DOCUMENT TITLE

ACCESS BNP ON DXI 9000 CLINICAL PERFORMANCE EVALUATION: SPECIMEN TESTING AND CLINICAL CONCORDANCE STUDY

PROJECT NAME
BNP FOR DXI 9000

PROJECT NUMBER
ID9016

PROTOCOL NUMBER
BNP-02-23

DHF DELIVERABLE NUMBER
2.7.2

REVISION
2.7

SPONSOR
BECKMAN COULTER, INC.
1000 LAKE HAZELTINE DRIVE
CHASKA, MN 55318

STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, the ethical principles that have their origin in the Declaration of Helsinki, the International Conference on Harmonization (ICH) consolidated Guideline E6 for Good Clinical Practice (GCP), and applicable regulatory requirement(s) listed below.

- Code of Federal Regulations 21 CFR, Part 11
- Code of Federal Regulations, Title 21, Part 50
- Code of Federal Regulations 21 CFR, Part 54
- Code of Federal Regulations, Title 21, Part 56
- Code of Federal Regulations 21 CFR, Part 812.140, Subpart G
- Code of Federal Regulations 45 CFR, Part 164
- Code of Federal Regulations 45, CFR, Part 160
- ETS No. 108: Convention for the Protection of Individuals with Regard to Automatic Processing of Personal Data
- ETS No.164: Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine.
- International Compilation of Human Research Protections
- General Data Protection Regulation (GDPR) (EU) 2016/679
- IVDR 2017/746

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List of Abbreviations

TERM	DEFINITION
A2	Access 2 Immunoassay System
AMR	Analytical Measuring Range
BNP	Brain natriuretic peptide
C	Celsius
CKD	Chronic Kidney Disease
CRF	Case Report Form
CLSI	Clinical and Laboratory Standards Institute
DxI 9000	DxI 9000 Access Immunoassay Analyzer
eCRF	Electronic Case Report Form
eTMF	Electronic Trial Master File
ED	Emergency Department
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HF	Heart Failure
ICH	International Conference on Harmonization
IFU	Instructions for Use
IRB	Institutional Review Board
IUO	Investigational Use Only
IVD	In Vitro Diagnostic
NP	Natriuretic peptide
NPA	Negative Percent Agreement
NT-proBNP	N-terminal-pro hormone BNP
OUS	Outside of United States
PPA	Positive Percent Agreement
QC	Quality Control
RLU	Relative light units
SOP	Standard Operating Procedure
US	United States

1. Key Roles and Contact Information

1.1. Sponsor Study Staff Contact Information

A list of the names and titles of Sponsor representatives is to be provided to the site as a separate document. Sponsor is Beckman Coulter, Inc at 1000 Lake Hazeltine Drive, Chaska, MN 55318. Main contact: (952) 448-4848.

1.2. Site Investigator Contact Information

A list of the names and titles of clinical investigators and the address and telephone number of study sites will be maintained in the sponsor master files and provided to investigators in the Investigator Site File. Beckman Coulter, Inc. will maintain an updated list, providing updates as required to the Investigator Site Files.

2. Study Synopsis

Full Title	Access BNP on DxI 9000 Clinical Performance Evaluation: Specimen Testing and Clinical Concordance Study
Short Title	ACCESS BNP CLINICAL PERFORMANCE EVALUATION: TESTING AND CLINICAL CONCORDANCE STUDY
Protocol Number	BNP-02-23
Protocol Version	2.7
Study Sponsor	Beckman Coulter, Inc. 1000 Lake Hazeltine Dr. Chaska, MN 55318
Study Purpose	The overall aim of the research is to establish clinical performance of the Access BNP Assay (**item number D06227)** on the DxI 9000 Access Immunoassay Analyzer (P/N C11137) on the intended use population and establish clinical concordance to Access BNP Assay (**item 98200, original formulation**) on the Access 2 Immunoassay system (P/N 81600N). The purpose of the protocol is to perform specimen testing (samples collected under the Access BNP Clinical Enrollment Protocol) to support clinical validation and the IUO Access BNP Assay's regulatory approval on the DxI 9000 Access Immunoassay Analyzer (P/N C11137). In addition, this protocol tests the samples using Access BNP assay (**item number 98200, original formulation**) on the Access 2 Immunoassay System (P/N 81600N) to perform a clinical concordance analysis to support global submissions, product launch and customer support materials.
Investigational Material/Product	IUO Access BNP for DxI 9000 Access Immunoassay Analyzer Reagent Pack (**item number D06227**)
Current State of the Art	Clinical practice guidelines[3,4,6] demonstrate the generally accepted state of the art as an aid in the diagnosis of congestive heart failure (also referred to as heart failure), assessment of severity of heart failure, and risk stratification of patients with heart failure, and in risk stratification of patients with acute coronary syndromes with other recommended tests.[3,4,6] The assessment of literature demonstrates that the currently marketed Access BNP assay is a useful tool to accurately measure BNP levels.
Study Objectives	The objective is to validate established diagnostic cutoff (rule-out) and assess HF severity on the IUO Access BNP Assay using the DxI 9000 Access Immunoassay Analyzer (P/N C11137). A method concordance analysis will be conducted between the IUO Access BNP (**item number D06227)** for DxI 9000 Access Immunoassay Analyzer (P/N C11137) and the Access BNP assay (**item number 98200;** original formulation) for Access 2 Immunoassay System (P/N 81600N).

Study Design	• This is a typical diagnostic device evaluation study with a single arm and no active intervention. • **A method concordance analysis** will be conducted between the IUO Access BNP assay (item number D06227) for DxI 9000 Access Immunoassay Analyzer (P/N C11137) and the Access BNP assay (Item number 98200, original formulation) for Access 2 Immunoassay System (P/N 81600N). • **A minimum of three (3) calibrator lots and a minimum of three (3) IUO reagent pack lots (Item number D06227) for DxI 9000 and three (3) IVD reagent pack lots (item 98200, original formulation) for Access 2 will be used in this study.** • Each site will be provided a minimum of one (1) unique calibrator lot and a minimum of three (3) individual IUO Access BNP assay reagent pack lots **(Item number D06227)** for DxI 9000 from Sponsor. The IUO reagent lots will be delivered at designated time points determined by Sponsor. • Each site will be provided a minimum of one (1) unique calibrator lot and a minimum of three (3) individual IVD Access BNP assay reagent pack lots **(item 98200, original formulation)** for Access 2 from Sponsor. The reagent lots will be delivered at designated time points determined by Sponsor.
Inclusion Criteria	• There are no inclusion criteria. Samples tested in this protocol are collected under BNP-05-24.
Exclusion Criteria	• Hemolysis observed 4+ per CLSIC56A grading scale. • Icterus (bilirubin) observed 4+ per CLSIC56A grading scale.
Target Population/ Sample Size	Specimens collected under BNP-05-24 protocol (titled: Access BNP Clinical Enrollment Study) are used. Approximately 1100 K2 EDTA samples with concentrations that span the analytical measuring range (AMR) are used in this testing study.
Site Requirements	A minimum of one (1) and approximately three (3) external testing sites that have a DxI 9000 Access Immunoassay Analyzer (P/N C11137) and Access 2 Immunoassay System (P/N 81600N).
Study Duration	The study duration for the clinical performance evaluation testing study is estimated to last approximately 12 months for sample testing and an estimated 3 additional months for data analysis.
Statistical Methods	All Statistical analysis will be performed by the Sponsor in accordance with **CLSI EP12** guideline. A separate statistical analysis plan will detail the analysis methods used. Primary endpoints of the study will be diagnostic accuracy between final clinical site diagnosis (HF and Non-HF) and IUO BNP assay testing results and severity assessment utilizing NYHA classification.

