UNIVERSITY OF NEW MEXICO

CLINICAL STUDY PROTOCOL

Compound Name:	^{13}C-Urea solution for nebulization
Protocol Number:	16-327 Amendment **#6**
Protocol Title:	A Phase 1, open-label, evaluation of a ^{13}C-urea breath test for the detection of urease-producing bacteria in patients with pneumonia in the emergency department.
Date of Protocol:	20 April 2018
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Co-Investigator:	Jon Femling, MD, PhD Department of Emergency Medicine Assistant Professor Emergency Medicine University of New Mexico Email: JFemling@salud.unm.edu
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INVESTIGATOR'S AGREEMENT

By signing below, I confirm that I have read this protocol and agree

- to assume responsibility for the proper conduct of the study at this site
- to conduct the study according to the procedures described in this protocol and any future amendments
- not to implement any deviation from, or changes to, the protocol without written approval from the Institutional Review Board or Independent Ethics Committee, except where necessary to eliminate an immediate hazard to subject(s)
- that I am aware of and will comply with all applicable regulations and guidelines

_____ _____

Investigator's Signature **Date**

_____

Investigator's Name (Print)

_____

Investigator's Title (Print)

Site Address: _____

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SYNOPSIS (PAGE 1 OF 4)

Name of Sponsor/Company: Justin T Baca, MD, PhD University of New Mexico	**Protocol Number:** 16-327
Name of Study Drug/Device: ^{13}C-Urea solution for nebulization	**Protocol Title:** A Phase 1, open-label, evaluation of a ^{13}C-urea breath test for the detection of urease-producing bacteria in patients with pneumonia in the emergency department.
Name of Active Ingredient: ^{13}C-urea	**Phase of Development:** 1

Objective: The primary objective of this study is to determine the ability of a ^{13}C-urea breath test to detect urease producing pathogens in patients with pneumonia as confirmed on sputum culture results.

Additional objectives of this study will include:

- Determine optimal times for post-nebulization breath collection
- Evaluate the safety and tolerability of the ^{13}C-urea breath test in patients with pneumonia

Methodology:

This is a Phase 1, open-label evaluation of an inhaled ^{13}C-urea breath test in the identification of urease positive bacteria in patients diagnosed with pneumonia in the emergency department (ED). This study will enroll up to 75 adult male and female subjects at two US sites in two cohorts.

Adult subjects will be screened for participation in two dosing cohorts to be enrolled sequentially. Cohort A will enroll 15 subjects presenting to the study sites with pneumonia symptomatology that is planned for outpatient treatment. Following enrollment and review of the safety of dosing in Cohort A, subjects receiving a diagnosis of pneumonia in the ED that are planned for admission will be screened for enrollment in Cohort B. Eligible subjects will provide informed consent, medical history, and samples for laboratory testing (including pregnancy testing for females of childbearing potential) during screening. Subjects will be evaluated for their CURB-65 Pneumonia Severity Score, and Community-Acquired Pneumonia Severity Index (PSI) where required data are available.

Prior to breath test administration, Cohort B subjects will provide a oropharyngeal swab for bacterial identification via next generation sequencing (NGS) plus a sputum sample for bacterial culture and NGS; the sputum sample may be induced, or collected as part of initial ED workup if appropriate culture has been ordered. Subjects will undergo breath test with collection of baseline breath samples (up to 6), followed by complete nebulization of ^{13}C-urea solution. Immediately following the end of nebulization, breath collection bag samples will be collected at up to 6 time points with at least 1 minute intervals within 10 minutes post-nebulization. Exhaled breath bags will be centrally processed to determine the change in exhaled $^{13}CO_2$ levels. Vital signs, including resting blood pressure, resting pulse, respiratory rate, peripheral oxygen saturation, and temperature will be collected prior to, and following completion of, nebulization treatment. In the event of bronchospasm, during or following nebulization, albuterol rescue will be available for treatment at the discretion of the investigator.

Once required tests are complete, the subject will be treated/followed according to standard institutional protocol/practice. The subjects' clinical course will be followed for at least 24 hours, where possible, to document the final diagnosis and outcome.

SYNOPSIS (PAGE 2 OF 4)

Name of Sponsor/Company: Justin T Baca, MD, PhD University of New Mexico	**Protocol Number:** 16-327
Name of Study Drug/Device: ^{13}C-Urea solution for nebulization	**Protocol Title:** A Phase 1, open-label, evaluation of a ^{13}C-urea breath test for the detection of urease-producing bacteria in patients with pneumonia in the emergency dept.
Name of Active Ingredient: ^{13}C-urea	**Phase of Development:** 1
Number of subjects to be enrolled: This study will enroll up to 75 adult male and female subjects in two dosing cohorts.	
Number of study sites: 2	**Study country location**: United States

Criteria for inclusion: Subjects must meet all of the following criteria to be considered eligible for the study:

Cohort A Only:

1. be a man or woman age 18-70, inclusive
2. have suspected bacterial pneumonia on presentation to the study site based on clinical signs and symptoms
3. be expected to be treated on an outpatient basis in the opinion of the treating clinician at time of enrollment OR a definitive decision to admit has not been made, and the subject meets ALL of the following criteria; Pulse <125 beats per minute, Systolic blood pressure ≥100 mmHg, Respiratory rate ≤24 breaths per minute, Temperature >35C, and Temperature <40C. Subjects who are expected to be placed in an ED observation unit are eligible if they meet all of the defined vital sign criteria

Cohort B Only:

4. be a man or woman age 18-85, inclusive,
5. have a diagnosis of suspected bacterial pneumonia on presentation to the study site based on findings of a positive chest x-ray or advanced radiology and clinical signs and symptoms
6. be planned for admission to the hospital ward/floor in the opinion of the treating clinician at time of enrollment
7. be capable of providing a spontaneous or induced sputum for analysis

Both Cohorts A and B:

8. be capable of completing the breath test according to the clinical judgement of the investigator
9. be able to understand the study procedures, agree to participate in the study program, and voluntarily provide written informed consent

Criteria for exclusion: Subjects who meet any of the following criteria will be excluded from the study:

1. have a known allergy to urea or any excipient in the nebulized solution
2. be pregnant or have a positive urine pregnancy test
3. have evidence of active oral infection, such as abscess or dense exudate, that requires antibiotic therapy
4. have known diagnosis of cystic fibrosis or bronchiectasis
5. have a known or suspected acute asthma exacerbation on presentation
6. have received treatment with oral or intravenous (IV) antibiotics in the preceding 2 days prior to screening, unless antibiotic failure is suspected

   OR

   Cohort A: have received treatment with oral or IV antibiotics greater than 6 hours prior to breath test

   Cohort B: have received treatment with oral or IV antibiotics greater than 4 hours prior to breath test
7. have an acute illness or other condition that, as determined by the investigator, would preclude participation in the study

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Name of Sponsor/Company: Justin T Baca, MD, PhD University of New Mexico	**Protocol Number:** 16-327
Name of Study Drug/Device: ^{13}C-Urea solution for nebulization	**Protocol Title:** A Phase 1, open-label, evaluation of a ^{13}C-urea breath test for the detection of urease-producing bacteria in patients with pneumonia in the emergency department.
Name of Active Ingredient: ^{13}C-urea	**Phase of Development:** 1
Investigational product: ^{13}C-urea solution for nebulized administration	
Reference therapy: NA	
Duration of treatment: Enrolled subjects will receive a single nebulized dose of ^{13}C-urea with subsequent breath collection. Subjects will complete all study evaluations during a single ED visit.	

Criteria For Evaluation

Efficacy:

Efficacy endpoints will include the following:

- Evaluate the exhaled $^{13}CO_2$ levels at each time point post-nebulization
- Evaluate the relationship between the exhaled $^{13}CO_2$ levels at each time point and subject's causative pathogen based upon sputum culture or NGS
- Evaluate the sensitivity and specificity of the ^{13}C-urea breath test for urease producing pathogens
- Evaluate the positive and negative predictive value of the ^{13}C-urea breath test for urease producing pathogens
- Correlation of the ^{13}C-urea breath test with CURB65 in subjects with sputum cultures positive for urease pathogens
- Correlation of the ^{13}C-urea breath test with PSI in patients with sputum cultures positive for urease pathogens
- Univariate association of all study variables with urease pathogen caused pneumonia
- Multi-variate logistic regression model for urease pathogen caused pneumonia utilizing study variables that are significant in univariate analysis to compute odds ratio for each model variable and overall explanation of variance by model

Safety:

Safety endpoints will include:

- Incidence of adverse events (AEs)
- Incidence of death, transfer to ICU, or intubation

SYNOPSIS (PAGE 4 OF 4)

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Statistical methods:

Sample size determination:

The sample size for this study was selected based on prior exposures and existing safety data. Descriptive statistical analysis of results are planned as findings from this study will be used in order to appropriately determine the sample size requirements for future definitive evaluations of inhaled ^{13}C-urea in an adequately powered study of efficacy in this population.

Study populations:

Intent-to-Treat (ITT) Analysis Set: The ITT set will include all subjects who receive nebulized ^{13}C-urea. The ITT analysis set is also referenced as the Safety Analysis Set. Safety analysis will be performed using the ITT set.

Modified Intent-to-Treat (mITT) Analysis Set: The mITT subjects include all ITT subjects who provide at least one post-dose breath sample. Efficacy analysis will be performed on the mITT analysis set.

Efficacy analysis:

Breath test exhaled $^{13}CO_2$ results at each time point will be presented for the overall population for determination of optimal breath sampling time. Relationship between the exhaled $^{13}CO_2$ at each time and subject's pneumonia diagnosis and pathogen (determined by sputum culture or NGS) will be examined. The time point where exhaled $^{13}CO_2$ can reliably predict the presence of urease positive pathogen in subjects with pneumonia diagnosis will be considered as the optimal time for ^{13}C-urea breath test sampling.

The proportion of subjects with breath test positive/negative determination will be compared to sputum culture or NGS results when available. Correlation analyses will be performed where sufficient data are available.

Safety analysis:

The Medical Dictionary for Regulatory Activities (Version 18 or higher) will be used to classify all AEs with respect to system organ class and preferred term. AEs will be summarized for the safety analysis set.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
BMI	body mass index
BP	Blood Pressure
CFR	(United States) Code of Federal Regulations
ºC	degrees Centigrade
CRF	case report form
ED	Emergency department
ºF	degrees Fahrenheit
G	Gram
GCP	Good Clinical Practice
GMP	Global Medication Performance
H	Hour
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	Intravenous
Kg	Kilogram
m^2	square meters
Mg	Milligram
mL	Milliliter
NF	National Formulary
Ng	Nanogram
pH	negative log of hydrogen ion concentration
SAR	suspected adverse reaction
SAE	serious adverse event
US	United States
USP	*United States Pharmacopeia*

1. INTRODUCTION

Rapid identification of respiratory pathogens remains a global concern affecting initiation of therapy, and pharmacotherapy selection. The lack of rapid point of care tests that can provide clinicians with some indication of which pathogen(s) is causing disease may lead to selection of inappropriate therapy, in particular to overuse of broad spectrum antibiotics.

