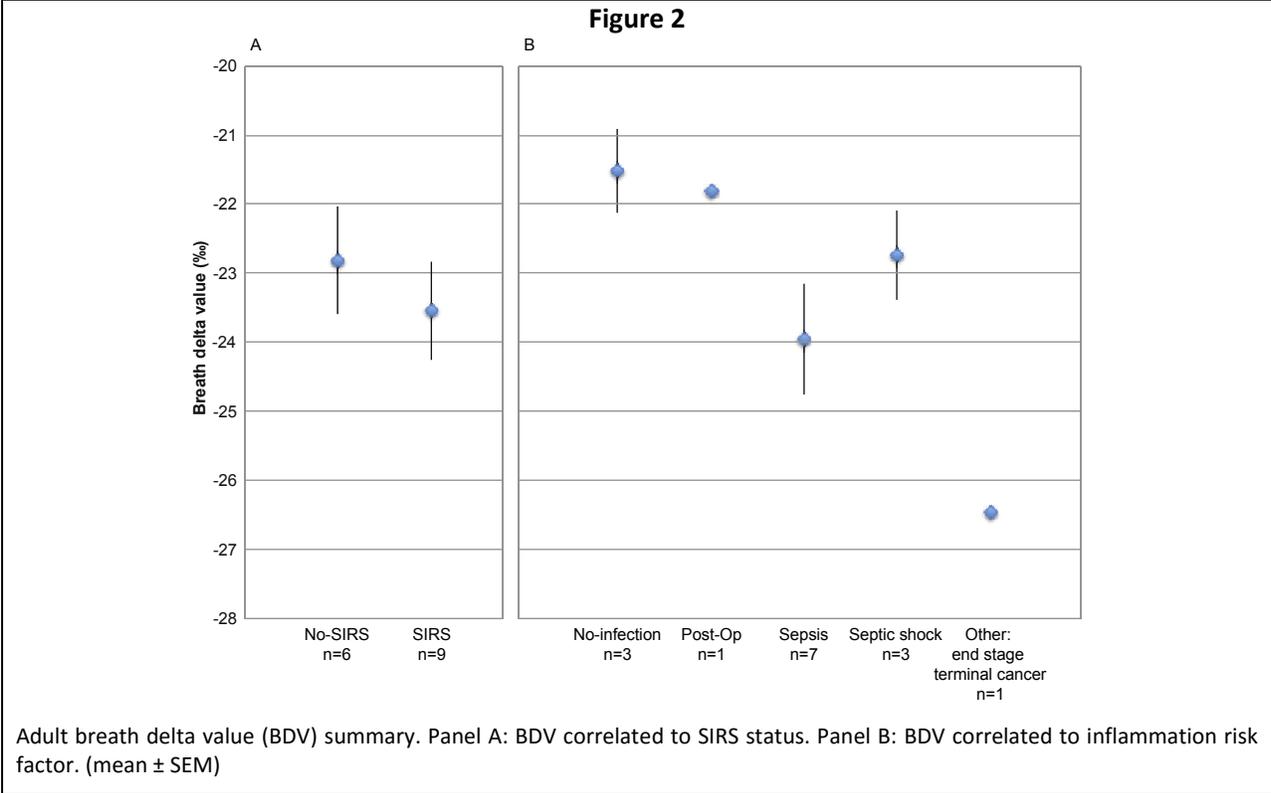
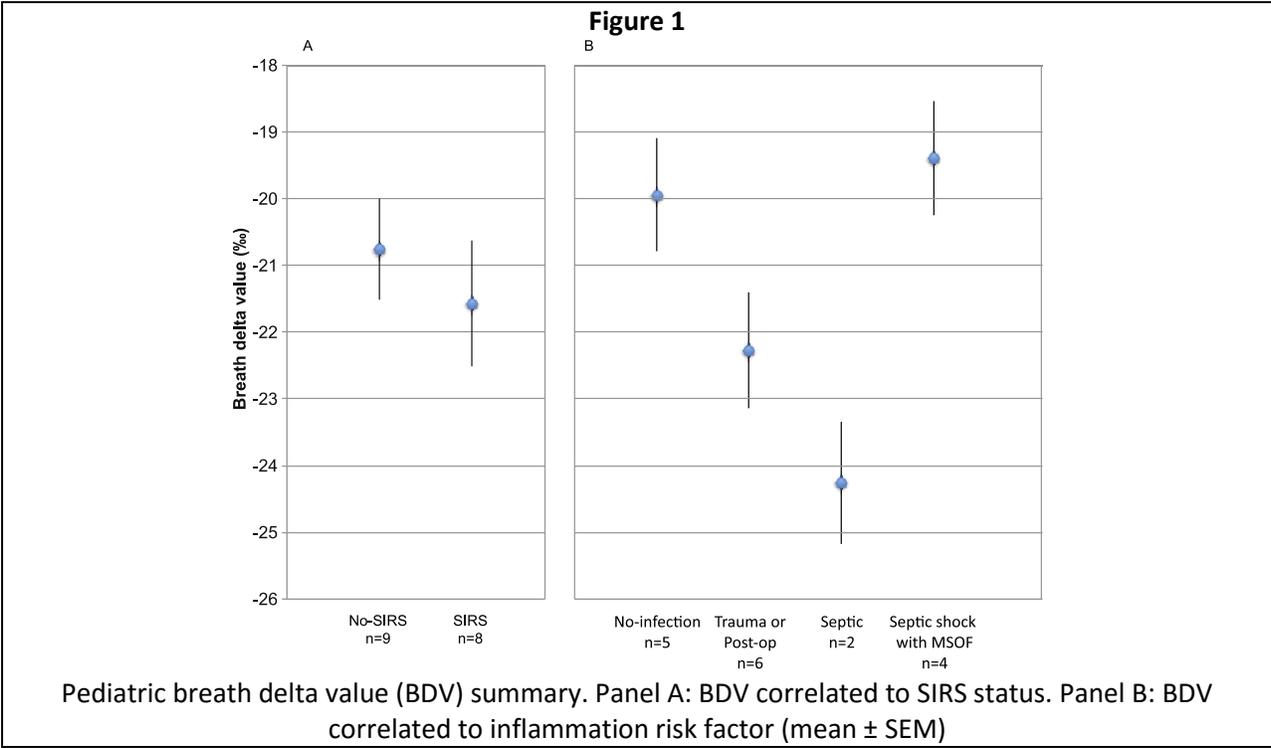
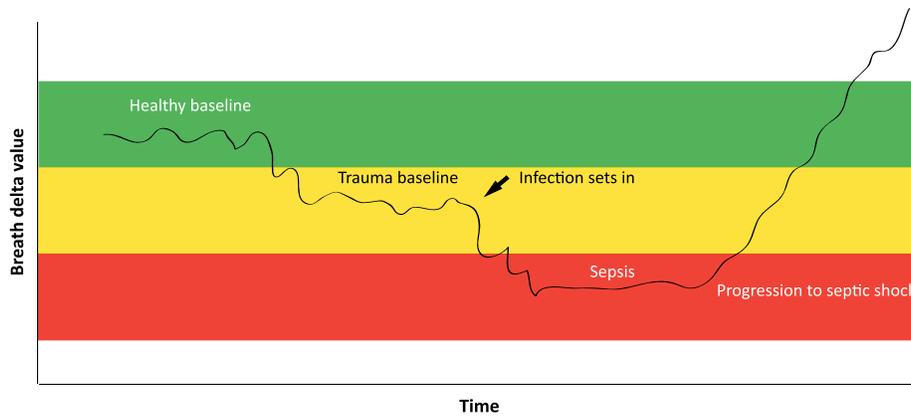
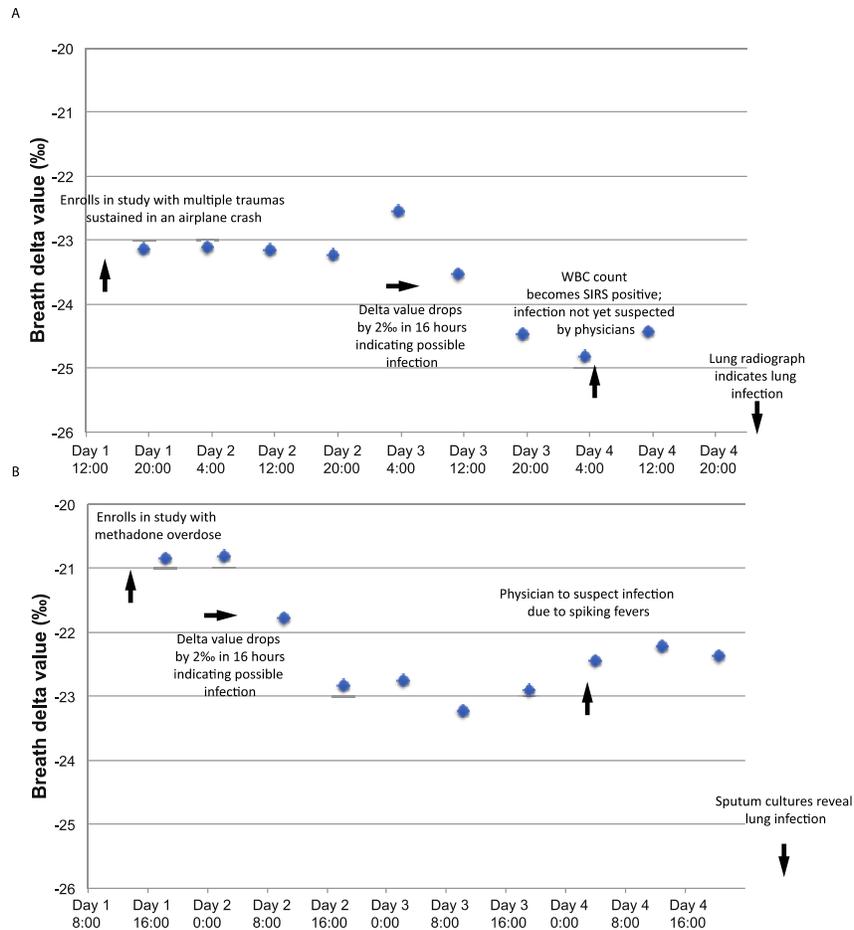