	Method concordance will be established using the valid testing data from the investigational device Access BNP Assay for DxI 9000 Access Immunoassay Analyzer (P/N C11137) against the currently marketed Access BNP Assay for Access 2 Immunoassay System (P/N 81600N). Positive percent agreement (PPA) and Negative percent agreement (NPA) will be calculated using the data.

3. Introduction

3.1. Background

Heart Failure (HF) is a complex medical condition that often exists with other comorbidities. Further, symptoms of HF are nonspecific making HF diagnosis difficult. B-type natriuretic peptide biomarkers (BNP and NT-proBNP) are standard-of-care biomarkers measured at patient presentation in the emergency department (ED) for patients presenting with signs and symptoms suggestive of heart failure. The release of natriuretic peptides (NP) appears to be in direct proportion to ventricular volume expansion and pressure overload. The plasma concentrations of both hormones are increased in patients with asymptomatic and symptomatic left ventricular dysfunction, permitting their use in supporting a clinical diagnosis of HF (but not used alone for this purpose). NPs are well established for use in diagnosis of patients with HF and are included in standard of care guidelines for both the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) and European Society of Cardiology (ESC).[1,2,3,4]

In the Breathing Not Properly Study, B-type natriuretic peptide test accurately diagnosed HF in patients presenting to the ED with dyspnea, with a sensitivity of 90% and specificity of 76% at a cutoff of 100 pg/mL.[5] The current commercialized Access BNP assay (primary predicate) for the Beckman Coulter Access Family of Immunoassay Systems has similarly established that BNP results less than or equal to 100 pg/mL are representative of normal values in patients without heart failure.

The Access BNP assay is being redesigned for transference to the DxI 9000 Access Immunoassay Analyzer (P/N C11137).

Specimen collected under BNP-05-24 “Access BNP Clinical Subject Sample Collection Enrollment Study Protocol” are to be tested on the investigational-use-only (IUO) Access BNP on Beckman Coulter DxI 9000 Access Immunoassay Analyzer (P/N C11137).

3.2. Study Purpose

The overall aim of the research is to establish clinical performance of the Access BNP Assay (**item number D06227)** on the DxI 9000 Access Immunoassay Analyzer (P/N C11137) on the intended use population and establish clinical concordance to Access BNP Assay (**item 98200, original formulation**) on the Access 2 Immunoassay system (P/N 81600N).

The purpose of the protocol is to perform specimen testing (samples collected under the Access BNP Clinical Enrollment Protocol) to support clinical validation and the IUO Access BNP Assay's regulatory approval on the DxI 9000 Access Immunoassay Analyzer (P/N C11137). In addition, this protocol tests the samples using Access BNP assay (**item number 98200, original formulation**) on the
Access 2 Immunoassay System to perform a clinical concordance analysis to support global submissions, product launch and customer support materials.

3.3. Subject Risk Benefit Analysis

3.3.1. Potential Risks

There are no potential known risks to the subjects during the testing phase of the clinical study.

The testing will be performed using de-identified clinical samples that will be supplied to the testing sites by the Sponsor. The testing is performed on samples containing only a unique sample identification number. Subject information such as names or addresses are not included. Protected health information (PHI) will not be revealed to the sponsor and will not be a part of the barcode label.

3.3.2. Potential Benefits

There is no direct benefit to the subject from participating in the study.

Potential benefits to the subject may include knowing that their contribution could benefit medical science in the future and could facilitate the development of accurate blood tests for detecting and monitoring disease. No results obtained from any analyses will be used to determine or alter the patient's treatment or care.

3.4. Investigational Device Description

IUO Access BNP for DxI 9000 Reagent Pack Description (item number D06227):

The Access BNP test is a two-site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with mouse monoclonal anti-human BNP antibody-alkaline phosphatase conjugate and paramagnetic particles coated with mouse Omniclonal anti-human BNP antibody. BNP in human plasma binds to the immobilized anti-BNP on the solid phase, while the mouse anti-BNP conjugate reacts specifically with bound BNP.

After incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of analyte in the sample. Analyte concentration is automatically determined from a stored calibration.

3.5. Comparator Device Description

The comparator device(s) are:

Beckman Coulter

- **Assay:** Access BNP (**Item Number 98200**, original formulation)
- **Instrument:** Beckman Coulter **Access 2** Immunoassay System (P/N 81600N)

Materials required for the comparator test(s) and analyzer model(s) used shall be in accordance with the manufacturer instructions for use.

3.6. Intended Use

Reagent (Item Number D06227)	The Access BNP test is intended for use with the Beckman Coulter DxI Access Family of Immunoassay Systems for the *In Vitro* quantitative measurement of B-type natriuretic peptide (BNP) in plasma specimens using EDTA as the anticoagulant. The test is intended to be used for the following indications: • as an aid in the diagnosis of congestive heart failure (also referred to as heart failure) • as an aid in the assessment of severity of congestive heart failure • for the risk stratification of patients with acute coronary syndromes • for the risk stratification of patients with heart failure
Reagent (Item number 98200, original formulation)	The Access BNP test is intended for use with the Beckman Coulter Access Family of Immunoassay Systems for the In Vitro quantitative measurement of B-type natriuretic peptide (BNP) in plasma specimens using EDTA as the anticoagulant. The test is intended to be used for the following indications: • as an aid in the diagnosis of congestive heart failure (also referred to as heart failure) • as an aid in the assessment of severity of congestive heart failure • for the risk stratification of patients with acute coronary syndromes • for the risk stratification of patients with heart failure

Calibrators (Item Number 98202)	The Access BNP Calibrators are intended to calibrate the Access BNP test for the quantitative determination of BNP levels in human EDTA plasma using the Beckman Coulter Access Family of Immunoassay Systems.
QC (Item Number 98201)	The Access BNP QC Controls are intended for monitoring the performance of the Access BNP test using the Beckman Coulter Access Family of Immunoassay Systems.

3.7. Training and Proficiency

Study Protocol training is conducted by Sponsor with study site personnel prior to site initiation to develop familiarity with the investigational assay testing procedure(s) and data export/transfer procedure(s). This may also involve performing instrument startup, quality control, sample preparation, sample handling and testing. Investigation-use only (IUO) Instructions for Use (IFU) documents are to be reviewed and all steps of the study are to be understood prior to the study start.

Sites are to run through all procedural steps to ensure understanding of the study requirements. Data collected during familiarization is not used in the study analysis.
Site personnel are expected to have proficiency in clinical laboratory standard operating procedure (SOP).

3.8. Investigational Sites

The research is expected to be at a minimum of one (1) and a target of three (3) external testing sites.