Urease producing organisms represent a virulent set of pathogens which may be present in respiratory infections including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Identification of the presence or absence of urease activity may allow clinicians to narrow the spectrum of suspected infections, confirming the need for antimicrobial therapy and influencing therapy selection.

The breath test is based upon a modification of existing breath testing technology that detects and monitors a biomarker associated with peptic ulcers. The mechanism of action for detecting urease-containing bacteria in the lungs is the same as for detecting another urease-containing bacterium, *Helicobacter pylori*, in the stomachs of patients with peptic ulcers.

For the intended indication, inhaled ^{13}C-urea will be catalytically hydrolyzed into ^{13}C-carbon dioxide ($^{13}CO_2$) if urease is present in the bacterial pathogens causing the pneumonia (**I**).

$$^{13}CO(NH_2)_2 + 3H_2O \xrightarrow{\text{urease}} {}^{13}CO_2 + 2NH_4OH \qquad \textbf{(I)}$$

CO_2 contains predominantly ^{12}C but a small amount of ^{13}C is present. Since the urea is produced with ^{13}C-urea all CO_2 generated by hydrolysis with urease will be $^{13}CO_2$. By measuring the ratio of $^{13}CO_2/^{12}CO_2$ in exhaled breath samples before and after inhalation of ^{13}C-urea, the presence of urease-containing bacteria can be detected. In the absence of urease-containing bacteria in the lungs, the isotopic ratio of ^{13}C /^{12}C in the baseline and test breath samples will be approximately the same. If urease-containing bacteria are present within the lungs, the concentration of exhaled $^{13}CO_2$ will be increased measurably in the test breath sample relative to baseline. It is anticipated that urease-producing bacteria can be detected within 5 minutes of initiating the breath test.

The University of New Mexico conducted a proof of concept study of the ^{13}C-urea Breath Test in 3 normal volunteers and 3 Cystic Fibrosis (CF) patients with known pseudomonas colonization. Two doses of isotonic ^{13}C-urea (20 mg and 50 mg) were used. All doses were tolerated well with no significant AEs. The exhaled $^{13}CO_2$ was higher in the CF subjects with both doses but separation was better with the 50 mg dose. In this study the first breath samples were taken at 5 minutes after ^{13}C-urea nebulization and results rapidly declined in the next samples. These results suggest that breath sampling should be started sooner after the completion of nebulization of the ^{13}C-urea

Mycobacteria tuberculosis contains urease and ^{13}C-urea breath test was shown to detect TB in a rabbit model. Subsequently a study has been initiated in patients with suspected TB infection in South Africa. This study has evaluated the administration of a 50 mg nebulized dose of ^{13}C-urea with subsequent breath collection and analysis for exhaled $^{13}CO_2$ concentrations. This study has administered doses of ^{13}C-urea to over 100 subjects for TB screening. Doses were well tolerated

with a low incidence of mild AEs and no nebulization treatments requiring premature discontinuation.

The current study is proposed to expand the experience of ^{13}C-urea testing in patients with a diagnosis of pneumonia in the ED. This study will evaluate the tolerability of dosing in this population, as well as the correlation of testing results with bacterial culture observations.

2. STUDY OBJECTIVE

The primary objective of this study is to determine the ability of the ^{13}C-urea breath test to detect urease producing pathogens in patients with pneumonia as confirmed on sputum culture results.

Additional objectives of this study will include:

- Determine optimal times for post-nebulization breath collection
- Evaluate the safety and tolerability of the ^{13}C-urea breath test in patients with pneumonia

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

This is a Phase 1, open-label evaluation of an inhaled ^{13}C-urea breath test in the identification of urease positive bacteria in patients diagnosed with pneumonia in the emergency department (ED). This study will enroll up to 75 adult male and female subjects at two US sites in two cohorts.

Adult subjects will be screened for participation in two dosing cohorts to be enrolled sequentially. Cohort A will enroll 15 subjects presenting to the study sites with pneumonia symptomatology that is planned for outpatient treatment. Following enrollment and review of the safety of dosing in Cohort A, subjects receiving a diagnosis of pneumonia in the ED that are planned for admission will be screened for enrollment in Cohort B. Eligible subjects will provide informed consent, medical history, and samples for laboratory testing (including pregnancy testing for females of childbearing potential) during screening. Subjects will be evaluated for their CURB-65 Pneumonia Severity Score, and Community-Acquired Pneumonia Severity Index (PSI) where required data are available.

Prior to breath test administration, Cohort B subjects will provide a sputum sample for bacterial culture; sample may be induced, or collected as part of initial ED workup if appropriate culture has been ordered. Subjects will undergo breath test with collection of baseline breath samples (up to 6), followed by complete nebulization of ^{13}C-urea solution. Immediately following the end of nebulization, breath collection bag samples will be collected at up to 6 time points with at least 1 minute intervals within 10 minutes post-nebulization. Exhaled breath bags will be centrally processed to determine the change in exhaled $^{13}CO_2$ levels. Vital signs, including resting blood pressure, resting pulse, respiratory rate, peripheral oxygen saturation, and temperature will be collected prior to, and following completion of, nebulization treatment. In the event of bronchospasm, during or following nebulization, albuterol rescue will be available for treatment at the discretion of the investigator.

After administration of nebulized ^{13}C-urea solution, subjects will be monitored at the study site for any adverse events. Adverse events directly related to nebulizer administration would be expected to present within minutes of nebulization. Evidence shows that, generally, symptoms of anaphylaxis appear between 1-15 minutes after exposure to an agent. Symptoms of anaphylaxis may present as lightheadedness, dizziness, difficulty breathing, wheezing, hives, swelling under the skin, rashes, and/or nausea. The study team will monitor subjects for 20 minutes after nebulization for any adverse events. All subjects will receive standard care in the hospital setting prior to and after completion of study procedures. Subjects will sign and be given a copy of the consent form which has contact information and instructions that they should return to the emergency department if they suspect they are suffering from any symptoms related to the nebulization. The instructions in the consent form also specify that, if returning to the emergency department at the corresponding study site with symptoms they believe are due to the study, they should bring the consent form with them to the ED and ask that the study site Principal Investigator be immediately called and notified on their return. In Cohort A, all safety observations prior to discharge, and in Cohort B the clinical course over the hospital admission,

will be reviewed and submitted to the safety monitoring committee as described below in Sections 7 and 8.

Once required tests are complete, the subject will be treated/followed according to standard institutional protocol/practice. The subjects' clinical course will be followed for at least 24 hours, where possible, to document the final diagnosis and outcome.

3.2. Rationale for Study Design and Control Groups

This study will evaluate the safety and accuracy of the use of the ^{13}C-urea breath test in identifying urease positive organisms in newly diagnosed cases of pneumonia in the ED. Urease producing organisms represent a virulent set of pathogens which may be present in respiratory infections including *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Staphylococcus aureus*. Use of the ^{13}C-urea breath test may allow for rapid determination of bacteria presence that could provide guidance to future antibiotic selection. In this study, the ^{13}C-urea breath test observations will be compared to bacterial identification upon sputum culture.

3.3. Clinical Study Sites

There are two clinical sites for this study:

- Site 01 = University of New Mexico Health Sciences Center, 2211 Lomas Blvd NE, Albuquerque, NM
    - Subject recruiting done at UNM Sandoval Regional Medical Center and Main Emergency Departments (Cohort A, B) and co-located Urgent Care clinic (Cohort A only)
    - Study procedures done in exam room at ED (Cohort A, B) or adjacent Clinical & Translational Sciences Center (Cohort A only, ~100 yards from ED/Urgent Care Clinic)
- Site 02 = Henry Ford Hospital, 2799 W. Grand Blvd, Detroit, MI
    - Subject recruiting done at ED (Cohort A, B)
    - Study procedures done in ED exam room (Cohort A, B)

4. STUDY POPULATION

4.1. Inclusion Criteria

Subjects must meet all of the following criteria to be considered eligible to participate in the study:

Cohort A Only:

1. be a man or woman age 18-70, inclusive
2. have suspected bacterial pneumonia on presentation to the study site based on clinical signs and symptoms
3. be expected to be treated on an outpatient basis in the opinion of the treating clinician at the time of enrollment OR a definitive decision to admit has not been made, and the subject meets ALL of the following criteria; Pulse <125 beats per minute, Systolic blood pressure ≥100 mmHg, Respiratory rate ≤24 breaths per minute, Temperature >35C, and Temperature <40C. Subjects who are expected to be placed in an ED observation unit are eligible if they meet all of the defined vital sign criteria

Cohort B Only:

4. be a man or woman age 18-85, inclusive,
5. have a diagnosis of suspected bacterial pneumonia on presentation to the study site based on findings of a positive chest x-ray or advanced radiology and clinical signs and symptoms
6. be planned for admission to the hospital ward/floor in the opinion of the treating clinician at the time of enrollment
7. be capable of providing a spontaneous or induced sputum for analysis

Both Cohorts A and B:

8. be capable of completing the breath test according to the clinical judgement of the investigator
9. be able to understand the study procedures, agree to participate in the study program, and voluntarily provide written informed consent

4.2. Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from participating in the study:

1. have a known allergy to urea or any excipient in the nebulized solution
2. be pregnant or have a positive urine pregnancy test
3. have evidence of active oral infection, such as abscess or dense exudate, that requires antibiotic therapy
4. have known diagnosis of cystic fibrosis or bronchiectasis
5. have a known or suspected acute asthma exacerbation on presentation
6. have received treatment with oral or intravenous (IV) antibiotics in the preceding 2 days prior to screening, unless antibiotic failure is suspected

OR

Cohort A Only:

have received treatment with oral or IV antibiotics for > 6 hours prior to breath test

Cohort B Only:

have received treatment with oral or IV antibiotics for > 4 hours prior to breath test

7. have an acute illness or other condition that, as determined by the investigator, would preclude participation in the study

4.3. Discontinuation of Subjects

4.3.1. Procedures for Withdrawal

A subject may be discontinued from the study by the investigator at any time if either determines that it is not in the subject's best interest to continue. Subjects who withdraw consent or who are discontinued from the study before completing all protocol-specified assessments should be evaluated by the investigator before leaving the hospital. The date and the primary reason for discontinuation will be recorded on the subject's case report form (CRF).

4.3.2. Replacement of Subjects

Subjects who do not complete ^{13}C-urea nebulization and all scheduled breath sample collection time points may be replaced in this study.