Figure 3



Illustrative representation of the breath delta value (BDV) paradigm during infection, progressing from healthy to trauma through infection to septic shock. Based on preclinical animal data, the paradigm predicts changes in BDV due to trauma, infection, and septic shock.

Of the 32 subjects enrolled in the proof-of-principle trial, two subjects (age 14 and 15 years) who were not initially suspected to have infection developed infection during the study or within 72 hours of completing the study. In these two cases, the BDV trended downward—which, according to the paradigm in Figure 3, indicates ongoing acute phase response -- 36 to 48 hours before an infection was diagnosed (Figure 4). There were no other cases that exhibited this trend. Data from these two cases suggest that the BDV may be a leading indicator of infection in critically ill patients. To confirm and further investigate these preliminary findings, this study is proposed to determine if the BDV is an early indicator of infection in critically ill adult ICU subjects.

Figure 4

The breath delta value (BDV) may have been an early indicator of the onset of infection in two pediatric cases of ventilator associated pneumonia. Panel A: pediatric case 4 was enrolled with multiple trauma sustained in an airplane crash. The BDV was stable for 48 hours after hospital admission. On day 3 of the study, the BDV decreased 2 delta values during 16 hours. Physicians suspected progression of infection the following day due to spiking temperature and a low white blood cell count. The infection (*Serratia marcescens*) was confirmed by lung radiograph and sputum cultures 24 hours later. Panel B: pediatric case 15 was enrolled in hypoxic shock due to a methadone overdose. The BDV was stable for the first 16 hours of hospital stay. On day two of the study, the BDV decreased by 2‰ during 16 hours. A spiking temperature curve alerted physicians to a possible infection 24 hours later. After a further 24 hours, sputum cultures revealed *S. aureus* lung infection.

Animal studies

Butz et al published results of two experiments in mice evaluating $^{13}\text{CO}_2/^{12}\text{CO}_2$ as a biomarker of lipopolysaccharide (LPS) induced acute phase response (6). In the first experiment, four mice were injected with sterile saline, and five mice were injected with LPS; each injection was given intraperitoneally. In the second experiment, three mice were injected with dexamethasone (DEX), three mice with LPS, and three mice with sterile saline. Stable isotopes of blood amino acids and carbon in exhaled CO_2 were monitored. An increase in the relative isotopic mass of serum alanine, proline, and threonine was observed three hours after LPS injection. BDV began to decrease immediately after the administration of LPS and became significantly less than BDV of the control (saline) animals within 2.5 hours after injection. A similar BDV decrease was not observed with DEX treatment. These changes in BDV are indicative of systemic inflammation and have been replicated in other animal models (leghorn chickens, rats, and swine) using live bacteria (13). The authors concluded that acute phase protein synthesis, typical of systemic inflammation, caused the changes observed in plasma amino acids and in exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV. These results were confirmed in a porcine cecal ligation and puncture model of sepsis (14). From these studies, exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV may be an early indicator of the acute phase response due to endotoxemia.

In another experiment, Assadi-Porter et al administered a live viral agent to chickens via drinking water. Approximately 80 hours after starting the experiment, the first significant decline in $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV occurred. A partial recovery period was observed, followed by a sudden and more profound decrease. The cycle of partial recovery and subsequent decrease was then periodically repeated. The authors concluded that the rate of change in $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV, and the repeated spikes in $^{13}\text{CO}_2/^{12}\text{CO}_2$, may help differentiate between viral and bacterial infections (15).

These studies suggest that exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV may be a potential early indicator of systemic infection in humans.

2.0 Objectives

2.1 Primary objective

To define variation of BDV in critically ill adult subjects admitted to ICUs.

Hypothesis to be tested:

- Null hypothesis: BDV in critically ill adult ICU subjects is equal to BDV in non-infected adult ICU subjects.
- Alternative hypothesis: BDV in critically ill adult ICU subjects is different from BDV in non-infected adult ICU subjects.

2.2 Secondary objective

To determine the magnitude of BDV change in subjects who are subsequently diagnosed with severe infection.

Hypothesis being tested

- Null hypothesis: BDV in non-infected critically ill adult ICU subjects does not decrease significantly before a clinical diagnosis of severe infection.
- Alternative hypothesis: BDV in non-infected critically ill adult ICU subjects decreases significantly before clinical diagnosis of severe infection.

Hypothesis being tested

- Null hypothesis: BDV in the exhaled breath of critically ill adult ICU subjects is not a marker for response to treatment for infection.
- Alternative hypothesis: BDV in the exhaled breath of critically ill adult ICU subjects is a marker for response to treatment for infection.

2.3 Tertiary objective

- Compare positive and negative predictive value of SIRS/MEWS criteria with the BDV.

2.4 Specific aims

- To determine the positive and negative predictive value of $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV determination as an indicator of infection in critically ill adult ICU subjects.
- To determine baseline variability of exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV in a population of critically ill adult ICU subjects.
- To determine the response to treatment using the exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV in a population of critically ill adult ICU subjects.

3.0 Study design and rationale

This is a multi-center prospective study to assess exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV as an indicator of infection in critically ill adult ICU subjects. Critically ill adult ICU subjects who are not suspected of having an infection at the time of ICU admission will be enrolled as study subjects. Up to 100 Subjects will be enrolled from 8-10 centers across the United States.