The participating sites shall have:

- Ability to perform testing on the Beckman Coulter DxI 9000 Access Immunoassay Analyzer (P/N C11137) and Beckman Coulter Access 2 Immunoassay System (P/N 81600N).
- Appropriately trained staff to perform study testing requirements.
- A refrigerator, -20°C and -70°C freezer (or colder) to store reagents, calibrators, QC material, and study specimens within the specific temperature requirements of each product instruction for use**.**

3.9. Study Duration

The study duration is estimated to last approximately 12 months for sample testing. However, study sample testing is officially complete when the site completes testing of the sample count provided by Sponsor. This may be more or less than the estimated 12 months. Data analysis may take another estimated 3 months.

4. Source Documents and Access to Source Data/Documents

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Study staff will permit authorized representatives of Beckman Coulter, Inc. and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

Source documents may include instrument files, maintenance logs, temperature logs, etc. Instrument files may be captured as outlines in section 5.2 while paper-based logs may be maintained by the site and uploaded electronically. Study staff and sponsors staff will have controlled access.

5. Data Handling and Record Keeping

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate instrument data source documentation.

5.1. Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, which will ensure that they are accurate and complete. Unanticipated problems must be reviewed by the investigator or designee.

5.2. Data Capture Methods

Data is to be directly captured from the laboratory instruments that perform testing. Where possible, the site is to electronically transfer laboratory test result instrument data to Beckman daily or as agreed upon by Sponsor. Data extraction and transfer instructions are to be provided during site initiation training and retained as study-specific documentation in the Investigator Site File (ISF).

An electronic data capture system is not planned for this study. If one is required because the testing site cannot provide an output of laboratory test results directly from the instrument, it is to be password protected with appropriate data quality checks for an electronic data system. If an additional data capture system is required (e.g., eCRF), site personnel shall receive proper training coordinated by the Sponsor.

5.3. Types of Data

Data collected is laboratory information on assay test performed on the Beckman Coulter DxI 9000 Access Immunoassay Analyzer (C11137) and Beckman Coulter Access 2 Immunoassay System (P/N 81600N) as required by this protocol.

5.4. Study Records Retention

Study records will be maintained for at least three years post market approval.

6. Ethics/Protection of Human Subjects

6.1. Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

6.2. Institutional Review Board/Independent Ethics Committee

The protocol, and other applicable study materials will be submitted to the IRB/IEC for review and approval. Approval of both the study and the protocol must be obtained before site is initiated. As applicable, amendments to the protocol will be reviewed for required submission to the IRB/IEC for review and approval before the changes are implemented in the study.

6.3. Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor. Site personnel do not have access to medical standard of care test results or other subject identifying data beyond a sample identification (ID) number for the purposes of conducting this protocol required testing procedures. Only subjects that consented to this use of the collected samples have been included for testing.

The samples used in this testing protocol are collected under protocol BNP-05-24 Access BNP Assay Clinical Subject Sample Collection Enrollment Study Protocol. Samples that will be

included in analysis after testing are from subjects that were appropriately consented and have not been withdrawn or discontinued, met study inclusion/exclusion criteria, and provided sufficient specimen sample volume required for testing.

Samples collected under protocol BNP-05-24 are anonymized to this protocol's testing sites and in compliance with regulations on data privacy and patient confidentiality.

7. Objectives

7.1. Study Objectives

The objective is to validate established diagnostic cutoff (rule-out) and assess HF severity on the IUO Access BNP Assay using the DxI 9000 Access Immunoassay Analyzer (P/N C11137).

A method concordance analysis will be conducted between the IUO Access BNP (**item number D06227)** for DxI 9000 Access Immunoassay Analyzer (P/N C11137) and the Access BNP assay (**item number 98200;** original formulation) for Access 2 Immunoassay System (P/N 81600N).

7.2. Study Endpoints

Primary endpoints of the study will be diagnostic accuracy between final clinical site diagnosis (HF and Non-HF), IUO BNP assay testing results and severity assessment utilizing the NYHA class.

8. Study Design

This is a typical diagnostic device evaluation study with a single arm and no active intervention.

- Approximately 1100 patient samples (K2 EDTA) collected under BNP-05-24 protocol (titled: Access BNP Clinical Enrollment Study) with concentrations that span the analytical measuring range will be tested.
- Samples are tested with the IUO Access BNP assay **(item number D06227)** on Beckman Coulter DxI 9000 Access Immunoassay Analyzer (P/N C11137) and Access BNP assay (**item number 98200; original formulation)** on Beckman Coulter Access 2 Immunoassay System (P/N 81600N) one (1) run per day, one (1) replicate per run.
- Testing will be performed at a minimum of one (1) and approximately three (3) external testing sites that have the Beckman Coulter DxI 9000 Access Immunoassay Analyzer (P/N C11137) and Access 2 Immunoassay System (P/N 81600N).

- **A minimum of three (3) calibrator lots and a minimum of three (3) IUO reagent pack lots (Item number D06227) for DxI 9000 and three (3) IVD reagent pack lots (item 98200, original formulation) for Access 2 will be used in this study.**
- Each site will be provided a minimum of one (1) unique calibrator lot and a minimum of three (3) individual IUO Access BNP assay reagent pack lots **(Item number D06227)** for DxI 9000 from Sponsor. The IUO reagent lots will be delivered at designated time points determined by Sponsor.
- Each site will be provided a minimum of one (1) unique calibrator lot and a minimum of three (3) individual IVD Access BNP assay reagent pack lots (Item 98200, original formulation) for Access 2 from Sponsor. The reagent lots will be delivered at designated time points determined by Sponsor.

9. Study Requirements

9.1. Familiarization Testing

Purpose: Familiarization testing is performed by study personnel prior to starting study testing in order to develop familiarization with the investigational assay testing and data submission procedures to Sponsor. Results of this testing are used to confirm that quality control acceptance criteria are being met for the assay on the Access DxI 9000 Access Immunoassay Analyzer at the site.

Procedure:

- Familiarization testing includes calibrating the assay and running commercial QC on a minimum of one (1) lot of QC as instructed by Sponsor.
- Run QC in **replicates of two (2) at each QC level each day, over a minimum of three (3) days** on Beckman Coulter Access DxI 9000 Access Immunoassay Analyzer.
- Samples are run on minimum of one (1) calibrator lot and minimum of one (1) reagent lot at the site.
- Verify that Quality Control samples are within the ranges provided by Sponsor.
- Following completion of the familiarization testing, Sponsor is to review the data and notify the laboratory when to proceed with the study testing.

Familiarization results may be used to fulfill the familiarization study requirement in other protocol(s) that the site participates in (or vice versa) if the same instrument and personnel are used and the familiarization procedure requirements outlined in this protocol are met.

9.2. Instrument Maintenance and Qualification

If applicable, instrument installation and installation qualification/operational qualification/performance qualification (IQ/OQ/PQ) are to be performed for each Beckman Coulter DxI 9000 Access Immunoassay Analyzer (P/N C11137).

Each day prior to study testing, all required routine maintenance must be completed and documented according to manufacturer's required instructions and laboratory policies for all instruments and/or equipment utilized in the study.