4.4. Lifestyle Guidelines

4.4.1. Confinement

All study related procedures will be performed in the ED or following hospital admission in a single visit lasting less than approximately four hours. The duration of hospitalization following breath test completion for disease management will be at the discretion of the healthcare facility.

4.4.2. Diet

Subjects will not be allowed food or drinks for 15 minutes before the start of nebulization through completion of the final breath sample.

5. TREATMENTS

5.1. Administration of Study Medication

Administration of ^{13}C-urea solution will be completed using an Aerogen Aeroneb Solo nebulizer device, within 60 minutes following reconstitution with 3 mL of sterile water for injection. The solution will be nebulized until the full dose has been administered, with subjects breathing normally through their mouths using the nebulizer mouthpiece. The start and stop time of nebulization will be recorded in the subject CRF. The time nebulization is completed or discontinued will be considered Time 0 for post-dose breath sample collection.

5.2. Identity of Study Medication

Lyophilized ^{13}C-urea will be provided in 50 mg single-dose vials for reconstitution using 3 mL of sterile water for injection. Inactive ingredients include: Lactose Monohydrate, Tromethamine, and Dilute Hydrochloric Acid (to adjust pH).

5.3. Method of Assigning Subjects to Treatment Groups

All subjects will receive a single 50 mg dose of inhaled ^{13}C-urea.

5.4. Selection of Doses

The dose of ^{13}C-urea has been selected based on clinical use in a subset of subjects. Administration of 50 mg inhaled ^{13}C-urea has been well tolerated in health volunteers and cystic fibrosis patients, while producing detectable levels of $^{13}CO_2$ in subjects with cystic fibrosis with known bacterial infections (Raissy et al., 2016) (IDE #G120190; unpublished). The safety and efficacy of the administered dose of ^{13}C-urea has also been well tolerated in an evaluation of 97 subjects with and without TB infection in an ongoing study conducted by Avisa Pharma in South Africa (Unpublished, Avisa Pharma).

5.5. Selection of Timing of Dose

Administration of ^{13}C-urea will be conducted prior to, or within 4 hours following, initiation of antibiotic therapy in subjects with a pneumonia diagnosis in the ED. In these subjects it is anticipated that adequate infection burden will be present to produce measurable levels of $^{13}CO_2$ upon breath analysis within 10 minutes post-nebulization (Raissy et al., 2016). Timing of optimal post-dose breath collection for $^{13}CO_2$ analysis will be evaluated during this study.

5.6. Blinding and Unblinding of Study Medications

All study participants will receive ^{13}C-urea, no blinding or dummy doses will be utilized in this study.

5.7. Treatment Compliance

Study personnel will oversee administration of ^{13}C-urea while subjects are present in the ED or hospital. The exact date and start and stop times of nebulization will be recorded, as well as the times of post-dose breath collection.

5.8. Drug Accountability

The investigator (or designee) will sign for the investigational product (IP) when it is received. The IP (^{13}C-urea) must be handled and stored as described and administered only to those subjects formally entered into the study.

At the completion of the study, and after reconciliation of all delivery and usage records, any unused IP will be returned or destroyed per written instructions.

5.9. Packaging, Labeling, and Storage

^{13}C-urea will be provided in single use glass vials for reconstitution prior to nebulization. Non-blinded labels will be utilized for this study, to include product identifier, lot number, expiration, and a disclaimer for investigational use.

Prior to reconstitution, ^{13}C-urea will be stored within a range of 15ºC to 30ºC (59ºF to 86ºF). Once reconstituted, ^{13}C-urea solution will be utilized within 60 minutes.

All study medication will be stored in an area with restricted access. A temperature log or chart will be maintained to monitor the environment.

5.10. Prior and Concomitant Medications

All medications taken by subjects within 7 days prior to receiving ^{13}C-urea will be recorded on the CRF. If a medication is started to treat an AE, the medication must be recorded on the Concomitant Medications page of the CRF.

5.11. Concomitant Interventions and Procedures

All interventions or procedures within 7 days prior to receiving the ^{13}C-urea, whether diagnostic or therapeutic, will be recorded on the CRF, along with time, date, and reason for the intervention or procedure. If an intervention or procedure is implemented to treat an AE, the event must be recorded on the AE page of the CRF, along with all relevant information.

6. STUDY PROCEDURES

Schedules of study procedures for overall study assessments and day-of-dosing assessments are provided in Appendix A.

6.1. Efficacy Assessments

6.1.1. Sample Collection

Baseline breath collection will be completed within 30 minutes prior to nebulization using up to six breath collection bags. Sufficient exhaled breaths to fill the bag will be collected at each defined time point. Breath collection will be by direct exhalation into a bag, or by a breath capture device attached to the nebulizer.

Up to six post-nebulization breath samples will be collected within 10 minutes after completing the nebulized dose, at intervals of 1 minute or greater. Breath collection will be by direct exhalation into a bag, or by a breath capture device attached to the nebulizer.

Samples will be collected within ±1 minute of the scheduled post-dose time. Actual times will be recorded for all samples as the time of first collected exhaled breath.

Sputum samples will be collected by trained study personnel per standard clinical practice within 15 minutes of the breath test. In brief, subjects will be asked to take several deep breaths and then produce a forceful cough. The subject will be encouraged to expectorate into a sample cup, and the sputum sample will be sent for culture. If the subject has difficulty producing a sputum sample, nebulized saline may be used to loosen secretions and encourage sputum production.

A oropharyngeal swab will be collected per standard clinical practice, and the sample will be preserved for microbiome analysis. A portion of the collected sputum sample will be preserved for microbiome analysis.

Sample collection will be performed according to GCP in a room in the emergency department or Clinical and Translational Sciences Center (adjacent to UNM-ED, Cohort A only).

6.1.2. Processing of Breath Samples

Breath collection bags will be immediately sealed following collection and labeled with the subject number and collection time point until processed. Breath samples will be centrally processed, and resulting levels will be recorded in the subject CRF.

The breath testing will be performed by Metabolic Solutions, a CLIA accredited laboratory (CLIA ID #30D0970292) at their facility in Nashua, NH. Breath samples from each study subject will be labeled with the de-identified study subject ID, the date and time of collection, and a study ID. The breath samples from a study subject will be shipped overnight via FedEx in an approved and appropriately labeled shipping container. One container is used for each subject and will hold the 300 ml breath bags collected from the subject. Metabolic Solutions will measure the CO_2 concentrations in each bag and also the $^{13}CO_2$ change over baseline for the post-nebulization breath bags. They will also properly dispose of the breath bags after measurement. The Metabolic Solutions measurements will be tied to the de-identified study

subject ID and collection date/time in the subsequent report sent to the PI for entry into the subject's case report form.

6.1.3. Processing of samples for microbiome analysis

A oroopharyngeal swab and a portion of the collected sputum sample will be preserved with a nucleic acid protectant that also neutralizes infectious organisms, such as Zymo Biosciences DNA/RNA shield or equivalent. We will conduct microbial sequencing to determine the presence of microorganisms and their relative abundance. To this end, total nucleic acid (DNA and/or RNA) will be isolated to allow for microbiome analysis by sequencing. Microbiome sequencing will be done at UNM laboratories under direct supervision of the study investigators. Sequencing will be conducted on a next generation sequencing system, such as the Illumina MiSeq sequencer. The microbiome content will be determined through bioinformatic analysis of the sequencing reads with a taxonomic database such as the Greengenes Consortium Database, Kraken database or a similar microbiome classification database. These samples with be labeled with the de-identified study subject ID, the date and time of collection, and a study ID. Raw sequence data generated from the sequencer in the format of a .fastq file and labeled only with the study number and no identifying data may be transferred, stored, and analyzed using web and/or cloud-based solutions.

6.2. Demographic and Baseline Characteristics Assessment

6.2.1. Demographics

Demographics information will be collected during screening visit including age, sex, ethnicity, race, weight, height, BMI. CURB-65 Pneumonia Severity Score, and/or Community-Acquired Pneumonia Severity Index (PSI) may be determined when required data are available.

6.2.2. Medical History

The investigator or designee will document each subject’s medical history during the screening visit. Documentation of medical history should report tobacco use and diagnostic criteria for healthcare associated pneumonia, including hospitalization within 90 days, residence in nursing home or long term care facility, recent visit to hospital or hemodialysis unit, or receipt of IV antibiotics, chemotherapy or wound care within 30 days. When available ED diagnostic chest x-ray should be reported in the medical history and included in the subject file.

6.2.3. Clinical Laboratory Tests

The investigator or designee will document results of laboratory tests collected in the course of routine care during the subject’s hospitalization. These results will be collected on the CRF.

6.2.4. Physical Examination

The investigator or designee will perform a physical examination (HEENT, cardiovascular, respiratory, gastrointestinal, neurological, dermatologic, and musculoskeletal systems) during the screening visit. The oral cavity should be examined for evidence of oral infection (i.e. abscess) as part of the physical examinations (examination by a dentist is not required).

The Principal Investigator may perform a physical examination (the extent of which is determined by the study investigator) at any time during the study if indicated by change in a subject's medical condition.

6.3. Safety Assessments Description

6.3.1. Clinical Laboratory Tests

During screening, subjects will have samples collected for testing as follows:

Sputum:

- Bacterial culture (only required for Cohort B)

Urine

- Pregnancy test (female subjects)

Laboratory tests do not need to be repeated if samples have already been collected as part of the presenting or admission workup for the current ED visit or hospitalization.

6.3.2. Vital Sign Measurements

Resting vital signs will include resting blood pressure, resting pulse, respiratory rate, peripheral oxygen saturation, and temperature; temperature will only be required for vital signs collected for screening. Resting tests must be obtained after resting (seated/supine) for ≥ 5 minutes.

Vital signs including blood pressure, heart rate, respiratory rate and peripheral oxygen saturation will be recorded prior to nebulization, and following completion of sample collection. Subjects will have continuous pulse oximetry monitoring during sample collection and urea nebulization, and any change in oxygen requirement will be recorded.

Actual times will be recorded in the subject CRF.

6.3.3. Follow-Up Contact

After 48 hours post breath test administration, all subjects will receive a follow-up safety contact. The safety contact should be completed between 48 and 96 hours post breath test administration. For subjects who have been discharged, follow-up contact will be performed via telephone contact (up to 3 attempts) by a study investigator or designee. For subjects who remain inpatient at the hospital, the follow-up will be performed in person. Safety follow-up will include a chart review as well as discussion with the subject regarding their treatment course and current status.