This study is needed to determine the rate of infection detection of the Canary device. Logistic considerations include the definition of an infection and collection of data needed to diagnose infections consistent with the Centers for Disease Control (CDC) standards (16), and determination of onset timing of infection by clinical standards and by BDV. The BDV, as determined by the Canary™, will be retrospectively determined from exhaled breath samples, and will not be available or used clinically to establish or confirm the infection.

Effectiveness parameters will be estimated to determine the sample size required for the subsequent pivotal study. Parameters to be estimated include: timing of the infection onset, the lead time offered by various BDV thresholds, sensitivity of the BDV threshold, specificity of not reaching the BDV threshold, the receiver operating curve as a function of the BDV threshold, and the positive and negative predictive values based on hypothetical infection incidence. Models for infection outcome (Y/N) will be analyzed for the impact of clinical and on study covariates. The corresponding performance of a conventional best practices control (BPC) will be compared to determine if the Canary can identify infections earlier in the clinical course than BPC. Several time windows will be tested to determine the timing of and criteria for aligning a monitoring test result with BPC or clinical reference standard result. This pilot study will be used to formulate strategies to help control the Type I error (“false positive”) associated with multiple times when BDV could become positive. The pilot study will assess the robustness of the effectiveness outcomes across a broad spectrum of possible infection types.

The BDV will be analyzed to determine the range of exhaled $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV in adult subjects. The $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV will be compared with current clinical standard scores, physiologic measurements and biomarkers (e.g. vital signs, WBC, CRP, PCT), and any interventions -- such as antibiotic administration -- will be noted for the purpose of assisting in understanding variation of BDV. With the exception of CRP and PCT (require study specific blood samples), these data will be obtained from the medical records of patients who agree to participate as research subjects. To assess the hypothesis that BDV is a leading indicator of infection, BDV determined as a sustained decrease of $^{13}\text{CO}_2/^{12}\text{CO}_2$ BDV of approximately 2.0‰ or more over a 24 hour period will be compared with a diagnosis of infection in the following 72 hours.

3.1 Research subjects

3.1.1 Inclusion criteria

- 1) age 18 years or older
- 2) critically ill patient admitted to the Intensive Care Unit (ICU)
- 3) enrolled within 48 hours of ICU admittance (see Enrolled definition in section 6.1)
- 4) expected duration of hospital stay at least 120 hours (five days) from time of study enrollment
- 5) subject/LAR speaks a language of which the IRB has approved a consent form

3.1.2 Exclusion criteria

- 1) known or suspected infection at time of enrollment
- 2) known use of systemic antibiotic, antimicrobial and/or antifungal therapy within the seven (7) days prior to hospital admission
- 3) currently active cancer, defined as receiving treatment or intend to receive treatment within hospital stay for cancer (including but not limited to: radiation, chemotherapy, systemic orals, etc)
- 4) if not intubated, unable to cooperate with providing a breath sample

- 5) expected death within 24 hours of enrollment or lack of commitment to aggressive treatment by family/medical team (e.g., likely to withdraw life support measures within 24 hrs of screening)
- 6) female who is pregnant or lactating (negative serum or urine pregnancy test results within 48 hours of enrollment or to be performed during screening)
- 7) prisoner
- 8) known participation in an investigational and interventional research study within 30 days prior to enrollment, not approved in advance by sponsor (note: to be eligible, any interventional treatment must have ended at least 30 days ago)
- 9) Individuals who are directly affiliated with sponsor or study staff, or their immediate families. Immediate family is defined as spouse, domestic partner, parent, child, or sibling whether legally adopted or biological.
- 10) Any patient that is deemed unfit for study participation, per the Investigator's discretion.

Protected populations

Impaired cognitive ability

Potential subjects meeting eligibility criteria are likely to be cognitively impaired due to pharmacological sedation and severe illness. Permission for participation will be sought from the potential subject's legally authorized representative (LAR).

All subjects consented by substituted judgment will be assessed daily for capacity to consent prior to discharge from the hospital that is the study site. Subjects who were originally consented by substituted judgement and who regain capacity will be approached for re-consent. (see 4.3 Consent of subjects who regain capacity)

Prisoners

Due to the complexity of state and federal requirements governing the participation of prisoners in research, patients who are prisoners will not be considered for participation in this trial. In the unlikely event that a subject becomes a prisoner while participating in this study, study procedures will stop. Such subjects will be considered 'Withdrawn by Investigator'.

Pregnancy

Pregnancy tests (urine or serum) are performed as part of routine clinical care for women of childbearing potential in this subject population. Results used to determine pregnancy status will be no more than 48 hours old. Patients who are known to be pregnant will be excluded from participation in this study. A negative serum or urine pregnancy test is required for all women of childbearing potential prior to consent and enrollment in this study.

4.0 Subject recruitment and consent

4.1 Identification of potential subjects

Potential subjects will be identified by members of the clinical care team, who will receive instruction and training about the study, including purpose of the study and eligibility criteria.

A member of the study team will assess the potential subject's eligibility, as approved by the Institutional Review Board (IRB). Any protected health information used during the screening process of a potential subject will be the minimum necessary for the conduct of this study.

If informed consent is not obtained, all identifiable information will be destroyed at the end of the screening process. The criteria that excluded the patient from study participation will be recorded, but no identifiers will be retained.

4.2 Recruitment and consent

Determining capacity

Subjects who are conscious and appear to possess capacity to consent will view consent information with a study team member. This consent process will include a review of specified questions with the coordinator (e.g., what is the study purpose? What are the study procedures? Does this study involve treatment?). If the subject is unable to provide appropriate answers, despite review with the study team member, or if the study team member is not certain of the subject's decision making ability, then the study team will have a follow up conversation with the clinician investigator to confirm capacity. The communication should be documented in the subject's records.

Subjects assessed to be lacking consent capacity will be involved in consent discussions and asked to provide verbal 'assent' to the degree to which they are able. For subjects who are able to be involved in such discussions, they will be provided with an oral summary of the study in terms they are likely to understand. Subjects expressing dissent to participation will not be enrolled, regardless of surrogate consent.

If a subject is determined to lack the capacity to consent, a surrogate (e.g., family member) will be approached to discuss the study and to review the consent information. Surrogate priority will be:

Subject with no Research Power of Attorney, no Guardian, and no Healthcare Power of Attorney: If the potential subject is found to lack capacity, then the potential subject's next of kin may consent on behalf of the potential subject as a legally authorized representative (LAR). Any next of kin representative of the potential subject should be actively involved in the care of the subject. LAR order of priority is:

- a. spouse
- b. adult child
- c. parent
- d. adult sibling
- e. grandparent
- f. adult grandchild
- g. close friend

Recruitment of potential subjects

A study team member will approach subjects who are likely to be eligible for study participation, based on review of the potential subject's medical record and current clinical status. No study specific procedures will be performed prior to obtaining informed consent.

Informed consent process

A member of the study team will conduct the informed consent discussion with the potential subject or surrogate in a location where a private conversation may be held. The study team member will explain the study procedures, that the purpose of the study is to evaluate a new infection monitor, and that treatment of the potential subject is not the purpose of the study. Any study team member may conduct the informed consent discussion and obtain informed consent. A physician investigator will be available to address consent related questions. Coercion will be prevented by stressing that the potential subject or surrogate does not have to agree to participate, and that the care of the potential subject will not be affected by the decision to participate.

Enrollment

A subject will be considered enrolled in the study once they have been consented for participation and the first breath sample has been collected. See study definitions in section 6.1.