Per manufacturing instructions for the Beckman Coulter DxI 9000 Access Immunoassay Analyzer (P/N C11137) instruments:

- The user interface notifies you when maintenance tasks are due, provides instructions for performing the maintenance tasks, and contains a maintenance log to record that the maintenance was performed.
- Periodic maintenance includes the following types of maintenance:
    - Time-Interval Maintenance—Maintenance that is scheduled to occur after a specified period, such as daily, weekly, monthly, or yearly.
    - Test-Interval Maintenance—Maintenance that is scheduled to occur after a specified number of tests or operations.
- The system keeps a maintenance log of all completed maintenance tasks. Maintenance records may be saved or printed.

Additional maintenance may be required at the request of Sponsor. As applicable, to help maintain instrument performance and reliability, a trained service representative is to coordinate periodic maintenance activities for your analyzer in accordance with the terms of your service agreement.

9.3. Calibration

The commercial Access BNP Calibrators **(item number 98202)** are provided at six (6) levels: zero (0), and approximately 25, 100, 500, 2,500, and 5,000pg/mL. Refer to calibration card for exact concentrations.

Assay calibration data are valid up to 28 days. Run the calibrators in duplicate.

9.4. Comparator Method

The calibration and maintenance of the Access 2 Immunoassay System (P/N 81600N) should be performed per manufacturer's instructions.

9.5. Quality Control Analysis

- Commercial Access BNP QC **(item Number 98201)** is to be run in **replicates of two (2) at each QC level each day prior to performing study testing procedure**.

  - The results of the QC testing are to be evaluated against ranges provided by Sponsor.

- Commercial QC for the Access 2 Immunoassay instrument is to be run in **replicates of two (2) each day prior to performing study testing procedure**.

9.6. Quality Control Acceptance Criteria

- Site study staff must verify that all control levels meet acceptance criteria before analyzing any study samples.
  - **Do not thaw** any test samples until results of quality control have met specifications.
  - One of the two following scenarios must be true for each level of QC:
    - Both QC replicates are within acceptable ranges provided by sponsor.
    - The mean of two replicates and at least one of the two replicates are within the acceptable QC ranges provided by sponsor.

- If QC acceptance criteria is not met, notify and follow direction of Sponsor. Do not proceed to testing study procedure until directed by Sponsor.
  - When outside the acceptable range, the operator is to document any actions taken to have QC fall within range.
  - Documented information is to be provided to Sponsor.

9.7. Reagent/Product Handling and Storage

IUO ACCESS BNP for DxI 9000 **Reagent Pack (item Number D06227)** handling and storage:

Stability	
Unopened at 2 to 10°C	Up to stated expiration date
After opening at 2 to 10°C	28 days

- **Store upright.**
- **Refrigerate at 2°C to 10°C for a minimum of two hours** before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., a broken elastomer), discard the pack.

ACCESS BNP for Access 2 Immunoassay System Reagent Pack (Item Number 98200, original formulation) handling and storage:

Stability	
Unopened at 2 to 10°C	Up to stated expiration date
After opening at 2 to 10°C	28 days

- **Store upright.**
- **Refrigerate at 2°C to 10°C for a minimum of two hours** before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., a broken elastomer), discard the pack.
- Discard reagents if any discoloration is observed.

ACCESS BNP **Calibrator (Item Number 98202)** handling and storage:

- Provided ready to use.
- Store upright and freeze at -20°C or colder in a non-defrosting freezer away from the freezer door.
- Stable until the expiration date stated on the label when stored at -20°C or colder.
- Vial is stable at 2 to 10°C for 30 days after initial use or when removed from frozen storage.
- Signs of possible deterioration are quality control values out of range.
- Sponsor will provide exact concentrations.

ACCESS BNP **QC (Item Number 98201)** handling and storage:

- Provided ready to use.
- Store upright and freeze at -20°C or colder.
- Mix contents thoroughly by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at -20°C or colder in a non-defrosting freezer away from the freezer door.
- Vial is stable at 2 to 10°C for 30 days after initial use or when removed from frozen storage.
- Signs of possible deterioration are quality control values out of range.
- Sponsor will provide QC mean values and standard deviations.

9.8. Sample Handling and Storage

- Each testing site may receive shipments throughout each month from multiple sample collection sites. Upon receipt:
    1. Remove temperature data logger (e.g. DeltaTrak®) from shipment container
    2. Transfer samples upon receipt to -70 C (or colder) storage.
    3. Follow study guide for temperature data logger (e.g. DeltaTrak®) for data handling and upload.

- Frozen samples shall be stored frozen at -70°C (or colder) and remain in a frozen state until results of quality control specifications are met.
    - Record appropriate equipment temperatures on the Temperature Log.

- **Thaw samples only once, allowing to equilibrate to room temperature <u>for a minimum of 30 minutes prior to testing</u>.**
    - Complete the Daily Run Log
    - Do not test samples that meet Exclusion Criteria:
        - Hemolysis (hemoglobin) sample quality observation 4+ CLSI C56A grading scale.[6]

- Icterus (bilirubin) sample quality observation 4+ CLSI C56A grading scale.[6]

- **Testing must be completed on both DxI 9000 and Access 2 instruments within 60 days of the blood collection date and within 2 hours of removal from the -70 C (or colder) freezer on the same aliquot.**
    1. After specimen have thawed, they should be mixed by gently inverting several times.
    2. Centrifuge following the manufacturer's instructions for use. (Note: Standard recommended instruction is 3,000 RCF for 10 minutes).
    3. Following centrifugation, test specimens following this protocol's testing procedure **(*See section 11.3 Investigational Method*)**.
    4. Transfer each sample to appropriate instrument sample cups; follow assay requirements for minimum assay dead volumes and assay volume requirements.

- After testing and valid result confirmed, store aliquots with remaining thawed specimen in the -70 C (or colder) freezer.
    - Clearly mark partially used aliquots.
    - Sponsor will give direction on final disposition of the samples prior to the study close out visit.

9.9. Investigational Product Accountability

Sponsor is to document the location of all investigational materials from shipment of the devices to the testing sites until return or disposal. The testing site investigator is responsible for accountability of the investigational device at the site. Access to the investigational device/ product is controlled and use is to be limited to the clinical study only by authorized staff and in accordance with the protocol. The site is required to maintain the inventory of these materials provided and document the reconciliation of these materials. At the completion of this clinical study, all unused materials, supplies, and samples and all unused, used, expired, or malfunctioning investigational device (reagents or instruments) must be returned to Sponsor or disposed of according to instructions from Sponsor. If materials are disposed at the study site, the Investigator must provide Sponsor with a signed record of disposition. Sponsor may provide a log to the site. The investigator or designee is to keep records documenting the receipt, use, return and disposal of the investigational device. The records are to include the following (if applicable):