6.4. Assessments by Visit

6.4.1. Screening Procedures

Investigators and research staff from the Emergency Department will assist in the identification of subjects through the clinical site Emergency Departments or UNM-HSC Urgent Care clinic (part of the UNM site and located next to the ED, Cohort A only). ED Research staff are trained in the identification and referral of subjects for currently active research studies. Research staff will identify subjects by asking ED staff and providers if they are currently caring for any patients that meet the protocol case description and by evaluating potentially eligible subjects

with in Firstnet (UNM) or EPIC (HFH) who present with a reason for visit that would put them at reasonable suspicion for pneumonia (e.g. possible pneumonia, difficulty breathing, COPD etc.). Research staff will not discuss informed consent with potential study subjects or retain any PHI.

Research staff will not participate in the identification of subjects or any part of this study until they have completed required CITI training and are added as team members on the study.

A waiver of HIPAA authorization may be sought for screening purposes only, so that eligible subjects are evaluated and enrolled as soon as possible after arrival so as to complete study procedures prior to antibiotic administration. The waiver may be necessary for facilitating subject enrollment without interfering with antibiotic administration times.

Subjects meeting the eligibility criteria listed in Section 4 may be enrolled in the study after the nature and purpose of the protocol have been explained to them, and they have voluntarily granted written informed consent to participate. All subjects will undergo screening prior to ^{13}C-urea administration. The following safety-related procedures will be performed as part of screening for all subjects:

- Medical history (Section 6.2.2)
- Physical examination (Section 6.2.3)
- Clinical laboratory testing (Section 6.3.1)
- Vital signs (Section 6.3.2)
- Urine pregnancy testing (Section 6.3.1; where appropriate)
- Measurement of body weight and height and calculation of BMI (Section 6.2.3)

6.4.2. Breath Test Procedures

After successful completion of the screening procedures, subjects will undergo breath test administration. The following assessments will be conducted as part of the breath test procedures for all subjects:

- Breath sample collection (Section 6.1.1)
- ^{13}C-urea administration (Section 5)
- Vital signs (excluding temperature; Section 6.3.2)
- Monitoring of AEs (Section 7)

6.4.3. Follow-Up

All subjects will undergo follow-up 48 hours following breath test administration to include:

- Follow-up Safety Contact (Section 6.3.3)
- Monitoring of AEs (Section 7)

6.5. Appropriateness of Assessments

Study assessments are appropriate to evaluate the objectives of the study while monitoring the safety of ^{13}C-urea administration. Measurement of exhaled $^{13}CO_2$ levels has been widely utilized in the analysis of patients receiving oral ^{13}C-urea in other indications.

6.6. Clinical Stopping Rules & Safety Data Review

This study will be discontinued if it is determined that there is a significant safety risk posed towards study subjects. Potential safety risks will be evaluated on an ongoing basis during the study by the independent medical monitor. Clinical data will be presented to the independent medical monitor in cohorts of subjects as listed in the procedure (Section 8). The independent medical monitor will adjudicate AEs and determine whether enrollment may be continued for the study. Following enrollment of the 15 subjects in Cohort A, enrollment into Cohort B will not begin until the independent medical monitor performs an overall review of safety in the cohort. If the independent medical monitor determines that no safety concerns are present, and that no increased risk is apparent for subjects with advance symptomatology (Cohort B), then enrollment may proceed with the next study Cohort. The independent medical monitor will continue to review safety data in cohorts of subjects, however enrollment may continue during review. In the event an SAE is reported in a subject, enrollment should be halted until the event can be reviewed by the independent medical monitor, and determined whether enrollment should be continued.

7. ADVERSE EVENTS, SERIOUS ADVERSE EVENTS, AND SERIOUS SUSPECTED ADVERSE REACTIONS

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE, SAE, or Serious Suspected Adverse Reaction (SSAR) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

7.1. Definition of an Adverse Event

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE may be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study medication, whether or not considered causally associated with the use of the study medication. Any abnormal laboratory value deemed clinically significant by the investigator, regardless of causal relationship, must be reported as an AE.

Examples of an AE include the following:

- significant or unexpected worsening or exacerbation of the condition or indication under study
- exacerbation of a chronic or intermittent preexisting condition, including either an increase in frequency or intensity of the condition (e.g., abnormal physical examination finding)
- signs, symptoms, or clinical sequelae of a suspected interaction
- signs, symptoms, or clinical sequelae of a suspected overdose of the study medication or a concurrent medication (overdose per se should not be reported as an AE or SAE, unless nonserious or serious sequelae occur)

The following examples are not considered AEs:

- medical or surgical procedure (e.g., endoscopy, appendectomy), although the condition that leads to the procedure is an AE
- anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) (including laboratory values) present or detected at the start of the study that do not worsen
- the disease or disorder being studied, or expected progression, signs, or symptoms of the disease or disorder being studied, unless they become more severe or occur with a greater frequency than expected for the subject's condition

All AEs, whether volunteered, elicited, or noted on physical examination, and regardless of causality or seriousness, will be assessed and recorded on the CRF beginning after administration of study medication through approximately 2 hours after administration. SAEs (see Sections 7.2 and 7.3) will be assessed and recorded after administration of study medication through the last study follow-up (as described in Section 6.4.3).

7.2. Definition of a Serious Adverse Event

An SAE is defined as any event that meets the following criteria:

- It results in death or is life-threatening (i.e., presents an immediate risk of death from the event as it occurred). (This criterion is not intended to include an AE that, had it occurred in a more severe form, might have caused death.)
- It results in persistent or substantial disability or incapacitation. (This criterion is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, diarrhea, or sprained ankle.)
- It results in unplanned hospitalization.
- It results in prolongation of an existing hospitalization.
- It is a congenital anomaly or birth defect.
- It requires medical or surgical intervention to prevent any of the above outcomes.

Medical and scientific judgment should be exercised in determining whether an AE is serious when considering important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent any of the other outcomes listed. Examples of such medical events that may also be considered serious include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization.

Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline does not meet the definition of an SAE.

Social or convenience admission to a hospital or prolongation of a hospitalization for social or convenience reasons not associated with the occurrence of an AE does not meet the definition of an SAE.

7.2.1. Serious Adverse Events That Occur After Study Completion

If an investigator becomes aware of an SAE or death that occurs in a subject more than 48 hours after the subject receives study medication and that investigator considers the event to be related to the study medication, the investigator is obligated to report the SAE.

7.3. Definition of a Suspected Adverse Reaction (SAR)

A SAR is defined as any adverse event for which there is a reasonable possibility that the adverse event was caused by the study drug. For the purposes of safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.4. Definition of a Serious Suspected Adverse Reaction (SSAR)

A SSAR is any Suspected Adverse Reaction (SAR) that is determined to be serious, based on the outcomes of a SAE described in Section 7.2; i.e. death, life-threatening, causes or prolongs

inpatient hospitalization, causes a persistent of significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital abnormality/birth defect.

7.5. Recording and Evaluating Adverse Events and Serious Adverse Events

The investigator will attempt to establish a diagnosis of the event on the basis of signs, symptoms, or other clinical information. In such cases, the diagnosis, not the individual signs or symptoms, should be documented as the AE or SAE.

7.5.1. Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study, using his or her clinical judgment. The intensity of each AE and SAE recorded on the CRF should be assigned to one of the following categories:

- mild: an event that is easily tolerated by the subject, causes minimal discomfort, and does not interfere with everyday activities
- moderate: an event that is sufficiently discomforting to interfere with normal everyday activities
- severe: an event that prevents normal everyday activities

An AE that is assessed as severe should not be confused with an SAE. *Severity* is a term used to describe the intensity of a specific event, and both AEs and SAEs can be assessed as severe. 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 the subject's or event's outcome or on action criteria usually associated with events that pose a threat to a subject's life or functioning (see Section 7.2).

7.5.2. Assessment of Causality

The investigator is obligated to use his or her clinical judgment to assess the relationship between the study medication and the occurrence of each AE or SAE. The investigator will assess the relationship to the study medication by using the following criteria:

- **Definitely Related**: An AE has a strong temporal relationship to the study drug. The AE is most likely explained by study drug. Dechallenge and rechallenge (if possible) are positive. The AE is consistent with a known response to the study drug. Another etiology is unlikely or significantly less likely.
- **Probably Related**: An AE has a strong temporal relationship to the study drug. The AE is more likely explained by study drug than by another cause. Dechallenge (if performed) is positive.
- **Possibly Related**: An AE has a reasonable temporal relationship to study drug. The AE could have been due to another equally likely cause. Dechallenge is positive.
- **Not Related**: The subject did not receive the study drug **OR** the AE has no temporal relationship to study drug **OR** the AE has a much more likely alternate etiology **OR** the AE is due to an underlying or concurrent illness or effect of another drug.

Even in situations in which minimal information is available for the initial SAE report, it is important that the investigator always make an assessment of causality for every event before transmitting the SAE reporting form and AE CRF page(s) to the medical monitor. The causality assessment is one of the criteria used when determining regulatory reporting requirements. The investigator may change his or her opinion of causality in light of follow-up information and amend the SAE reporting form and AE CRF page(s) accordingly.

7.5.3. Assessment of Outcome

All SAEs must be followed until they are resolved, the condition stabilizes, the events are otherwise explained, or the subject is lost to follow-up. The investigator will assess the outcome of the event by using the following terms:

- **Resolved**: The event resolved or the subject recovered without sequelae. An event (either serious or nonserious) occurred and had an endpoint, and the subject experienced no restrictions. Examples include stent placement for coronary artery disease (a device implanted is not a sequela), an appendectomy (a scar is not a sequela), a postoperative wound infection, or an upper respiratory tract infection.
- **Resolved with sequelae**: The event has at least one secondary outcome that may result in permanent disability, functional limitation, or both. Such sequelae are usually limited to SAEs. Examples include hip replacement resulting in foot drop (foot drop is not the intended outcome but is a risk of surgery), stroke resulting in paralysis, or emboli formation after a bacterial infection resulting in a renal infarct and loss of renal function.
- **Not resolved**: At the end of the study, a nonserious event either has not changed in intensity or may not have recovered to baseline values, and the outcome is unknown. Examples include headache, low-grade fever, or nausea.
- **Unknown**: The subject has withdrawn from the study prematurely or is lost to follow-up, and the status of the event is unknown.
- **Death**

7.5.4. Assessment of Expectedness

For the purposes of safety reporting, adverse events and suspected adverse events should be assessed as being expected or unexpected. An AE or SAR is considered unexpected if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

7.6. Follow-up of Adverse Events and Serious Adverse Events

Nonserious AEs will be followed after the study visit, until an appropriate resolution can be documented.

After the occurrence of an AE or SAE, the investigator is required to follow each subject proactively and provide further information on the subject's condition. All AEs and SAEs

documented at a previous visit or contact are designated as ongoing and will be reviewed at subsequent visits or contacts.

SAEs will be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. The investigator will ensure that follow-up information includes results of any additional laboratory tests or investigations, histopathologic examinations, or consultations with other healthcare professionals that serve to clarify the nature of the event, the cause of the event, or both.