4.3 Consent of subjects who regain capacity

Enrolled subjects will be assessed for capacity to consent daily until they complete the study. Subjects who regain capacity during their stay in the study hospital will be approached for consent to use the data and samples collected during the study. Study records of data and samples collected from subjects who decline study participation will be destroyed, with the exception of any data used for safety analysis.

Subjects who provide informed consent after regaining capacity can withdraw from the study at a later time (See 6.12 Withdrawal of subjects).

Subjects who are contacted over the phone, will be assessed for capacity and consented over the phone if necessary. Subjects who do not regain capacity to consent prior to being discharged from the study hospital, but who later regain this capacity, may contact the study site and request that their study related data and samples be deleted or destroyed. If the data or samples have already been analyzed (including for safety purposes) or de-identified, then the data used for study purposes will not be removed.

5.0 Study procedures

5.1 Baseline

The baseline period is from time of hospital admission until the first breath sample collection. Demographic data, relevant medical and surgical history, admission reason to the ICU/critically ill status, concomitant medications, and vital sign data will be collected.

5.2 ICU/IMC Days

This initial period of study days begins the day a subject is consented thru either discharge from the hospital or from an IMC/step-down unit to a general care status/unit. A study day will equal one calendar day. Day 1 will start at the time of the first breath collection and end at 23:59. Day 1 will typically be less than 24 hours and fewer than 6 breath samples may be collected. Research samples will be obtained until (1) the subject has been discharged to general care status/unit or (2) the subject has been discharged from hospital or (3) 28 days of breath sample collection has occurred, whichever comes first.

- Assess for capacity to consent (if subject did not previously provide consent)
- Research breath sample every 4 hours
 - Vital signs recorded concurrently
- Research blood draw once per calendar day
- Medical record abstraction

5.3 Post ICU/IMC Days

This period of the study will last three days beyond a subject's discharge from the ICU/IMC. If a subject is discharged home, the subject's electronic medical record (EMR) will be reviewed for an additional three days beyond discharge* for any results, notes, procedures, or readmission information.

- Assess for capacity to consent (if subject did not provide consent and is still in the hospital)
 - Medical record abstraction
- *If subject has been in the study for 28 days then the post ICU/IMC days will take effect on Day 29.

5.4 Antibiotic Use Follow-up Days (3 days beyond last dose of antibiotics)

If the subject is on antibiotics when discharged from the ICU/IMC, they will be followed for the three Post-ICU/IMC days (as detailed in 5.3) and then daily until the last dose of antibiotics (from the initial prescriptions at time of discharge), followed by an additional three days. If the subject is discharged home, the EMR will be reviewed daily until three days beyond the final scheduled dose of antibiotics. The subject will be called on the third day beyond their last anticipated dose of antibiotics to inquire about their infection/readmission status since their original hospital discharge.

- Assess for capacity to consent
- Medical record abstraction
- Phone questionnaire

5.5 Breath sample collection

Breath samples will be collected upon enrollment and every four hours thereafter until the subject is (1) discharged home or (2) to general care unit/status or (3) 28 days of breath sample collection has occurred, whichever comes first. Each sample should be collected in four-hour intervals, calculated from the initial sample time, with a window of ± 1 hour. The target time for sample collection will be determined when the first sample is collected and will not be reset if a subsequent sample is collected late or early. Samples collected outside of the specified time interval will still be analyzed. NOTE: Day 1 can start immediately after the baseline data collection is complete, and may occur on the same physical day. Therefore, it is likely that Day 1 won't have all 6 breath samples because of the shortened duration after the consent process.

Some subjects may be discharged from the study hospital in less than five days. If subjects are withdrawn (e.g. are discharged or died) on or before completing Day 5, they will be replaced in the overall enrollment numbers and not used in the primary analysis, though the data and breath samples collected will still be kept. Subjects who withdraw after completing Day 5, will have their data remain as part of the primary analysis. See Table 1 in section 5.12.

Breath sample collection bags will be provided by Isomark. Any unused bags will be returned to Isomark. Training documents about breath sample collection and storage are detailed in Isomark's Lab Manual. Collected samples will be labeled with site number, subject number, subject initials, date and time of collection. Collected samples will be stored in a secure location at the study hospital until sent to Isomark.

Mechanically ventilated subjects

For mechanically ventilated subjects, an appropriately trained and qualified member of the subject's clinical care team or study team member will obtain expired breath from a side port adaptor in the expiratory limb of the subject breathing circuit. The gas sample will be obtained in a small gas sample bag. This method of sample collection allows inflation of a sample bag without any interference with operation of the mechanical ventilator or breathing circuit. The breath sample bag will inflate visibly during approximately 2-4 breath cycles. The bag will appear inflated, but not over inflated, after which time the sample bag will be disconnected and sealed.

Sampling from expiratory limb:

When sampling from the expiratory limb of the breathing circuit, the sample bag will be attached to a standard diameter, straight adaptor with a side port fitted with a normally closed valve which only opens when a sample bag is attached. The standard side port adaptor will be placed by a respiratory therapist in the expiratory limb of the breathing circuit proximal to the subject at the start of the clinical trial for the subject and will remain in place for the duration of the clinical trial for the subject or for the duration of mechanical ventilation support, or, if a breathing circuit is replaced anytime during the clinical trial for the subject, then a new adaptor will be placed.

Non-mechanically ventilated subjects

Non-mechanically ventilated subjects will be asked to inflate a breath sample bag by exhaling. Subjects should repeat exhalation until the bag appears visibly inflated, but not over inflated.

Subjects who are having difficulty breathing (Mask Sample Collection)

If the subject is not ventilated but has difficulty inflating a bag using the bag's mouthpiece, a mask collection option is available. Collecting a sample using the mask is performed by attaching a sample bag to the breathing mask via a connector. The mask is placed over the subject's nose and mouth during exhalation. The sample bag should appear visibly inflated, but not over inflated. If more than one breath is required to inflate the bag, the mask must be removed during inhalation.

5.6 Blood sample collection

Once daily during breath sample collection days, 3 mL of blood will be obtained in a green top, lithium heparin tube from subjects via an existing IV or arterial catheter. If subjects do not have an existing catheter, research samples may

be obtained via peripheral lines or venipuncture. Timing of the blood sample is not critical, but one sample is to be collected during each calendar day of the ICU/IMC Study Days period. Blood may be obtained from an artery or a vein. Collected samples will be affixed with a provided de-identified label.

To avoid sample collection from subjects who are anemic, if a subject's known hemoglobin concentration is less than 6.0 g/dL at any time, then blood collection will stop and will not resume until the subject's hemoglobin concentration has increased to greater than or equal to 6.0 g/dL. If blood sample acquisition is stopped due to hemoglobin concentration criterion, then other study activities will continue per protocol.

5.7 Blood sample processing, storage and analysis

Please see Isomark's Lab Manual for complete instructions. Whole blood samples will be spun down in a centrifuge. A minimum of 1.5 mL of plasma should be transferred to aliquot tubes. Plasma samples will be labeled with a duplicated label pre-printed with the subject number and the date/time of collection written. Remaining red blood cells (RBC) will be recapped in the lithium heparin tubes and stored in that tube. The plasma aliquot tubes will be kept frozen at the site for a minimum of 24 hours before batch shipping to Isomark's reference lab for later analysis of PCT and CRP. The RBC tubes will be kept frozen at the site for a minimum of 24 hours before batch shipping to Isomark for isotope ratio analysis. Following assay of these biomarkers, all samples will be exhausted or destroyed.

Study samples will be kept between -50° and -196° C until the time of analysis. The storage freezer must maintain constant temperature (i.e. must not be frost-free or defrost automatically). Study team members will report all occurrences of freezer temperature deviating outside of this range. Temperature logs from the freezer(s) will not be collected.