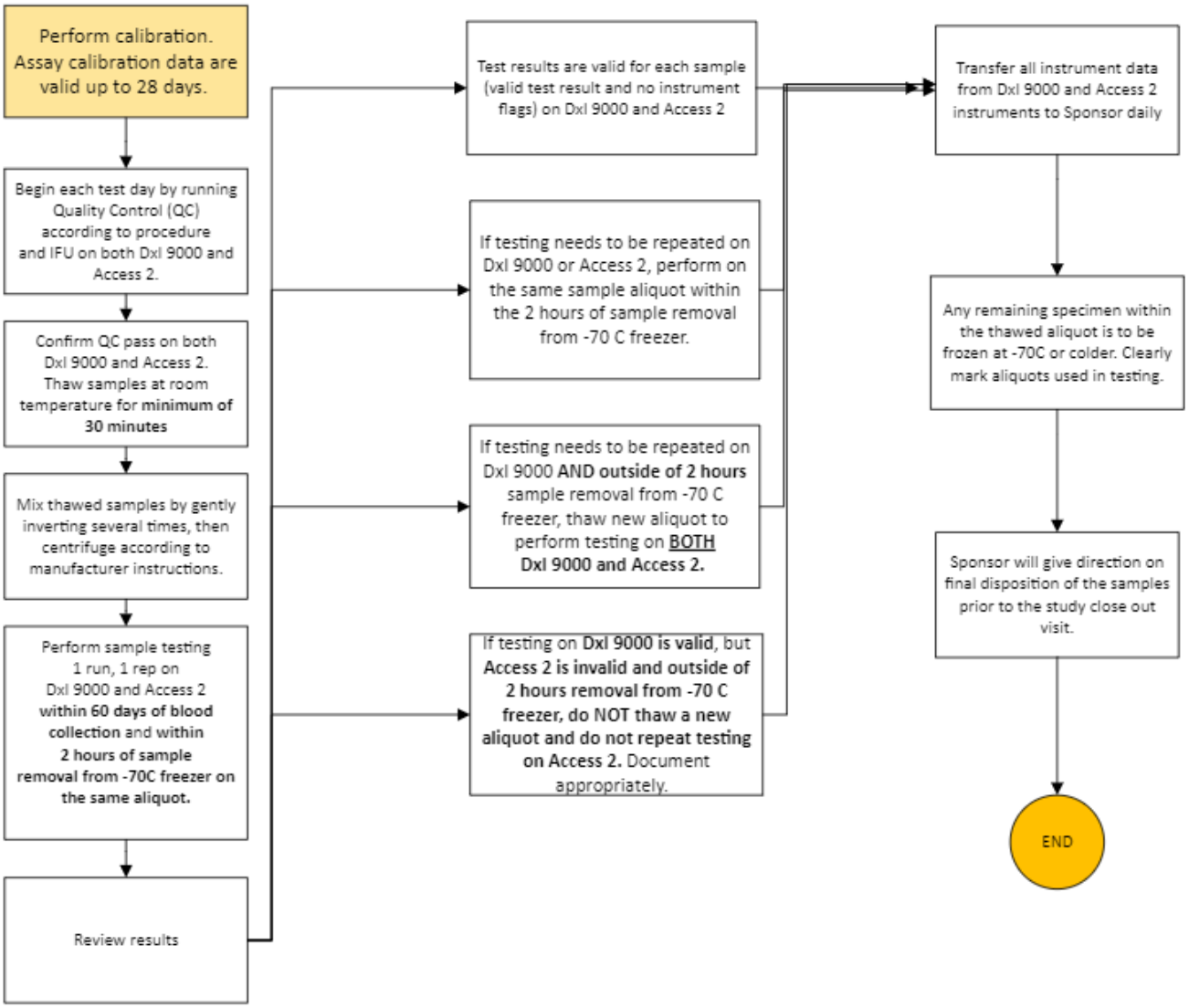
- Date of receipt
- Identification of each IVD medical device (e.g., batch number, serial number, or unique code)
- Expiry date
- Date or dates of use
- Date on which the investigational device was returned or disposed of, when applicable.
- Date of return of unused, expired, or malfunctioning product, when applicable.

9.10. Premature Study Termination or Suspension

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending, or terminating party. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB/IEC and will provide the reason(s) for suspension or termination.
Circumstances that may warrant termination include, but are not limited to:

- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination of futility.

10. Study Flow Chart



11. Study Testing

11.1. Sample/Isolate Requirements

Specimen were collected under protocol BNP-05-24 Access BNP Clinical Subject Sample Collection Enrollment Study Protocol from subjects presenting with a clinical suspicion of new onset heart failure or worsening symptoms suggestive of decompensated or exacerbated heart failure.

- K2 EDTA plasma samples are collected with IRB/IEC oversight and in accordance with local laws and regulations.
- Samples are expected to reasonably represent the U.S. demographic population.
- Samples shall be preserved in frozen state and stored frozen -70°C (or colder) until ready for testing (i.e., results of quality control specifications are met).
- Sponsor will monitor for sample concentration across the Analytical Measuring Range (AMR) with the Access BNP assay on the DxI 9000 Immunoanalyzer (i.e., 5 – 5000 pg/mL).

11.2. Comparator Method

The calibration and maintenance of the Access 2 Immunoassay System (P/N 81600N) should be performed per manufacturer's instructions.

11.3. Investigational Method

- Each site to receive K2 EDTA subject samples, collected under a separate protocol, for testing in approximately monthly shipments, or at other intervals determined by Sponsor, for the duration of the study.
- Testing must be completed on both the DxI 9000 Access Immunoassay Analyzer (P/N C11137) and Access 2 Immunoassay System (P/N 81600N) **within 60 days of the blood collection date and within 2 hours of removal from the -70 C (or colder) freezer on the same aliquot.**
- Refer to section 9.8 Sample Handling and Storage for a detailed instruction.
- Samples are tested with the IUO Access BNP assay on Beckman Coulter DxI 9000 Access Immunoassay Analyzer (P/N C11137) and Access 2 Immunoassay System (P/N 81600N).
- **Perform one (1) run, one (1) replicate for each specimen.**
    - Confirm test result **on DxI 9000 is valid for each study subject** (i.e., valid test value and no instrument flags).
    - **If testing needs to be repeated on DxI 9000 or Access 2, perform on the same sample aliquot within the 2 hours of sample removal from -70 C freezer.**

- **If testing needs to be repeated on DxI 9000 AND outside of 2 hours sample removal from -70 C freezer, thaw new aliquot to perform testing on BOTH DxI 9000 and Access 2.**
- **If testing on DxI 9000 is valid, but Access 2 in invalid and outside of 2 hours removal from the freezer, then do not thaw a new sample and do not test on Access 2. Document appropriately.**
- **NOTE:** Site shall electronically transfer all laboratory test data instrument file to Sponsor (Beckman) at **a minimum daily** or as agreed upon by Sponsor.

- Results shall **not** be invalidated, eliminated, or discarded by the site.
  - If Sponsor determines the test result(s) cannot be included due to the type of protocol deviation, re-testing shall be appropriately documented and repeated as part of the corrective action.

12. Assessment of Safety and Product Quality

To manage health and safety reporting and any product quality issues identified during the study, the clinical site is to consult and involve Sponsor. Cross functional teams that may get involved include but are not limited to the following: Clinical Affairs, Data Management, Service and Support, Supply Chain Management, Complaint Handling, Customer Technical Support, Product Development, and Regulatory Affairs. Reporting of issues shall follow internal Sponsor protocols and/or standard operating procedure(s). Methods to address product safety and quality issues is be documented in a log housed in Sponsor Master File and in the Investigator Site File (ISF).