New or updated information will be recorded on the originally completed SAE reporting form and CRF pages, with all changes signed and dated by the investigator. The updated SAE reporting form and CRF pages should be resubmitted within the time frames outlined in Section 7.7.

7.7. Prompt Reporting of Serious Adverse Events

Once the investigator determines that an event meets the protocol definition of an SAE, he or she must notify the medical monitor within 24 hours.

ANY SAE OR ANY OUTCOME OF DEATH DUE TO ANY CAUSE, WHICH OCCURS DURING THE COURSE OF THIS STUDY, REGARDLESS OF RELATIONSHIP TO STUDY MEDICATION, MUST BE REPORTED IMMEDIATELY (within 24 hours).

COMPLETE THE SAE DETAILS REPORTING FORM AND FORWARD BY E-MAIL TO THE MEDICAL MONITOR AND THE FOLLOWING CONTACT:

Mark Schuyler, MD
Medical Safety Monitor
Telephone: 505 925-4197
E-mail: mschuyler@salud.unm.edu

In the initial e-mail, the investigator must provide the following CRF pages, completed to the greatest extent possible:

- AE record
- medical history
- prior and concomitant medications

Also, the following documents are to be forwarded: any laboratory results, diagnostic test results, or medical reports relevant to the SAE.

E-mail transmission is the preferred method to transmit SAE information. In rare circumstances and in the absence of e-mail capacity, notification by fax or telephone is acceptable, with a copy of the SAE reporting form and CRF pages sent by overnight mail. Initial notification via telephone does not replace the need for the investigator to complete the SAE reporting form and CRF pages within the time frames outlined.

If the investigator does not have all information regarding an SAE, he or she must not wait to receive additional information before notifying the medical monitor of the event. The form must

be updated when additional information is received. Follow-up information received on all SAEs must be forwarded to the medical monitor by using the same procedure and timelines as for an initial report.

7.8. Regulatory Reporting Requirements

The investigator must promptly report all SAEs in accordance with the procedures detailed in Section 7.7, "Prompt Reporting of Serious Adverse Events." The investigator/medical monitor has a legal responsibility to notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the appropriate project contact for SAE receipt is essential so that SSARs that are either unexpected or observed with increasing occurrence, be reported and legal obligations and ethical responsibilities regarding the safety of other subjects are met.

An investigator letter is prepared for any SAR that is attributable to study medication, serious, and unexpected. The purpose of the investigator letter is to fulfill specific regulatory and Good Clinical Practice (GCP) requirements regarding the product under investigation.

The investigator, or responsible person according to local requirements, must comply with requirements related to the reporting of SAEs to the IRB or IEC.

8. SAFETY MONITORING PLAN

The Independent Safety Monitoring for Pneumonia Urease Positive Organism Screening Protocol (#16-327)

8.1. Background

A Safety Monitoring Committee (SMC), comprising the Independent Medical Monitor (or designee) and each Principal Investigator (or designee) will provide ongoing safety surveillance of the study. This committee will be responsible for monitoring study conduct and safety data.

The SMC will review the safety experience of each subject and the cumulative occurrences of adverse events. Through regularly scheduled and ad hoc meetings or teleconferences, the SMC will provide ongoing review of safety data to determine whether subject entry and treatment are appropriate, whether the protocol meets appropriate needs for safety monitoring and conduct, and whether continued study conduct is justified. The Clinical study site Principal Investigator will be notified accordingly when actions in response to safety concerns or protocol modifications occur and should ensure that the IRB is notified in a timely manner.

8.2. Purpose

Provide independent review and surveillance of adverse effects observed after subject exposure to a nebulized urea solution (test article) used as a tool to screen for urease positive organisms in subjects presenting with clinical signs and symptoms of pneumonia.

8.3. Assessment Personnel

A qualified Independent Medical Monitor will be a physician experienced in the assessment of lower respiratory infections, and use of nebulized solutions in such patients, and with sufficient training and experience to evaluate the safety associated with of use of the test article in these subjects.

For this program, one or more physicians from the institution who are not involved in the enrollment or assessment of study subjects, but who are Board Eligible or Board Qualified in EM, Critical Care, or Pulmonology will review subject case reports to evaluate adverse events which may or may not be related to administration of test article per the schedule described below, and advise on the safety of continued subject enrollment. For this study, Mark Schuyler, MD has agreed to be the independent medical monitor (Section 7.7).

8.4. Planned Review Process

Frequency: Greater than 100 subjects have had the test article administered to date without the occurrence of serious adverse effects (SAEs). Therefore, this protocol intends for the safety monitoring review to be performed in cohorts as follows:

- Subjects #1 through 3 = Group 1
- Subjects #4 through 6 = Group 2
- Subjects #7 through 10 = Group 3

- Subjects #11 through 15 = Group 4
- Subjects #16 through 20 = Group 5
- Subjects #21 through 25 = Group 6
- Subjects #26 through 30 = Group 7
- Subjects #31 through 35 = Group 8
- Subjects #36 through 45 = Group 9
- Subjects #46 through 55 = Group 10
- Subjects #56 through 65 = Group 11
- Subjects #66 through 75 = Group 12

Each group of subjects will be reviewed to assess whether any SAEs or other significant safety events have occurred. Safety review will be documented using the study safety review form provided in Appendix C.1, to be completed by the independent medical monitor (or appropriate designee) and acknowledged by the study investigator. If an SAE has occurred, the independent medical monitor will evaluate whether the observed SAE is definitely or possibly related to test article administration, or if there is no likely relationship between the SAE and test article.

9. STATISTICAL METHODOLOGY

9.1. Determination of Sample Size

The sample size for this study was selected based on prior exposures and existing safety data. Descriptive statistical analysis of results are planned as Findings from this study will be used in order to appropriately determine the sample size requirements for future definitive evaluations of inhaled ^{13}C-urea in an adequately powered study of efficacy in this population.

9.2. Study Endpoints

9.2.1. Efficacy Endpoints

Efficacy endpoints will include the following:

- Evaluate the exhaled $^{13}CO_2$ levels at each time point post-nebulization
- Evaluate the relationship between the exhaled $^{13}CO_2$ levels at each time point and subject's causative pathogen based upon sputum culture or NGS analysis
- Evaluate the sensitivity and specificity of the ^{13}C-urea breath test for urease producing pathogens
- Evaluate the positive and negative predictive value of the ^{13}C-urea breath test for urease producing pathogens
- Correlation of the ^{13}C-urea breath test with CURB65 in subjects with sputum cultures or NGS results positive for urease pathogens
- Correlation of the ^{13}C-urea breath test with PSI in patients with sputum cultures or NGS results positive for urease pathogens
- Univariate association of all study variables with urease pathogen caused pneumonia
- Multi-variate logistic regression model for urease pathogen caused pneumonia utilizing study variables that are significant in univariate analysis to compute odds ratio for each model variable and overall explanation of variance by model

9.2.2. Safety Endpoints

Safety endpoints will include the following:

- Incidence of all AEs
- Incidence of death, transfer to ICU, or intubation

9.3. General Considerations for Statistical Analysis

9.3.1. Analysis Datasets

Intent-to-Treat (ITT) Analysis Set: The ITT set will include all subjects who receive nebulized ^{13}C-urea. The ITT set is also referenced as the Safety analysis set. All safety analysis will be based on the ITT analysis set.

Modified Intent-to-Treat (mITT) Analysis Set: The mITT subjects include all ITT subjects who provide at least one post-dose breath sample. The mITT analysis set will be used for efficacy analysis.

9.3.2. Test Hypothesis and *P* Value Justification

No formal statistical hypothesis will be examined. When a p-value is produced from an analysis, the nominal p-value will be reported as is without adjustment for multiplicity.

9.3.3. Procedures for Handling Missing Data

Unless indicated otherwise, no imputation will be done for missing data. However, AEs with missing severity assessments will be tabulated as "severe," and AEs with missing relationship assessments will be tabulated as "related" for the purpose of analysis; and the missing data will be presented in data listing as is.

9.4. Study Population Summaries

Population summaries will be provided for the safety analysis set included in this study.

9.4.1. Disposition

The summary tables will provide frequency counts for subject disposition (all treated subjects, subjects who completed the study, subjects who discontinued from the study, and reason for discontinuation) for the study overall and for each cohort. Identification numbers for discontinued subjects will also be included in the tables.

Disposition in terms of number of subjects excluded from each analysis sets (ITT and mITT) will also be provided for the overall population and for each cohort.

9.4.2. Demographics

The demographic summary will include descriptive statistics for age, sex, race, weight, height, and BMI for the overall study population.

9.4.3. Protocol Violations

All protocol violations and deviations will be identified.

9.4.4. Treatment Compliance

All doses of study medication will be overseen by study personnel. The exact time of initiation and completion of nebulization will be documented within each subject's CRF. No formal summary of treatment compliance will be produced.

9.4.5. Prior and Concomitant Medications

All prior and concomitant medications will be tabulated for the overall study population. Prior and concomitant medications will be coded to the therapeutic drug classes and generic drug names using the World Health Organization (WHO) Drug classifications version 1Q2015 or higher.

9.5. Efficacy Analysis

Breath test exhaled $^{13}CO_2$ results at each time point will be presented for the overall population for determination of optimal breath sampling time. Relationship between the exhaled $^{13}CO_2$ at each time and subject's pneumonia diagnosis and pathogen (determined by sputum culture) will be examined. The time point where exhaled $^{13}CO_2$ can reliably predict the presence of urease positive pathogen in subjects with pneumonia diagnosis will be considered as the optimal time for ^{13}C-urea breath test sampling.

The proportion of subjects with breath test positive/negative determination will be compared to sputum culture results when available. Correlation analyses will be performed where sufficient data are available.

9.6. Safety and Tolerability Evaluations

All analyses will be provided for the overall group.

9.6.1. Extent of Exposure

All subjects will receive Study drug ^{13}C-urea solution via Aerogen Aeroneb Solo nebulizer device. The solution will be nebulized until the full dose has been administered, with subjects breathing normally through their mouths using the nebulizer mouthpiece.

No formal exposure summary will be prepared.

9.6.2. Adverse Events

The Medical Dictionary for Regulatory Activities (Version 18 or higher) will be used to classify all AEs with respect to system organ class and preferred term.

Three types of summaries will be produced for the AE summary:

1. an overall summary of AEs: number of subjects with at least one event and number of events for each severity for all AEs, and SAEs
2. a summary table of AEs and SAEs by system organ class and preferred term and severity
3. a summary table of AEs and SAEs by preferred terms in descending order of total incidence

AEs will be tabulated by cohort and for study overall. AEs that lead to premature discontinuation from the study or to death will be listed separately via data listings.