Clinical care providers for the study subjects and study team members will not have access to the PCT or CRP measurements until all study data and sample collection is complete. Subjects will not be informed of their individual PCT or CRP measurements.

5.8 Carbon isotope measurement

Measuring breath carbon isotope concentration

Please see Isomark's Lab Manual for complete instructions. Collected breath samples will be shipped to Isomark within 48 hours of final collection from a subject via a shipping service, such as UPS or FedEx, to Isomark's breath analysis lab. If multiple subjects are enrolled, then shipments should be made once per week. Isomark personnel will analyze the isotope ratio. Some of the gas sample will be withdrawn from the gas tight bag by the Canary. The ¹³CO₂/¹²CO₂ ratio in each sample will be determined from direct measurement. BDV will be calculated using Pee Dee Belemnite (PDB) as a standard reference:

$$BDV = \frac{(^{13}\text{CO}_2/^{12}\text{CO}_2 \text{ sample} - ^{13}\text{CO}_2/^{12}\text{CO}_2 \text{ PDB}) \times 1000}{^{13}\text{CO}_2/^{12}\text{CO}_2 \text{ PDB}}$$

where BDV is expressed as parts per mil (‰).

Results of carbon isotope measurement

Carbon isotope measurement in exhaled CO₂ is not part of standard medical treatment in any patient population. Clinical care providers for the study subjects and study team members will not have access to the carbon isotope measurements until all study data and sample collection is complete. Subjects will not be informed of their individual carbon isotope measurements.

5.9 Third parties

Isomark will be using reference laboratories for the analysis of CRP and PCT as well as for the red cell carbon isotope concentration. These laboratories will analyze study samples and provide results to Isomark personnel who will distribute to sites for documentation and data entry. If a third party were to require the study samples for another study related analysis, an amendment to this protocol would be submitted, and only de-identified information would be available to the third party.

Measuring C-reactive Protein and Procalcitonin

Study samples will be stored in the freezer until batch shipped to the central laboratory, Marshfield Laboratories, for biomarker analysis. Marshfield Laboratory has been contracted to analyze the CRP and PCT biomarkers for the collected blood. Blood samples will be shipped to the central laboratory overnight via Fed Ex. Marshfield Laboratory will document received date and time as well as time and date of analysis performed. Following assay of these biomarkers, all samples will be exhausted or destroyed, via biomedical waste procedures.

Measuring red blood cell carbon isotope concentration

Study samples will be stored in the freezer until batch shipped to the central laboratory, for carbon isotope analysis. Packed red cell samples will be shipped to the central laboratory overnight via a shipping service. The laboratory will document received date and time as well as time and date of analysis performed. Packed red cell samples will be combusted in an elemental analyzer and the carbon isotope ratio will be determined by isotope ratio mass spectrometry. All samples will be exhausted or destroyed, via biomedical waste procedures.

5.10 De-identification of samples

The master list will be destroyed two years after completion of study data analysis, and Isomark will notify the study site of this requirement at that time. After the master list is destroyed, samples will not be traceable to individual subjects.

5.11 Availability of test results

Data from laboratory tests will not be available in a timely manner, will not influence clinical care, and will not be revealed to the subject or subject's clinical care team.

5.12 Withdrawal of subjects

Table 1: Withdrawal of subjects				
Withdraw type	Specimens and data	Subject to be replaced?	Used for primary analysis?	Note
Infection Failure <ul style="list-style-type: none"> Received antibiotic therapy during 7 days prior to hospital admission 	All specimens and data will be kept.	Yes; will be labeled as a 'Screen Failure'	No	Screen failed subjects will be notified verbally of their withdrawal from the study.
Inclusion/Exclusion Failure <ul style="list-style-type: none"> Have not met inclusion/exclusion criteria after original screening (e.g. later known cancer treatment or abx use prior to hospital admission) 	All specimens and data will be kept.	Yes; will be labeled as a 'Screen Failure'	No	Screen failed subjects will be notified verbally of their withdrawal from the study.
By Investigator	All specimens and data obtained prior to withdrawing from study will be kept.	Yes	No	Subjects may be withdrawn from the study at any time at the discretion of the PI or the subject's attending physician with PI agreement. Withdrawn subjects will be notified verbally.
High frequency mechanical ventilation	Breath collection will be discontinued with high frequency ventilation. Development of infection will be monitored for 72 hours after the last breath sample is collected. All specimens and data will be retained in the study record.	No	No	High frequency ventilation is rarely used in adults because it has been shown to provide little or no benefit to subjects (17).

Table 1: Withdrawal of subjects				
Withdraw type	Specimens and data	Subject to be replaced?	Used for primary analysis?	Note
Surrogate who provided consent withdraws consent	All specimens and data obtained prior to subject withdrawing from study will be retained in the study record.	Yes, if withdrawn prior to Day 10	Yes, if withdrawn after Day 10	
Subject does not agree to participation when consent capacity regained	All data removed and samples destroyed unless already de-identified or used for safety analysis.	Yes	Yes, if samples and data de-identified or used for safety analysis	
Subject withdraws consent after initially providing consent	All specimens and data obtained prior to subject withdrawing from study will be retained in the study record.	Yes	Yes	
Unable to collect breath or blood samples through Day 5 (e.g., subject discharged or died, or continuous CPAP or BiPAP, or unable to obtain or provide breath sample after extubation)	All specimens and data will be retained in study record.	Yes	No	Medical records will be reviewed until discharge from the hospital or the IMC.
Sample collection outside of scheduled collections (e.g. extra samples-protocol deviation, samples outside of allowance window, etc)	All specimens and data will be retained in study record.	NO	Yes; subject will still be used in primary analysis, extra samples will NOT be used in primary analysis	Samples will be entered into EDC as extra samples, with note of their deviation.

6.0 Definitions

6.1 Study definitions

Enrollment

Subjects will be enrolled in the study as soon as possible after ICU admission, though not beyond 48 hours. A subject will be considered enrolled in the study if he/she meets all inclusion criteria, does not meet any exclusion criteria, and if he/she or a surrogate have provided informed consent and the first breath sample has been collected. A subject that later is withdrawn from the study will be included in the analysis following the criteria outlined in section 5.12.

Systemic Inflammatory Response Syndrome (SIRS)

SIRS is the presence of at least two of the following four criteria:⁽¹⁸⁾

- T >38°C or <36°C
- Tachycardia, i.e. HR > 90 beats/min
- Tachypnea, i.e. RR > 20 breaths/min
- Increased leukocyte count >12,000 cells/mm³ or decreased leukocyte count < 4,000 cells/mm³ (not secondary to chemotherapy induced leukopenia), or >10% immature neutrophils

Modified Early Warning System Criteria (MEWS):

The modified early warning system is a tool to help monitor patients and improve how quickly a patient experiencing a sudden decline receives clinical care. The scoring is based on:

- Respiratory rate
- Heart rate
- Systolic blood pressure
- Conscious level
- Temperature
- Hourly urine output (for previous 2 hours)

Antibiotic Use

It is intended that subjects should not have an infection at the time of enrollment. Additionally, subjects should not be on a known course of antibiotics or antimicrobials within the seven days prior to hospital admission to be eligible for study participation.

Subsequent to enrollment, a subject who is discovered to have received systemic continuous antibiotic therapy within the seven days prior to hospital admission will be considered an infection failure, and will be withdrawn from the study. Perioperative antibiotics administered as routine standard of care are permitted.

Source documents

Source documents are the medical record(s) of a patient who has agreed to participate as a study subject. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification of accuracy and completeness, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico technical departments involved in the clinical trial.

Source data

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.