The test device is an in vitro diagnostic device that does not come in contact with patients; no patient adverse reactions or complications are expected. If the investigational product results in the injury, death, or serious medical deterioration of the study staff, or if the potential for death, injury or serious medical deterioration exists, the Investigator must immediately contact Sponsor study site manager.

In the event the Principal Investigator identifies the test device does not meet safety or product quality expectations, they must immediately report findings to Sponsor study site manager.

For any report of product safety and quality issues, a report documented by study site staff is submitted to Sponsor study site manager to review and address.

13. Safety Warnings and Precautions

The Access BNP assay is **FOR INVESTIGATIONAL USE ONLY** on the DxI 9000 Access Immunoassay Analyzer (P/N C11137). The performance characteristics of this product have not been established. No clinical decision or patient notification may be made based upon results using this investigational use system.

Samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

14. Clinical Site Monitoring

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by Beckman Coulter®, Inc. The monitor will evaluate study processes and documentation based on the International Conference on Harmonization (ICH), E6: Good Clinical Practice guidelines (GCP).

Remote data monitoring is performed on instrument data (including test results, QC, and calibration data), temperature records, and service records. Remote data monitoring of instrument data is completed weekly at a minimum and approximately monthly for other records.

15. Regulatory and Administrative Requirements

15.1. Responsibility of Investigator

The obligations of the Principal Investigator(s) are as detailed in the Confidentiality Agreement, Clinical Studies Agreement, and the Investigator Protocol Signature Page.
In general terms, it is the responsibility of the Principal Investigator(s) to:

- Ensure that the clinical study is conducted in full conformance with all requirements of the clinical study protocol, study agreement, applicable ICH guidelines including Good Clinical Practice, 21 CFR 812.43 and other applicable FDA regulations, local and/or institutional regulations, and with all requirements of an Institutional Review Board (IRB)/ Independent Review Committee (IEC), if applicable.
- Keep any agreement, contract, or register that stipulates the responsibilities, attributions, and functions of all those involved in the clinical performance study.
- Maintain records of specimen/investigational product accountability and specimen/investigational product integrity.
- Disclose potential conflicts of interest, including financial, that could interfere with the conduct of the clinical performance study or interpretation of results as required by 21 CFR Part 54.
- Until either a letter of IRB/IEC approval or a letter stating IRB review and approval are not required has been provided to Sponsor.
- Provide any required updates of study progress to the IRB/IEC.
- Notify the IRB/IEC when the study is interrupted, discontinued, or completed.
- Ensure that the study is conducted in accordance with Good Clinical Practice guidelines.

- Comply with all applicable privacy laws, e.g., Health Insurance Portability and Accountability Act (HIPAA), Safe Harbor, etc.
- Ensure that study personnel are experienced with the Reference Method(s) used in this study.
- Ensure masking of samples, as required.
- Maintain an adequate internal quality control program and, if requested, to provide the sponsor with a copy of the laboratory's relevant calibration and maintenance procedures.
- Document relevant study-related communications.
- Permit the sponsor to monitor and audit study records (including source documents), if requested. Document review will be conducted in accordance with institutional requirements relating to the protection of study subject confidentiality, when applicable.
- Permit inspection of relevant study documents by regulatory agency personnel, as necessary.
- Immediately report any protocol deviation to the Sponsor and follow this initial report with any required documentation.
- Ensure study personnel are available during the study to answer questions and to provide requested information in a timely manner.
- Ensure study records are stored so that they are secure from damage (e.g., protected from water damage or fire).
- Ensure study records are stored in a way that maintains confidentiality (as specified in the Confidentiality Agreement and the Clinical Study Agreement).
- Ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

Retain the clinical study records until notified by Sponsor that the records may be destroyed or returned, postpaid, to Sponsor (refer to Code of Federal Regulations 21 CFR, 812.140, Records). If the Principal Investigator (or designate) needs to change custody of, or change the location of the clinical study records, the Principal Investigator (or designate) is responsible for contacting Sponsor to work out record retention logistics.

15.2. Responsibility of Sponsor

It is the responsibility of the Sponsor to:

- Provide the Investigator with a Clinical Study Protocol and any subsequent amendments.
- Provide the Investigator with a Clinical Study Agreement.
- Provide the Investigator with clinical samples as required.
- Provide the Investigator with QC values for QC lots.
- Make payments to the Principal Investigator or to the Investigator's institution according to the Clinical Study Agreement.
- Conduct auditing and monitoring of the site; reviewing the monitoring report(s) and following up any action(s) required in the monitoring report(s) taking prompt action to secure conformity with all clinical performance study requirements.
- Maintain accountability of IVD medical devices under investigation throughout the clinical performance study

- Document correspondence with all parties involved in the clinical study protocol including, when applicable, ethics committees and regulatory authorities
- Ensure that appropriate mechanisms are in place to cover the liability of the sponsor for the study (e.g., insurance)
- Provide study-specific training to the site study personnel prior to the study's initiation as needed.
- Review the clinical study protocol with the Investigator and the study personnel prior to the study's initiation.
- Provide the reagents and materials listed in the appropriate section of this clinical study protocol.
- Maintain the integrity of any study samples that are also required for the standard of care.
- Take prompt action to secure conformity with all study requirements.
- Ensure that IRB/IEC approval or waiver is obtained prior to study initiation at the site.
- Provide the Investigator with a final report.

15.3. Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff.

As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly. These practices are consistent with investigator and sponsor obligations in ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

No testing results shall be invalidated, eliminated, or discarded by the site. All deviations from the protocol must be addressed in source documents, reported to sponsor in a timely manner, and reported to the local IRB/IEC in accordance with IRB/IEC requirements. It is the responsibility of the Principal Investigator to determine if the IRB/IEC should be notified of this event.

15.4. Study specific or unique noncompliance concerns

Any noncompliance issue impacting the rights, welfare, and safety of human subjects are classified as a Major noncompliance and will be escalated following Beckman Coulter's standard operating procedure(s) and or Corrective Action Preventive Action (CAPA) process.

15.5. Changes to Protocol

In the event the study protocol is amended, Beckman Coulter will provide the Principal Investigator with the protocol amendment(s) and a revised protocol. It is the responsibility of the

Principal Investigator to determine if the protocol amendment(s) should be submitted for IRB approval.

Protocol amendments that affect the subject rights, safety, or welfare must be submitted to and approved by the IRB. The sponsor must be notified of the approval, in writing, prior to implementation.

Protocol amendments that are unrelated to subject rights, safety, or welfare may be submitted to the IRB at the discretion of the Principal Investigator. The Principal Investigator or designee must notify the sponsor, in writing, when he/she has determined that IRB approval is not required, prior to implementation.

15.6. Reconciliation of Study Material, Supplies and Samples

Sponsor is to supply enough quantities of study materials, supplies, and samples to enable the site to complete the study. The site is required to maintain the inventory of these materials provided and document the reconciliation of these materials.