9.6.3. Clinical Laboratory Tests

The study will not have routine clinical safety laboratory tests. Bacterial culture results will be summarized for the study overall.

9.6.4. Subgroup Analyses for Safety Endpoints

No subgroup analysis is planned for safety endpoints.

9.7. Interim Evaluation

No interim analysis is planned for this study.

10. STUDY ADMINISTRATION

10.1. Regulatory and Ethical Considerations

10.1.1. Regulatory Authority Approval

The investigator will obtain approval to conduct the study from the appropriate regulatory agency in accordance with any applicable country specific regulatory requirements before any site may initiate the study in that country.

10.1.2. Ethical Conduct of the Study and Ethics Approval

This study will be conducted according to GCP; US 21 Code of Federal Regulations (CFR) Part 50 (Protection of Human Subjects); US 21 CFR Part 56 (IRBs); US 21 CFR Part 54 (Financial Disclosure); International Conference on Harmonization (ICH) Guidance for Industry, E6 GCP: Consolidated Guidance; the Nuremberg Code; and, where applicable, the principles of the Declaration of Helsinki (Recommendations guiding Medical Doctors in Biomedical Research Involving Human Subjects).

10.1.2.1. Ethics Committees

The investigator is responsible for ensuring that this protocol, the site's informed consent form, and any other information that will be presented to potential subjects (e.g., advertisements or information that supports or supplements the informed consent form) are reviewed and approved by the appropriate IRB or IEC. The investigator agrees to allow the IRB or IEC direct access to all relevant documents. The IRB or IEC must be constituted in accordance with all applicable regulatory requirements.

If the protocol, the informed consent form, or any other information that the IRB or IEC has approved for presentation to potential subjects is amended during the study, the investigator is responsible for ensuring that the IRB or IEC reviews and approves, where applicable, these amended documents. The investigator must follow all applicable regulatory requirements pertaining to the use of an amended informed consent form, including obtaining IRB or IEC approval of the amended form, before new subjects consent to take part in the study using the new version of the form. IRB or IEC approval of the consent forms must be obtained in addition to the approval given for the clinical study. Regulatory review and approval may be required in some countries before IRB or IEC approval can be sought.

10.1.2.2. General Considerations

The ethical standards defined within GCP are intended to ensure the following:

- Human subjects are provided with an adequate understanding of the possible risks of their participation in the study, and they have a free choice to participate or not.
- The study is conducted with diligence and in conformance with the protocol in such a way as to ensure the integrity of the findings.
- The potential benefits of the research justify the risks.

10.1.3. Informed Consent

The informed consent form must reflect the required elements of informed consent specified in 21 CFR Part 50.25. The final informed consent form must be approved by the IRB or IEC. If any new information becomes available that might affect subjects' willingness to participate in the study, or if any amendments to the protocol require changes to the informed consent form, the IRB or IEC must provide written approval of any revisions to the informed consent form in advance of its use.

Consenting will occur according to GCP and clinical site practices in the emergency department or Clinical and Translational Science Center (adjacent to UNM-ED, Cohort A only).

Investigators must provide subjects with all the information necessary to make an informed decision about their participation in the study, including the nature and intended purpose of the study, possible benefits, and possible risks.

All information in the informed consent form should be provided in a language (whether written or spoken) that is as nontechnical as practical and that is understandable to the subjects.

Before written informed consent is obtained, the subject should be given ample time and opportunity to inquire about the details of the study. All questions must be answered to the satisfaction of the subject (or his or her legally authorized representative).

This study requires administration of nebulized urea within 4 hours of administration of antibiotics. Subjects will be told that if they wish to participate in the study, they will need to decide to participate within 3 hours of receiving antibiotics in the emergency department in order to participate in the study. The study procedures will not delay or interfere with antibiotic administration.

Before a subject undergoes procedures specific to the protocol, the informed consent form must be signed and dated by the subject (or his or her legally authorized representative) and any other signatories as required by the IRB or IEC.

If a subject (or legally authorized representative) cannot read, a short form approved by the IRB or IEC may be used. Only the short form itself is to be signed by the subject or the representative. However, the witness shall sign both the short form and a copy of the summary, and the person actually obtaining the consent shall sign the copy of the summary in accordance with 21 CFR 50.27 (b2).

After all required signatures have been obtained, a copy of the informed consent form should be provided to the subject, and the original must be kept on file at the site and made available for review. Documentation of the informed consent discussion must be noted in the subject's case history.

10.1.4. Investigator Reporting Requirements

The clinical site investigators are responsible for completing and maintaining adequate and accurate CRFs and source documentation. Source documentation constitutes original records (first point of entry, either hard copy or electronic), which may include progress notes, medication administration records, operation reports, laboratory reports, discharge summaries, and so on. All CRFs should be completed contemporaneously in their entirety and stored in a confidential and locked location.

All patient records relating to the present study will be kept in a locked cabinet in the locked ED Research Offices within the UNMH Emergency Department (2211 Lomas Blvd NE, Suite 1303 and 1304) and Henry Ford Hospital Emergency Department (2799 W. Grand Blvd, Detroit, MI 48202), as appropriate. Only approved study personnel will have key access to the research materials. All study materials with PHI will be de-identified prior to transmission to the study sponsor. The link between research records and patient identifiers will be destroyed prior to archiving the study records. We intend to archive study records in a locked cabinet in the IDTC file room at 700 Camino de Salud NE after study closure. Henry Ford Hospital will archive study records in a locked cabinet located in the Emergency Department research office at 2799 W. Grand Ave. after study closure.

Study information will be entered in an Excel spreadsheet located on a dedicated laptop housed at the UNM Emergency Department Research Office. The database will only be accessible to IRB approved study staff and will not contain subject identifiers.

10.2. Study Monitoring

The site investigator is responsible for ensuring the proper conduct of the study with regard to subject protection, ethics, protocol adherence, site procedures, and integrity of the data. Qualified study monitors will review all aspects of the study according to Good Clinical Practices and for compliance with applicable government regulations. The investigator agrees to allow monitors direct access to all site information, clinical data, supplies and dispensing and storage areas during monitoring visits. The site investigator and staff are responsible for being available during monitoring visits for consultation. See Appendix E for details of the study monitoring plan.

10.3. Quality Assurance

A regulatory authority or an IRB representative may visit the study site at any time during the study or after completion of the study to perform audits or inspections. The purpose of an audit or regulatory inspection is to examine systematically and independently all study-related activities and documents to determine whether these activities were conducted according to the protocol, GCP, ICH guidelines, and any other applicable regulatory requirements. It is the site investigators' responsibility to inform the Principal Investigator immediately in the event of an audit notification.

10.4. Study and Site Closure

If the investigator or officials from regulatory agencies discover conditions arising during the study that indicate that the study should be halted or that the study site should be closed, this action may be taken after appropriate consultation between the study investigators. Conditions that may warrant termination of the study include, but are not limited to, the following:

- discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled in the study
- submission of knowingly false information from the research facility to the study monitor or regulatory agencies

- failure of the investigator to comply with GCP (e.g., ICH guidelines, regulatory agency guidelines)
- insufficient adherence to protocol requirements or an unacceptably high rate of missing, erroneous, or improperly collected data
- evidence from the blinded data of sufficient technical problems with the study that one could believe with a high degree of certainty that subjects are being exposed to the investigational drug without a realistic expectation of evaluable data
- a decision on the part of the investigator to suspend or discontinue testing evaluation or development of the product
- failure of the investigator to enroll subjects into the study at an acceptable rate

10.5. Records Retention

10.5.1. Health Insurance Portability and Accountability Act of 1996

The investigator agrees to comply with all applicable federal, state, and local laws and regulations relating to the privacy of subjects' health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR Parts 160 and 164 (the Health Insurance Portability and Accountability Act of 1996 privacy regulation). The investigator shall ensure that study subjects authorize the use and disclosure of protected health information in accordance with the privacy regulations of the Health Insurance Portability and Accountability Act.

10.5.2. Financial Disclosure

Financial disclosure is not required for this study.

10.5.3. Access to Original Records

Regulatory authorities expect that monitors, auditors, and representatives of national and international government regulatory agency bodies have access to original source documentation (see examples in Section 10.1.4) to ensure data integrity. "Original" in this context is defined as the first documentation of an observation and does not differentiate between hard-copy and electronic records.

10.5.4. Archiving of Study-Related Documents

Records related to this clinical study must be retained either for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

10.6. Provision of Study Results and Information to Investigators

The investigator and study staff will have access to the research results and will be able to link the results to a particular subject. The investigator and study staff will be directed to hold this information confidentially.

10.7. Subject Tracking

Drug accountability logs, a subject identification log (to be retained by the investigator only), and a subject enrollment log will be used to track subject participation in the study.

11. REFERENCES

Raissy, H. H., Timmins, G., Davies, L., Heynekamp, T., Harkins, M., Sharp, Z. D., & Kelly, H. W. (2016). A Proof of Concept Study to Detect Urease Producing Bacteria in Lungs Using Aerosolized ^{13}C-Urea. *Pediatric Allergy, Immunology, and Pulmonology*, *29*(2), 68–73. http://doi.org/10.1089/ped.2015.0619

APPENDIX A. SCHEDULES OF STUDY VISITS AND PROCEDURES

Table 1: Schedule of Study Procedures

Procedure	Screening	Treatment			Follow-Up 48 Hours Post Breath Test
		Pre-Treatment	Nebulization	0-10 Minutes Post-Nebulization	
Informed Consent	X				
Inclusion/Exclusion Evaluation	X				
Demographics and Medical History	X				
Physical Examination	X				
Sputum Collection	X[b]				
Oroopharyngeal Swab	X[b]				
Urine Pregnancy Test	X				
Breath Sample Collection		X[a]		X[a]	
Vital Signs	X	X	X	X	
^{13}C-urea Administration			X		
Safety Contact					X
Prior and Concomitant Medication	<---------- X ---------->				
Adverse Event Monitoring			<---------- X ---------->		

a Up to 6 samples collected pre- and post-nebulization
b Only required for Cohort B

APPENDIX B. INVESTIGATOR OBLIGATIONS

As an investigator, you are responsible for ensuring that the study is conducted according to the protocol, the signed Statement of Investigator, and all applicable regulations.

Debarment

Individuals ineligible to conduct or be involved with clinical studies, including those ineligible as a result of debarment under the Generic Drug Enforcement Act of 1992, will not be allowed to conduct or work on this study.

Institutional Review Board

You are required to obtain initial and continuing review and approval by an IRB or IEC that complies with the requirements specified in 21 CFR Part 56. Before initiating the trial, you must have written approval from the IRB or IEC for the protocol, informed consent form, subject recruitment procedures (e.g., advertisements), and any other written information to be provided to the subjects. You must submit the Investigator's Brochure and any updates to the IRB or IEC for review. The IRB or IEC must also provide written approval of any amendments to the protocol that affect the conduct of the study and any changes to the informed consent form in advance of use. If the duration of the study is longer than 1 year, re-approval by the IRB or IEC must be obtained on a yearly basis (or at more frequent intervals if required by the IRB or IEC).