Infection

Any known or suspected infection, per the discretion of the investigator or attending physician(s) should be documented. If the subject has a known or suspected infection prior to consent/enrollment, the subject will not be eligible for study participation. If the subject has a known or suspected infection after enrollment, the principal findings leading to an infection diagnosis, clinical suspicion notes, shall be documented.

7.0 Data analysis and statistical considerations

7.1 Sample size determination

For trauma patients admitted to an ICU, the mean overall nosocomial infection rate is 6 to 18%. (9, 19, 20) The incidence rate of infection could be greater for all surgical ICU patients. Data collected to date indicate that a sustained decrease in BDV of 2.0‰ or more correlates with the onset of infection when the subject is used as his-her control. The average intra subject standard deviation (SD) across time points in 32 mechanically ventilated subjects previously studied was 1.16‰, regardless of the underlying medical conditions. The inter subject SD is expected to be 1.5‰ at most. Using the BDV measurements during the breath sample monitoring period (breath sample assessments every 4 hours), the expected overall SD of the mean BDV measurements (across time points) is less than 1.5‰.

Furthermore, during the monitoring period, the mean difference in the BDV between subjects who are diagnosed with a nosocomial infection and subjects not diagnosed with an infection is expected to be 2.0‰ or more (primary hypothesis). This difference will be considered to be the minimum difference with clinical importance.

Assuming that 5% of the subjects will have a confirmed nosocomial infection over the monitoring period and an overall standard deviation of 1.5‰, a total sample size of 100 subjects (5 infected and 95 non-infected) is required to detect a minimum clinically-important difference of 2.0‰ with at least 80% power at the two-sided 5% significance level.

If the actual rate of nosocomial infections is 10%, then the proposed total sample size of 100 subjects will provide 98% power to detect a mean difference of 2.0‰. Assuming SD to be 1.5‰ (at most) for the change in BDV before and after the diagnosis of an infection is made, a total sample size of 100 subjects and an infection rate of 5%, a mean decrease of 2.0‰ prior to and after the confirmed infection will be detected with 79% power at the one sided 5% significance level (secondary hypothesis).

If the rate of nosocomial infections is 10%, then the mean decrease of 2.0‰ will be detected with 99% power. The proposed sample size of 100 subjects is sufficient to provide accuracy in estimating the specificity of the BDV analyzer. That is, with a sample size of 100 and assuming that 95% of the subjects will have no confirmed nosocomial infection, the specificity will be estimated with a standard error of less than 5%, and the 95% confidence interval of the estimated specificity will be no greater than 20%.

To account for a missing value rate -- due to study withdrawal (see 5.12 Withdrawal of subjects) or infection failure of up to 10%, the total enrollment target is 110.

The goal sample size of 100 subjects will be achieved through implementation of the protocol at more than one approved study site.

7.2 Analysis plan

Descriptive statistics will be generated. For continuous variables, descriptive statistics may include the number of subjects from whom data was used in the calculation, mean, standard deviation, median, minimum, and maximum; frequencies and percentages may be displayed for categorical data.

A 2-sample t-test will be used to compare the averaged (across time points) BDV between subjects who are diagnosed with an infection, without an infection, with sepsis and without sepsis. A linear mixed effects model with subject specific random effects will be used to compare BDV measurements between infected and non-infected and septic and non-septic subjects. This model will be also used to estimate the intra- and inter-subject variability of the BDV measurements. A paired t-test will be used to compare the BDV measurements prior to and after a diagnosis of infection or sepsis. Fisher's exact test and the Kappa statistic will be used to evaluate the relationship between a positive indication of infection by the BDV and a diagnosis of infection or sepsis in the subsequent 72 hours. Sensitivity, specificity, negative predictive value, positive predictive value and corresponding 95% confidence interval will be calculated.

7.3 Missing data plan

The number of missing samples will be minimized, but some samples will be missed due to clinical care logistics. A complete case analysis will be the primary analysis. An imputation based analysis will be a secondary analysis. Specifically, the last observation carried forward (LOCF) and multiple imputation method based on the Markov Chain Monte Carlo technique will be utilized to perform the imputation based analyses. A sensitivity analysis will be conducted to compare the results of the complete case analysis with the results of the imputation based analysis.

7.4 Interim analysis plan

At each completion of 25 subjects, an interim analysis will be done to ensure subject safety and to assess preliminary positive and negative predictive value of the BDV infection indicator. If at least one subject did not develop an infection during the study period, then additional enrollment will be stopped, and inclusion criteria will be reviewed. Subject safety will be assessed. If any significant risks to subject safety are identified, then additional enrollment will be stopped, and safety considerations will be assessed.

8.0 Risks and benefits of study participation

8.1 Potential risks

This study involves minimal risk to individual subjects. Other than the minor risks related to blood sample acquisition and confidentiality of protected health information, there are no significant risks of participation in this study.

Risks associated with breath sample collection

The isotopic gas analyzer system used in this study, the Isomark Canary, is an investigational medical diagnostic device, exempt from IDE requirements in accordance with 21 CFR as described in Section 13 of this protocol. There will be no increased risk of infection, since for mechanically ventilated subjects gas sampling will be only on the expiratory mechanical ventilator circuit tubing and there will be no break in the ventilator circuit and for non-ventilated subjects gas sampling will be performed by inflating a single-use sample bag. The intervention has no impact on respiratory physiology measurements, because the gas sampling and the analysis are performed in vitro.

Risks associated with blood sample acquisition

Blood may be collected via existing IV or arterial catheter. No IV or arterial catheters will be placed for study purposes. Risk of collecting blood samples can include infection or mild anemia. There is theoretical risk of an embolus resulting from the collection of blood from an arterial catheter, but such occurrences are rare in routine clinical practice.

Risks associated with loss of confidentiality

There is a risk that information recorded about patients who agree to participate as study subjects will be shared with people who would not normally have access to this information.

Unknown risks

This study may involve risks to the subject that are presently unforeseeable. Subjects or their LARs will be informed as soon as possible if any information is discovered that may affect the subject's health, welfare, or decision to be in this study.

8.2 Mitigation of potential risks

Mitigation of risks associated with blood draw

To minimize the risk from blood draws, blood samples will be collected by trained clinical care or study team members. Subjects with low hemoglobin levels will not be required to collect study samples until hemoglobin concentration has increased to greater than or equal to 6.0 g/dL.

Mitigation of risks associated with loss of confidentiality

The confidentiality of study subjects will be protected by using coded subject information and password protected electronic files and by storing study data in locked rooms. Access to electronic files will be limited to the study team. The study sponsor, Isomark, will have access to subject data that is de-identified. Access to identifiable data will be limited to study team members of the study hospital. Any paper records will be maintained in locked rooms, and access to these records will be limited to the study team members.

8.3 Potential benefits

Potential benefit to an individual subject

There is no direct benefit to the subject.

Potential benefit to society

The potential benefits of the research include earlier diagnosis of infections. If this indicator accurately discriminates infection in subjects with SIRS secondary to critical illness, it will be extremely valuable in the diagnosis and follow up of every seriously ill patient.

8.4 Risk vs benefit

There is minimal risk to participating subjects. Participation in this study will not affect subjects' clinical care. There is no anticipated direct benefit to subjects, but potential benefit is that future patients may be afforded a new, more timely and accurate method of detecting onset of serious infection as a result of this study.

9.0 Adverse events and unanticipated problems

9.1 Definitions

Definitions are guided by the International Conference on Harmonization and the United States Code of Federal Regulations [21 CFR § 312.32].

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or study subject who is administered a pharmaceutical product or medical device that does not necessarily have a causal relationship to this treatment or device. An AE could be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of an investigational medical product, whether or not considered related to the investigational medical product. This definition includes illnesses or injuries, and exacerbations of pre-existing conditions.