At the completion of this clinical study, all unused materials, supplies, and samples must be returned to the sponsor or disposed of according to instructions from the sponsor. If materials are disposed at the study site, the Investigator must provide the sponsor with a signed record of disposition.

16. Statistical Considerations

The number of K2 EDTA individual subject samples used in this testing study will be approximately 1100.

All valid testing results of samples collected under protocol BNP-05-24 Access BNP Clinical Subject Sample Collection Enrollment Study Protocol from subjects presenting with a clinical suspicion of new onset heart failure or worsening symptoms suggestive of decompensated or exacerbated heart failure will be included in analysis.

Primary endpoints of the study will be diagnostic accuracy between final clinical site diagnosis (HF and Non-HF), IUO BNP assay testing results and severity assessment utilizing the NYHA class.

Method concordance will be established using the valid testing data from the investigational device Access BNP Assay **(item number D06227)** for DxI 9000 Access Immunoassay Analyzer (P/N C11137) against the currently marketed Access BNP Assay **(item number 98200, original formulation**) for Access 2 Immunoassay System (P/N 81600N). Positive percent agreement (PPA) and Negative percent agreement (NPA) will be calculated using the data.

The objective is to validate established diagnostic cutoff (rule-out) and assess HF severity on the IUO Access BNP Assay **(item number D06227)** using the DxI 9000 Access Immunoassay Analyzer (P/N C11137). Corresponding sensitivity and specificity at this cutoff will be established for the intended use population. Diagnostic parameters (i.e., sensitivity, specificity, NPV, and PPV) will be

defined by subjects' HF status as indicated by the IUO assay(s) relative to subjects' clinical HF diagnosis. Additionally, correlation between Access BNP assay results and severity (NYHA class) will be evaluated using the Jonckheere-Terpstra test. Point estimates, event counts, and a 2-sided 95% score confidence interval will be reported for all relevant endpoints.

In addition to the endpoint results, descriptive statistics such as mean median, standard deviations are to be reported. The results are used to support regulatory submissions for the Access BNP assay in all geographic regions, including but not limited to the European Union and United States (US).

The following requirements are strictly followed to prevent and minimize potential biases:

- Any DxI 9000 results deemed valid per the instruments' instruction for use cannot be excluded for any reasons other than documented human errors.
- Access 2 valid result can only be excluded from analysis in the case a retest is needed on DxI 9000 and outside of the 2-hour window of sample removal from the -70C (or colder) freezer. In this case a new sample is thawed and tested on both DxI 9000 and Access 2. The first Access 2 valid result would be excluded from analysis.
- Discrepancy resolution information may be obtained on individual cases for learning purpose only. Such information is not to be reported in any forms of estimation or statistics.

17. Study Materials

The study materials and supplies that will be provided by Beckman Coulter are:

- Testing Specimen/Samples
- IUO Reagent kits for Beckman Coulter DxI 9000 Access Immunoassay Analyzer*
- IVD Reagent kits for Beckman Coulter Access 2 Immunoassay System
- IVD Calibrators
- IVD Quality Controls
- Clinical Study Binder – eTMF Florence
- USB Flash Drives (if applicable)
- DxI 9000 consumables (as needed)

* Inventory of Investigational Use Only (IUO) material must be maintained, and reconciliation procedures provided by Sponsor after study completion must be followed.

Basic lab equipment and supplies are not provided by sponsor such as centrifuges, pipettors, lab coats and gloves. Such supplies are part of site lab operation at the oversight of the Primary Investigator.

18. Supplemental Materials Provided

As applicable, these documents may be provided to the investigational site by Sponsor to support the conduct of the protocol, however, are not considered part of the protocol. Modifications to these documents may be done and do not require an amendment to the protocol.

- Study Guide (if applicable)
- IUO Instructions for Use document **
- Material Safety Data Sheets
- Instrument Operator's Manuals
- Biosafety Precautions
- Laboratory Handling

** A change to IUO Instructions for Use document would prompt a protocol review to be conducted by Sponsor and if deemed necessary, shall require protocol amendment.

19. Literature References

1. Mueller T, Gegenhuber A, Poelz W, et al. Diagnostic accuracy of B type natriuretic peptide and amino terminal proBNP in the emergency diagnosis of heart failure. *Heart* 2005; 91:606-612.
2. Friedewald V, Burnett J, Januzzi J, Roberts W, et al. The Editor's Roundtable: B-Type Natriuretic Peptide. *The American Journal of Cardiology*. www.AJConline.org
3. Heidenreich PA, Bozkurt B, Aguilar D, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. J Am Coll Cardiol 2022;Apr 1.
4. Piotr Ponikowski, Adriaan A. Voors, Stefan D. Anker, et. al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur J Heart Fail.* 2016 Aug;18(8):891-975.
5. Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, et al. Breathing Not Properly Multinational Study Investigators. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl JMed*. 2002; 347:161–167.
6. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. J Am Coll Cardiol 2022;Apr 1.

APPENDIX A: Investigator Protocol Signature Page
PROTOCOL SIGNATURE PAGE

Protocol: Access BNP on DxI 9000 Clinical Performance Evaluation: Specimen Testing and Clinical Concordance Study

Protocol Number: BNP-02-23

Version: 2.7

Site Name: _____

The signature below attests that I have read and understand the contents of this protocol (or revisions to the protocol) and will adhere to the study protocol requirements as presented including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. Federal Regulations and ICH guidelines.

Investigator Printed Name: _____

Investigator Signature: _____

Date (dd/mmm/yyyy): _____

BECKMAN COULTER	**Access BNP Clinical Performance Evaluation: Testing and Clinical Concordance Study**	Document BNP-02-23 Protocol Version: 2.7

Content Change Abstract

Revision Level	Date	Author Name	Change Description
1.7	1/19/2024	Janicia Harris	Initial Release
1.13	6/18/2024	Polly Robar	• Cover Page: Updated DHF deliverable number and sponsor address. Removed “Assay” from the document title. • Updated the Long Title on header and title page for purposes of better accuracy. Short title and protocol ID remains unchanged. • List of Abbreviations: Added AMR, CKD, and eTMF. • Throughout document: -Corrected name of Access 2 from Analyzer to Immunoassay System -Clarified EDTA sample type if K2 EDTA -Changed source of samples from HF-01-19 to BNP-05-24. -Updated the number of samples to approximately 1100 per BNP-05-24. -Updated from Original Formulation to Formulation as per K033383. -Refer to the Access BNP assay as the “currently marketed Access BNP” for clarification. • Section 2 Study Synopsis -Updated the purpose statement for improved clarity of the research purpose to validate a comparison of results. -Study Duration has been extended to 12 months to accommodate a fresh blood draw sample requirement. -Target Population updated. Samples to be tested will now be from a prospective blood draw study (BNP-05-24 protocol ID). Samples will continue to be frozen in the -70C or colder temperature requirement. Testing will be in parallel within the sample stability claim of 60 days (-20C). -Updated inclusion and exclusion criteria. No inclusion criteria as samples come from BNP-05-24. Exclusion criteria is testing samples with HIL observation of 4+ hemolysis or icterus.