You must provide reports of all SAEs from your site to the IRB or IEC. You are also responsible for providing the IRB or IEC with Safety Reports of any SAEs from any other study conducted with the study medication.

Confidentiality and Safety of Subjects

You are responsible for protecting the rights, safety, and welfare of subjects under your care and for the control of the drug(s) under investigation.

You are responsible for keeping a record of all screened subjects, including full names and last known addresses. All subjects will be identified on the CRFs by initials and subject numbers. Demographic information including date of birth, sex, and race will also be recorded on the CRFs. Confidentiality of subject data will be maintained in accordance with local laws.

In addition to your responsibilities for reporting AEs identified during the course of a subject's participation in the study, you must also report any SAEs that occur within 30 days after the last dose of study medication (regardless of relationship to study medication) and any serious adverse drug reactions (SAEs for which you consider that there is a reasonable possibility that the study medication caused the response) that you become aware of at any time (even if the event occurs more than 30 days after the subject's last exposure to study medication). This obligation is in addition to any protocol-specified requirement for reporting AEs occurring after the last dose of study medication. Please refer to Sections 7.7 and 7.8 of this protocol for contact information and SAE reporting requirements.

Study-Related Records

You are required to maintain complete and accurate study documentation in compliance with current GCP standards and all applicable federal, state, and local laws, rules, and regulations related to the conduct of a clinical study.

You are required to make all study documentation promptly available for inspection, review, or audit at your study site upon request by the investigator or any appropriate regulatory agencies.

Accountability of the Investigational Product

You or your designee (i.e., the pharmacist) is responsible for accountability of the investigational product at the site. You or your designee must maintain records of the product's delivery to the site, inventory at the site, use by each subject, and the return/destruction of any unused product. These records must include dates; quantities; batch, serial, or lot numbers; and expiration dates (if applicable).

You should ensure that the investigational product is used only in accordance with the protocol.

APPENDIX C. STUDY-SPECIFIC INFORMATION

Appendix C.1: Safety Review Form

Protocol 16-327 - Safety Review Form

Date Reviewed: _____

Subjects Reviewed: _____

Reviewer: _____

Were any significant safety observations identified? ☐ Yes (describe below) ☐ No

Subject ID	Description of event	Serious (Y/N)	Intensity	Relationship to treatment

Intensity: Mild, Moderate, Severe
Relationship to treatment: Not related, Possibly related, Probably related, Definitely related

Other safety observations to report? ☐ N/A

_____

_____

_____

Determination of review: ☐ Safe to proceed with enrollment

☐ Additional review required, hold enrollment

☐ Other, Describe: _____

_____

Reviewer Signature / Date

_____

Investigator Acknowledgement, Signature / Date

APPENDIX D. BMI CALCULATION

Body Mass Index Calculations

Body Mass Index

Weight in kilograms / (height in meters)^2

Meters = inches × 0.0254; kilograms = pounds × 0.45

Example:

For a man who weighs 165 pounds and is 71 inches tall:

165 × 0.45 = 74.25 kg

71 × 0.0254 = 1.8 m

74.25 / (1.8 × 1.8) = 22.9 kg/m^2

APPENDIX E. CLINICAL MONITORING PLAN

A Phase 1, Open-label Evaluation of a ^{13}C-Urea Breath Test for the Detection of Urease-producing Bacteria in Patients with Pneumonia in the Emergency Department

Clinical Monitoring Plan

Version 1.1, May 22, 2017

Study #: NCT03100760

Table of Contents

1 Introduction to the Monitoring Plan

This study specific monitoring plan is intended to ensure consistency in the scope of work performed between the University of New Mexico Health Sciences Center-Emergency Medicine (UNMHSC-EM) department, as the responsible party for Clinical Operations, and the investigative sites. A Clinical Research Organization (CRO) is being retained to conduct the clinical data monitoring for UNMHSC-EM and all other clinical sites. This monitoring plan is written to ensure and verify that the study will be conducted according to the study protocol, FDA/ICH Regulations and Guidelines, applicable regulatory requirements, and all State and Local laws.

2 Regulatory Documents

Monitoring activities will follow previously established CRO SOPs and Work Instructions, and contractual requirements, unless identified to be in conflict with UNMHSC-EM SOPs. If new SOPs are released, they will supersede the previous versions unless otherwise documented by the Study Manager. Instructions for completion of the case report forms (CRF) will be part of site training.

CRO will be responsible for the collection of each site's regulatory documents for the remainder of the study.

Specific requests in the aforementioned Work Instructions may be altered as needed by UNMHSC-EM to address current study needs. A site's Regulatory File includes but is not limited to the following documents:

♦ IRB/EC approved Informed Consent Documents and Protocol Approval Letters,

♦ Investigator Agreements,

♦ Investigator and co-Investigator CVs and current licensure,

♦ Signed protocol signature pages including any amendments,

♦ Clinical Laboratory Certification and Accreditation, e.g. CAP, CLIA, laboratory normal ranges, and the CV and license of the Lab Director,

♦ Sample Case Report Form, and

♦Monitoring documentation including confirmation of visits, follow-up letters to investigative sites, and monitoring reports. *Note: With the exception of the Initiation Visit Report, all Monitoring Visit Reports are filed only with the Master Site File at UNMHSC-EM and not in the sites' Regulatory Binder.*

The following additional documents will be collected from each site in addition to the documents listed above and submitted to UNMHSC-EM:

♦ A copy of the Delegation Log (Signature and Responsibility)

♦ A copy of the Clinical Supplies Receiving and Return logs

♦ A copy of the Specimen Shipment Log

♦ A copy of the Screening and Enrollment Log and all updates

♦ Safety monitoring reports

♦ Reports of Serious Adverse Events

3 Study Initiation Activities

3.1 Timing

CRO will conduct Site Initiations for the selected sites. If new sites are added, CRO will also conduct those Site Initiations. The Site Initiation will be scheduled once budgets are agreed upon, contracts executed, IRB approval and supplemental investigational device exemption (IDE) approval has been obtained and the IRB approval letter provided to UNMHSC-EM.

3.2 Pre-Initiation Communication

An CRO representative will contact the site to confirm the appropriate staff members who should be in attendance during the Site Initiation. The Site Initiation will be scheduled at a time in which all potential study staff members are able to attend. A letter confirming the Site Initiation including the agenda will be sent to the site within 5 working days of the Site Initiation.

Prior to the Site Initiation it will be confirmed that the site is able to send and receive e-mail.

3.3 Site Initiation Activities

Site Initiation activities and discussions include, but are not limited to:

- ♦ a discussion of the Investigator's responsibilities,
- ♦ the informed consent process,
- ♦ enrollment expectations,
- ♦ protocol review (including inclusion/exclusion criteria) and study procedures,
- ♦ regulatory document requirements,
- ♦ maintenance of all study logs,
- ♦ clinical supplies accountability,
- ♦ local privacy law compliance (e.g. HIPAA) and pre-study authorization (US sites only),
- ♦ source document requirements,
- ♦ CRF completion and query resolution,
- ♦ safety monitoring reporting, and
- ♦ serious adverse event reporting.

The CRA will be responsible for the following tasks during the Site Initiation:

- ♦ Review of protocol,
- ♦ Review of good clinical practices,
- ♦ Train the site study personnel on study procedures,
- ♦ Review the site's regulatory and study files,
- ♦ Documentation of the roles, responsibilities, and changes in staff (Delegation Log (Signature and Responsibility),
- ♦ Verify the clinical supplies storage conditions are adequate,
- ♦ Verify site has received all study specific documentation and supplies,
- ♦ Verify that site is adequately trained on procedures regarding third party vendors and couriers who will be shipping specimens

- ♦ Review study material resupply procedures, and
- ♦ Review specimen collection resupply procedures.

3.4 Site Initiation Reports

The Initiation Report must be prepared by the CRA who conducted the Site Initiation. The report shall be submitted to the UNMHSC-EM Study Manager in a timely manner (suggested within 10 working days) from the date of the Site Initiation.

3.5 Follow-up Communications

The CRA will send a follow-up letter to the site within 5 working days following the Site Initiation. This letter will highlight the activities of the Site Initiation, and resolution to any unanswered questions that were raised during the visit. Outstanding regulatory issues will also be addressed in the letter. A copy of the Initiation Visit Report will be included with the letter.

4 Monitoring Activities

4.1 Initial Site Visit

The first monitoring visits for Cohort A and B are targeted to be performed after a site enrolls their first 3 subjects into each cohort. This initial visit is intended to ensure that the study is conducted according to the protocol and that the CRFs are being completed accurately.

4.2 Periodic Site Visits

The CRA will schedule the second site visit after the last subject is enrolled for Cohort A at each site and has completed the study through the patient follow-up call.

The CRA will schedule the second site visit for Cohort B after approximately 15 patients have been enrolled at a given site and then again for a final visit once all subjects have been enrolled in Cohort B and the CRFs completed through the patient discharge from the hospital.

Additional monitoring visits can be scheduled based on the study site enrollment rates with monitoring visits occurring at a frequency established by UNMHSC-EM and agreed to by the sites, according to the sites' enrollment rate and the number of open cases.

4.3 Telephone Contacts and Documentation

Telephone or e-mail communication with each site will be performed initially on a weekly basis until it is apparent to the CRA the site has become comfortable with study procedures, after which, contact should be made no less than every 2 weeks and the CRA has not identified any site specific problems that may warrant more frequent contact. Site Coordinators are encouraged to call the monitor with any questions whatsoever to ensure compliance with protocol. The monitor should document any conversations the CRA believes is significant to the study timeline, regulatory considerations, protocol adherence, subject safety, and any other issues team members should be made aware of.

E-mail correspondence with a site regarding significant study-related issues, e.g. topics of discussion directly related to the conduct of the study, should be copied to the Study Manager and any other UNMHSC-EM personnel as applicable. Significant study-related e-mails will be maintained in UNMHSC-EM Master Files. UNMHSC-EM clinical staff will print the final string of email correspondence for filing in NCT03100760 Master Files.

When generating e-mail communication documenting significant study-related issues, a new e-mail shall be generated for each specific issue with the appropriate subject identified in the "RE:" section of the e-mail header, beginning with the study name in capital letters, e.g. "RE: NCT03100760, Specimen Shipment". E-mail strings may be utilized as long as the subject matter does not change.

4.4 Pre-Visit Communication

As sites reach the enrollment goal that will trigger a monitoring visit, an CRA (or delegate) will contact the site to schedule a monitoring visit. A letter confirming the visit will be sent to the site within 5 working days of the visit.