Serious adverse event (SAE)

A serious adverse event (SAE) is any untoward medical occurrence that:

- results in death
- is life threatening (i.e. the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred)
- requires or prolongs hospitalization
- results in persistent or significant disability or incapacity (i.e. the event causes a substantial disruption of a person's ability to conduct normal life functions)
- causes a congenital anomaly or birth defect, or
- is an important and significant medical event (e.g. allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or development of drug dependency or drug abuse) that, based upon appropriate medical judgment, may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes defining SAE.

Unanticipated adverse device event (UADE)

An unanticipated adverse device event (UADE) is any serious adverse effect on health or safety, any life threatening problem or death caused by or associated with a medical 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 a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety or welfare of clinical trial subjects.

9.2 Severity assessment

The PI for the study site and study's independent medical monitors will determine the severity of each AE using the following terms and definitions:

Mild: An AE that was transient and required no special treatment, and did not interfere with usual or normal activities

Moderate: An AE that interfered with usual or normal activities but was ameliorated by therapeutic measures

Severe: An AE that was intense or debilitating, and which interfered with usual or normal activities. Recovery was aided by therapeutic measures. Discontinuation of investigational medical product administration or use might have been required.

Life threatening: An AE that may result in immediate risk of death from the event as it occurred and requires immediate intervention to prevent the outcome of death (This AE will be recorded as a SAE).

Death: An AE that results in death of the subject (This AE will be recorded as a SAE).

ICU subjects are expected to have SAEs related to their medical condition. Those expected SAEs associated with the underlying disease are to be reported in an expedited manner if the PI or co-investigator of the study site is unable to rule out the involvement of the Canary. SAEs listed below are expected and will be recorded in the study record, but will not be reported in an expedited manner to Isomark:

urinary tract infection; acute kidney injury; acute renal failure; acute lung injury; adult respiratory distress syndrome; hypotension; metabolic acidosis; hypothermia; coagulation dysfunction; decreased platelet count; diffuse intravascular coagulation; thromboembolic event (pulmonary embolus; deep vein thrombosis; cerebral infarction); fat embolism; myocardial infarction; decreased cardiac output with cardiac arrest; hepatic injury; severe sepsis or septic shock; bacteremia; pleural effusion; abdominal compartment syndrome; repeated surgeries; amputation; cardiorespiratory insufficiency

This clinical trial involves ICU patients with comorbidities, surgical, procedural and pharmacologic interventions or severe injuries. The expected mortality rate is approximately 35%. Death of a patient who is a study subject due to his or her injuries or other known medical conditions is not unexpected. Death is not an efficacy endpoint of the study protocol.

9.3 Causality assessment

A study independent medical monitor and study PI will determine the relationship of AEs to this study protocol using the following scale:

- Definite = AE is clearly related to the study procedures
- Probable = AE is likely related to the study procedures
- Possible = AE is possibly related to the study procedures
- Unlikely = AE is doubtfully related to the study procedures
- Unrelated = AE is clearly not related to the study procedures

9.4 Procedures for recording and reporting AEs, SAEs

AEs to be recorded in the study record

AEs that occur after informed consent is obtained and which are determined to be definitely, probably, or possibly related to study procedures through the end of study participation will be recorded. All SAEs will be recorded.

Reporting timeframes

Each AE will be assessed to determine if it meets the criteria for an SAE. If an SAE occurs, expedited reporting will follow IRB policies.

All deaths and other SAEs must be reported by the study site within 24 hours of becoming aware of the event to Isomark, with the exception of SAEs that are specifically attributed to underlying disease.

Role of Isomark

Isomark will report to the investigators of the study site(s) all SAEs that are unexpected and associated with use of the Isomark Canary, or UADEs.

Isomark assumes responsibility for appropriate reporting of SAEs to regulatory agencies. Sites should follow their individual reporting guidelines to their local regulatory agencies.

The PI, the study independent medical monitor, and the sponsor will be the primary data and safety advisory group for the study.

The study will include interim review of actual and projected subject accrual rates, subject demographics, and a number and severity of AEs. The PI, the study independent medical monitor, and the sponsor will collaboratively determine actions of continuation, modification, or termination of the study.

10.0 Data Monitoring

The PI, the study independent medical monitor, and the sponsor will be the primary data and safety advisory group for the study.

The study will include interim review of actual and projected subject accrual rates, subject demographics, and a number and severity of AEs. The PI, the study independent medical monitor, and the sponsor will collaboratively

determine actions of continuation, modification, or termination of the study. Refer to Isomark's Data Management Plan (DMP) for additional details.

10.1 Endpoint assessment, adjudication

An endpoint assessment and adjudication committee (EAC) of three clinicians will review study recorded infections and sepsis diagnoses to determine accuracy of protocol specified criteria and to determine and develop criteria for BDV clinical use. None of the EAC will have been directly involved with care of the patient who is a study subject. Information to be reviewed may include clinical laboratory, pathology or imaging data, autopsy reports, physical descriptions, and other data deemed relevant. The EAC will be blinded to BDVs, and will not know if BDV changed for a particular subject. EAC members will receive de-identified study records, and will not receive study subject PHI.

Each EAC member will independently review study subjects' data to determine their infection status. The decision of the subject's infection status will be recorded in the EAC Infection Status case report form. The EAC's charter will outline their role as an EAC member, guidelines for completing the Infection Status case report form, and definitions that should be utilized and followed for the purposes of this study protocol.

11.0 Administrative requirements

11.1 Data record and management

Study team will be provided with secure access to and training using Overture Electronic Data Capture (EDC) System to assure accurate, error free data acquisition for each study subject. A specific data set will be recorded and completed for each study subject. The PI is responsible to ensure the accuracy, completeness, and timeliness of the data recorded. The PI or designated representative shall complete the data set using Overture within 2 calendar weeks after the information is available and the subject has completed the study.

Overture is a clinical trial database software that will provide the electronic data management system for this study to acquire, store, edit, manage, and export study data for analysis.

The study team will manage study data with Overture. Access to Overture is restricted to those who have been provided access by the database administrators. User access requires supervisor approval, completion of HIPAA and Human Subjects Protection training, and completion of role based training in Overture. Users' access is limited to protocols for which they have responsibility for protocol, subject, or data management. Within those protocols, the ability to view and modify data is restricted based on the user's location and role in the conduct of the research project (e.g. sponsor staff does not have the privilege to view identifiable subject information).

The technical components of Overture are managed by Isomark administrative staff. Information Technology team (for server maintenance, software upgrades, etc.), security and software support is provided by Isomark LLC (sponsor).

All communication between the users and Overture occurs via a secure internet browser with individually assigned user controls. This ensures that data passing between the client and Overture is protected from unauthorized data access. Overture fully supports compliance with 21 CFR Part 11.

Overture is hosted on a physically and logically secured COTS (commercial off the shelf) server running Microsoft SQL Server software. Data is exported from Overture with indirect identifiers (i.e. with subject number) for statistical and data monitoring purposes in an MS Excel, SAS, or similar analysis program format. At study completion, all data will be exported and data within Overture will be eliminated.

11.2 Ethical considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 812 and International Conference on Harmonization guidelines), applicable government regulations, and institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator, and a copy of this decision will be provided to the sponsor before commencement of this study.

All types of reportable events (non-compliance, UADEs, SAEs) will be recorded and reported following IRB policies.

All subjects for this study will be given a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the governing IRB for the study. The formal consent of a subject, using the IRB approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and the investigator designated research professional obtaining the consent.

11.3 Subject confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). All subjects will sign an informed consent document and HIPAA authorization that names specific privacy and confidentiality rights. Study data will be maintained per Federal and State data policies.

Protected Health Information (PHI) may not be disclosed to the sponsor, Isomark, LLC. To protect subject privacy, data provided to Isomark will be de-identified with the exception of dates and times. Please refer to the informed consent form for this study for further details specifying use of PHI in this study.

11.4 Data quality and management

Monitoring of this study will be conducted by the sponsor and independent clinical monitors. Study monitoring activities will include: (1) a site qualification visit; (2) a study startup meeting, referred to as the Site Initiation Visit; (3) interim monitoring visits to directly review study materials (regulatory files, consent forms, case report forms, device accountability records); (4) a final study close out visit. Monitoring plan details are outlined in Isomark's Clinical Monitoring Plan.

The research personnel based at the study site, assigned clinical study monitors and Isomark, LLC will complete Site Initiation Visit procedures after IRB approval and before enrollment of any subjects in the study. The Site Initiation Visit will include a summary of all training, monitoring activities and expectations during the study.

Interim monitoring activities will utilize the risk based monitoring approach described in the FDA draft guidance document. Following this approach, data directly supporting the primary analysis of this study will be 100% monitored and 25% of the remaining data for all subjects will be reviewed. Data from additional subjects may be reviewed if significant deficiencies are identified.

11.5 Source documents

Source documents will contain source data for the study. Specified source data will be recorded in the Overture EDC by manual transcription or other automated method that may become available. The study subject data set specifies the data to be recorded for each subject.

11.6 Subject study data set

If a datum is not applicable to the individual subject case, then this will be documented as 'N/A'.

Baseline Data:

- Informed consent obtained
 - NOTE: The study team will assess the capacity of each potential subject to consent daily and at every study encounter until capacity for consent is regained or until the subject is discharged from the study hospital.
- Demographic data
 - NOTE: The study team will verify SOC pregnancy test is negative for women of childbearing potential.
- Infections Signs and Symptoms (SIRS and MEWS data)
- Medical history and current clinical diagnoses
- Medical record abstraction, including but not limited to:
 - smoking history
 - vital signs
 - clinical laboratory analyses
 - concomitant medications

ICU/IMC Study Days:

- Assess for capacity to consent
- Breath samples
- Blood sample collection
- Vital signs
- Urine output
- Record standard of care lab values (e.g. CBC, bacterial cultures, PCT,etc)
- Diagnostic imaging
- Catheters, drains, lines
- Bedside procedures, interventions
- Surgical procedures
- Medications
- Nutrition status, support
- Mechanical ventilatory support
- Duration of ICU/hospital stay
- Medical history/diagnoses
- Adverse events

Post ICU/IMC Study Days:

- Assess for capacity to consent
- Readmission information
- Vital signs
- Urine output

- Record standard of care lab values (e.g. CBC, bacterial cultures, PCT,etc)
- Diagnostic imaging
- Catheters, drains, lines
- Bedside procedures, interventions
- Surgical procedures
- Medications
- Nutrition status, support
- Mechanical ventilatory support
- Duration of ICU/hospital stay
- Medical history/diagnoses
- Adverse events

Antibiotic Use Follow-up Days:

- Assess for capacity to consent
- Surgical procedures
- Bedside procedures, interventions
- Diagnostic imaging
- Standard of care lab values (e.g. CBC, bacterial cultures, PCT, etc.)
- Antibiotic information

11.7 Record retention

All study data will be retained by Isomark and the study site for a minimum of seven years after completion of the study. After seven years, paper documents will be destroyed. Electronic data will be deleted.

11.8 Investigator compliance

The investigator study team, i.e. study site and Isomark LLC, will conduct the trial in compliance with the protocol approved by the IRB. Changes to the protocol will require written IRB approval prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects.

11.9 Subject cost and payment

Cost

Subjects will not incur costs due to their participation in this study. Laboratory testing done as part of the study will be paid by the sponsor, Isomark, LLC.

Payment

Subjects will not be paid for participation in this study.

11.10 Quality Control

Study personnel will complete sample collection and sample analysis records to ensure proper sample collection analysis and data reporting for each subject enrolled.

- When a new subject is enrolled in the study, the study team member will label and complete the sample collection records with the subject's study number.
- The study team member will notify Isomark personnel by email that a new subject is enrolled.
- At each subsequent breath collection time, a study team member will label the breath sampling equipment with the subject study number, site number, date and time when each sample was collected. The study team member will complete and initial the relative collection records within the Case Report Form (CRF). If the sample is

obtained late, then the reason will be documented, e.g. subject in MRI. Each breath sample will be collected as close as possible and not exceeding one hour prior to or after the designated time for the sample collection.

- At each blood collection time, a study team member will label blood sampling equipment with collection date and time. Isomark will provide pre-printed labels with each subject's study number. The study team member will complete and initial the blood sample collection record, accounting for the time of collection, time of spinning, and time of freezing. The total time between collection and freezing cannot exceed four hours. If a sample is not collected on a given day, it will be considered missing data.
- A study team member will collect a breath sample every four hours and a blood sample every 24 hours for the duration that the subject is in the ICU or IMC and initial the sample collection records at each time-point.
- Collected breath samples and the original copies of the corresponding sample collection records will be shipped to Isomark within 48 hours of final collection from a subject via a shipping service, such as UPS or FedEx, to Isomark's breath analysis lab. If multiple subjects are enrolled, then shipments will be made once per week.
- Blood samples and the original copies of the corresponding blood sample collection records will be placed in an Isomark-provided container and shipped to Isomark's contracted reference lab via an Isomark-approved carrier. The blood samples are processed by the reference lab and the results are sent to the sites, and will need to be entered into the EDC by the site staff. After the results have been successfully received, the lab will dispose of any remaining blood samples.
- Each time breath samples are received, Isomark personnel will check the samples for complete label information and compare the samples to the EDC, noting any anomaly. Isomark personnel will then update the corresponding sample check-in record with sample arrival, inspection, and condition information, dating and initialing where instructed.
- At the beginning of each day on which samples are analyzed, the sample analysis technician will ensure the Canary is within calibration specifications by analyzing a standard sample of known isotopic composition and initial the sample analysis record.
- Breath samples are to be analyzed within 21 days of the time of collection. Isomark personnel will note the time and date of sample analysis on the sample analysis record and initial.
- Data will be recorded on the sample analysis record and initialed by the technician.
- After breath sample analysis is complete, the technician will ensure the remaining breath is exhausted (by expelling all remaining sample from the sample bag) or destroyed (by disposal as biomedical waste in the case of plasma samples) and will initial the sample analysis record.
- After breath analysis is complete, Isomark personnel will enter the results into the EDC.

11.11 Funding source

This study is sponsored by Isomark, LLC.

12.0 FDA Investigational Device Exemption (IDE) information

This study will be conducted in accordance with the regulations outlined in 21CFR812.

- a. As per §812.2(c)(3), the study involves a diagnostic system (see below) which is itself non-invasive, does not require invasive sampling, does not introduce energy into the subject, and requires the confirmation of the presence or lack of SIRS via standard physical examination and laboratory testing (white blood count and differential). As such, this study/this device is deemed IDE exempt.
 - i. The physical devices used in this study consist of:
 - commercially-available ventilator tubing and adaptors
 - gas-tight bags
 - Isomark isotopic carbon ratiometer for performing the carbon isotope ratio diagnostic measurement
 - Pee Dee Belemnite as a reference gas sample.

Each of these is used in accordance with its design intent, is legally owned by the sponsor, Isomark LLC, and does not require any additional labeling or special shipping controls. For purposes of complying with §812.5, computers with which applicable analysis software is used will be labeled 'Caution: Investigational Device. Limited by Federal Regulations to Investigational Use'.

- b. This study is being reviewed by Western IRB (WIRB) or the site specific local IRB.
- c. For this study, the PI will maintain records of this study as per § 812.140.

13.0 References

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