4.5 In House Monitoring Activities

The Study Manager will have access to e-mailed PDFs of the site(s)' CRFs. The CRF PDF does not contain any Protected Health Information (PHI). The Study Manager should review subject data online routinely

throughout the course of the study. Prior to each site visit, the CRA should ensure the site staff complete all appropriate visit pages.

In house monitoring activities may include but are not limited to the following:

♦ Query status: CRAs will check the query status and discuss this with the sites as required,

♦ Withdrawals: Study Manager will track the number of subjects withdrawn from each site,

♦ Patients screened: Study Manager will review the screening and enrolment logs provided for their sites to ensure subjects are being screened and enrolled according to the protocol,

♦ Subjects enrolled: Study Manager will track number of subjects enrolled and confirm CRFs are being completed in a timely manner.

4.6 CRF Completion

Only the site staff listed on the Delegation Log (Signature and Responsibility) who have received required training and are authorized to enter data or make corrections to CRFs. The CRA will be responsible for ensuring that only the appropriate parties are making entries in the CRFs and that the data is accurate and complete.

The CRA will ensure that the site staff is instructed on how to complete the CRFs correctly.

4.7 CRF Guidelines

In addition to checking CRF pages against source documents, the CRA must review all data for completion and plausibility. If the quality of completion of CRFs is inadequate, this must be discussed with the Investigator and appropriate site staff.

Monitors will not be able to enter data into the CRF and will have access to the PDF versions for query/note generation and associated operational tasks only.

Following the review and source verification of CRFs, the CRA should indicate the CRF as ‘monitored’ by initialing and dating each monitored page of the CRF.

4.8 Source Document Requirements

Data entered onto CRFs must be verified against original source documents to confirm accurate, complete and legible reporting of valid data. Original source documents may be the electronic medical record at the site, or copies of select medical record documents maintained in a shadow chart, or a combination of both. Sites will use an inclusion/exclusion criteria checklist copied from the CRF when a study coordinator approaches a patient to determine eligibility for the trial and to ensure all the required presentation data are captured and documented. This checklist will be considered a source document for the required presentation data. Medical history may also be obtained on this Worksheet but must be verified with the subject's official medical record. If the subject does not have an established medical record at the study facility to verify against, it should be noted by the study site personnel in the study documents that the information was derived from a patient interview and is non-verifiable.

Additional source documents required by the study include but are not limited to:

- ♦ Study required specimen collection dates, times, and method of collection,
- ♦ Medication records including prior and prescribed antibiotics,
- ♦ Laboratory tests,
- ♦ Chest X-ray results,
- ♦ Prior non-drug therapies and procedures,
- ♦ Documentation of the Initial and Final Diagnoses, and
- ♦ Disposition of the patient

4.8.1 CRF as Source Document

The only CRF that will be considered as source data will be the NCT03100760 case report forms for Cohort A and Cohort B. It is the clinical site's option to use these CRFs as a source documents.

4.9 Source Document Verification (SDV)

Source Document Verification will include 100% verification of all data recorded for all patients enrolled in the study.

Each site is required to maintain records of each subject's case history. Case histories include the CRFs and supporting data including, for example, signed and dated consent forms and medical records including, for example, past medical history, progress notes of the physician, laboratory results, the

individual's hospital chart(s), and the nurses' notes. Any significant deficiencies will be reported to the Study Manager and documented by the CRA in the Monitoring Visit Report.

4.9.1 Adverse Event/Serious Adverse Event Reporting

This study is a non-interventional study. The only study related procedure the patient will experience is the urea breath test. Adverse events will be collected up until 48 hours post administration of the study drug and any noted adverse events from this time period will be followed till resolution.

A patient may experience an adverse event related to the urea breath test, which is defined as but not limited to coughing, dizziness, feeling faint or fainting. If a patient were to experience an adverse event, this must be recorded in the CRF. If the adverse event is deemed to be serious, the event must be reported by the site investigator or his/her designate to the safety monitor (Dr. Mark Schuyler of UNMHSC) and the study PI (Dr. Justin Baca of UNMHSC-EM) as soon as possible. In addition, notification shall be given in writing to the FDA and the site's IRB per the IRB's internal procedures.

Serious is defined as either:

- ♦ Death,
- ♦ Life-Threatening,
- ♦ Unanticipated Hospitalization (initial or prolonged),
- ♦ Disability,
- ♦ Congenital Anomaly, or
- ♦ Requiring Intervention to Prevent Permanent Impairment or Damage.

4.10 Subject Screening and Enrollment

All screened subjects will be listed on the Subject Screening and Enrollment Log. The log will be sent by the site research coordinator or his/her designate to the NCT03100760 Study Manager weekly either by fax or e-mail attachment.

4.11 Regulatory Binder

The CRO CRA will review the Regulatory Binder for completeness and/or additions during Monitoring Visits. The CRA should collect original signed protocol signature pages and the Investigator Agreement form, leaving copies of these documents in the site's Regulatory Binder.

In addition, if there has been a change in site personnel and/or personnel responsibilities, the CRA should collect a copy of the updated Delegation Log (Signature and Responsibilities) for NCT03100760's Master files.

The CRA should follow up with the site coordinator to ensure timely processing and submission of any amendments to the protocol, informed consent, or other study related documents. The Site Regulatory Binder should thus be checked on a regular basis to ensure the filing of appropriate approval documents.

4.12 Data Query Process

Queries for CRFs can be issued by a CRA. CRA queries are based on the on-site CRF review and use a paper query form. These queries should be answered by the site staff, source verified and closed by the CRA.

4.12.1 Query Tracking

Queries generated from CRFs will be tracked by the NCT03100760 Study Manager. The CRA will follow-up with the site to ensure queries are followed-up and closed in a timely manner.

4.12.2 Query Status

There are **five categories** of query status for the CRFs, and these can be tracked by the CRA and the Study Manager for each site or for all sites. CRAs are able to generate manual queries, perform SDV and close queries. The five categories are:

♦ Open – Site: Queries with this status should be sent to the site for review or action.

♦ Open – CRO: Queries are set to this status after the site user responds to a query by adding a comment, and the query is thus routed back to CRO for review or action.

♦ Accept As Is: Queries are set to this status by CRO personnel if the site has indicated that the data value will not be changed, since it is accurate and in accord with source documents, despite the validation failure. The query is considered closed and will no longer display on the form.

♦ Obsolete: Queries are set to this status by CRO personnel if CRO personnel deem that the query has been generated in error. The query is considered closed and will no longer display on the form.

♦ Data Changed: This status is assigned to the query when data on the CRF is changed such that the query conditions are no longer violated. The query is considered closed.

4.13 Protocol Deviations/Violations

Protocol non-compliance will be documented in the Monitoring Visit Report. Significant protocol violations will be communicated to the Study Manager in a timely manner. UNMHSC-EM will review sites with frequent protocol deviations/violations and if the situation cannot be resolved, the site may be terminated from the study.

4.14 Site Supplies

Initial shipment of supplies will occur after the site receives approval from their IRB, supplemental IDE approval is granted and an initiation visit has been scheduled. The Investigator or designated study site personnel verifying the receipt of the supplies must complete the Clinical Supplies Received Log and send it to the Study Manager. Sites will contact the Study Manager when resupply of supplies is required.

4.14.1 Accountability

The CRA will perform accountability of the supplies at each monitoring visit. Verification of the supplies includes the following:

♦ Confirm that the Clinical Supplies Received Log is on file at the site.

♦ Confirm that the Clinical Supplies Received Log and supply inventory are in agreement.

♦ Confirm that the Clinical Supplies Received Log was faxed/e-mailed to the Study Manager after each receipt of supplies.

4.15 Monitoring Visit Reports

The Monitoring Visit Report must be prepared by the CRA who conducted the Monitoring Visit and submitted to the Study Manager in a timely manner (suggested within 10 working days) from the date of the Visit. The final Monitoring Visit Report shall be submitted to the Sponsor representative by the Study Manager no longer than 15 working days after the date of the visit.

4.16 Follow-up Communications and Activities

The CRA will send a detailed follow-up letter to the site in a timely manner (suggested within 10 working days) following the visit. This letter will highlight the activities of the Monitoring Visit, any protocol deviations/violations noted, and suggested corrective actions/pending items. Outstanding regulatory issues will also be discussed along with suggested resolution of the issue(s). If any significant CRF or other problem is identified during monitoring, these must be reported to the Study Manager via e-mail and/or telephone as soon as possible.

5 Site Closure Activities

5.1 Timing

The Site Closure Visit will be scheduled after all CRFs have been completed including all post-discharge follow-up visits, and all data queries have been resolved and closed.

5.2 Pre-Visit Communication

Prior to the Site Closure Visit the CRA will confirm with the Study Manager that the site is suitable for closure. The CRA should review the previous visit report, interim communications and regulatory tracking log, noting any outstanding actions. The visit should be arranged with the Principal Investigator and any other appropriate study site personnel by sending a visit confirmation letter. The confirmation letter should request any follow-up issues/documents required for resolution at the visit. Prior to the closure visit, the CRA should confirm that all CRF pages are complete. The CRA should also confirm with the Study Manager that all queries have been resolved.

5.3 Site Closure Visit Activities

During the visit, activities will include, but are not limited to, the following:

♦ Review and verify all subject CRFs have been completed,

♦ Resolve all outstanding data discrepancies and queries,

♦ Confirm the Principal Investigator has provided their signature for the CRFs,

♦ Perform final reconciliation of all supplies accountability records and coordinate return of the supplies or destruction of the supplies,

♦ Review the Regulatory Binder for completion and ensure all regulatory documents have been submitted to the NCT03100760 Master File,

♦ Confirm the End of Study Financial Disclosure Forms have been submitted to the NCT03100760 Master File,

♦ Confirm provisions for study document storage,

♦ Review and retrieve a copy of the study closure notification to the IRB/EC,

♦ Inform investigator of responsibilities (including record retention, inspections, financial disclosure, and publication policy)

Request that the investigator prepare, sign, and submit a final study report to the IRB/EC and remit a copy for the NCT03100760 Master File.

5.4 Follow-up Communications

The CRA will send a follow-up letter to the site in a timely manner (suggested within 10 days of the visit) following the visit. This letter will highlight the activities of the Termination Visit, protocol deviations/ violations noted, and suggested corrective actions. Outstanding regulatory issues will also be documented.

5.5 Site Closure Visit Report

The Site Closure Visit Report must be prepared by the CRA who conducted the Visit and submitted to the NCT03100760 Study Manager in a timely manner (suggested within 10 working days) from the date of the Visit. The final Visit Report shall be submitted to the Sponsor representative by the Study Manager no longer than 15 working days after the date of the visit.