Revision Level	Date	Author Name	Change Description
			-Study Design updated to a minimum of three (3) calibrator lot and three (3) IUO reagent pack lots will be used in this study. Each site will be provided a minimum of one (1) unique calibrator lot and minimum of three (3) individual IUO reagent pack lots from Sponsor. The IUO reagent lots will be delivered at designated time points determined by Sponsor. • Update to section 6.3 to reflect subjects are consented under the BNP-05-24 protocol for samples used in this testing study. • Update to section 7.2 Study End Points: "Results are compared against acceptance criteria." • Update to section 8 Study Design - see above and section 2 Synopsis. • Update to section 9.1 For familiarization testing, samples are run on a minimum of 1 calibrator lot and a minimum of 1 reagent lot at the site. • Update to section 9.5 Quality Control Analysis to state results of QC will be evaluated against ranges provided by sponsor, removed number of standard deviations. • Update to section 9.6 QC Acceptance Criteria. Requirement for both replicates of any QC level to fall within ranges provided by sponsor, or the mean of 2 replicates must include one replicate in range. • Update to section 9.8 Sample Handling and Storage Replaced section with new text to follow IFU for sample thaw at room temp for 30 minutes minimum and test within 60 days of collection and 2 hours of sample removal from freezer on both instruments on the same aliquot. Instructions for use of data logger. • Update to section 10.0 Study Flow Chart Replaced flow diagram to reflect updated process steps. • Update to section 11.1 Sample Requirements. To align with enrollment protocol BNP-05-24. No spiking, diluting, depleting, pooling or contrived samples. • Update to 11.3 Investigational Method, Method Comparison Study Procedure Replaced text in this section to include samples to testing sites at approximately monthly intervals, test on both assays on same aliquot

Revision Level	Date	Author Name	Change Description
			within 60 days of blood collection and 2 hours removal from -70C freezer. If re-test, must be within 2 hours of removal from freezer. If outside of 2 hours a new aliquot must be tested on both assays / instruments. • Update to section 16 Statistical Considerations. Updated protocol from HF-01-19 to BNP-05-24. Method concordance is to validate against the currently marketed assay. • Update to section 17 Study Materials listing study materials and supplies that will be provided by BEC. • Update to section 18 Supplemental Materials that will no longer be provided by BEC. • Update to section 19, added sixth literature reference. • Appendix A: Updated protocol name
Version 2.3	8/21/2024	Polly Robar	• Overall study changed from Method Comparison to a Clinical Performance Evaluation Testing study. • Updated the Full and Short Title on header and title page to reflect the change in study design. • Section 2 Study Synopsis -Updated the purpose statement to reflect the research purpose to perform specimen testing to support clinical validation and the IUO Access BNP Assay's regulatory approval on the DxI 9000 Access Immunoassay Analyzer. -Updated study objective to is to validate the established diagnostic cutoff (rule-out) and assess HF severity on the IUO Access BNP Assay using the DxI 9000 Access Immunoassay Analyzer. -Updated Study design to reflect min 1 calibrator lot used on this study. -Site requirements updated to target of 3 sites will be used for testing. -Update to Statistical Methods to reflect study end point is diagnostic accuracy between final clinical site diagnosis (HF or Non-HF) and IUO BNP assay testing results. • Update to section 3.2 Study purpose, see above from study synopsis.

BECKMAN COULTER	**Access BNP Clinical Performance Evaluation: Testing and Clinical Concordance Study**	Document BNP-02-23 Protocol Version: 2.7

Revision Level	Date	Author Name	Change Description
			• Section 3.5 Comparator Device Description removed as it is no longer needed. • Update to section 3.7 Investigational Sites target of 3 sites will be used for testing • Update to section 7.1 Study Objectives to validate established diagnostic cutoffs (rule-out cut-off) and ability to assess HF severity. • Update to section 7.2 Study Endpoints Removed Method Comparison and Method Agreement as they are no longer applicable. Changed endpoint to diagnostic accuracy between final clinical site diagnosis (HF or Non-HF) and IUO BNP assay testing results. • Update to section 8.0 Study Design to remove reference to method comparison study and related CLSI guideline, reflect the target of 3 external sites instead of 1, and removed reference to testing on the Access 2 instrument. Updated to minimum 1 calibration lot to be used on this study. • Removed section 9.4 Comparator Method for calibration and maintenance as it is no longer applicable. • Updates to section 9.7 Sample Handling and Storage to remove references to testing on both instruments on the same aliquot and any reference to the predicate Access 2 instrument. • Updated section 10 Study Flow Chart to remove reference to testing on Access 2. • Removed section 11.2 Comparator Method for calibration and maintenance as it is no longer applicable. • New section 11.2 Investigational Method updated to remove testing on the predicate assay. Now will be testing on DxI 9000 only, 1 run 1 rep... • Updated section 12 Assessment of Safety and Product Quality with minor text updates. • Updated section 16 Statistical Considerations to reflect new approach and analysis for this clinical performance testing study. Primary endpoints of the study will be diagnostic accuracy between final clinical site diagnosis (HF and Non-HF) and IUO BNP assay testing results.

Revision Level	Date	Author Name	Change Description
			• Updated section 17 Study Materials to remove reagent kits for Access 2 as it is no longer needed. • Updated Appendix A protocol signature page to reflect the new protocol title.
2.7	9/12/2024, 5:09:43 PM	Polly Robar	• Overall change to add Clinical Concordance analysis of DxI 9000 results vs. Access 2. • Updated the Full and Short Title on header and title page to reflect the change in study design. • Throughout document added part number for DxI 9000 (C11137) and Access 2 (81600N) • Section 2 Study Synopsis -Updated Study Purpose to reflect addition of clinical concordance analysis vs BNP assay testing on Access 2. -Updated Study Design to add Method Concordance -Updated Statistical Method to include Method concordance. • Updated section 3.2 Study Purpose to add clinical concordance. • Added section 3.5 Comparator Device Description for the Access BNP assay and Access 2 Immunoassay system • Updated section 3.8 Investigational site to include the ability to test on the Beckman Coulter Access 2 Immunoassay System. • Updated 5.3 Types of Data to include data collected from the Beckman Coulter Access 2 Immunoassay System. • Updated section 7.1 Study Objectives to include Method Concordance. • Updated section 7.2 Study Endpoints to include Method Concordance. • Updated section 8 Study Design to include testing on Access 2 Immunoassay System. • Added section 9.4 Comparator Method to note calibration and maintenance of Access 2 instrument should be performed per manufacturer's instructions. • Updated section 9.5 Quality Control Analysis to mention how commercial QC on Access 2 is to be run.

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Revision Level	Date	Author Name	Change Description
			• Updated section 9.8 Sample Handling and Storage to require that testing be completed on both DxI 9000 and Access 2 on the same aliquot. • Updated section 10 Study Flow to include testing on Access 2. A valid result on Access 2 is not required. • Added section 11.2 Comparator Method to note calibration and maintenance of Access 2 instrument should be performed per manufacturer's instructions • Updated 11.3 Investigational Method to mention repeat testing on Access 2 is not required if valid result on DxI 9000. • Updated section 16 Statistical Considerations: - Any DxI 9000 results deemed valid per the instruments' instruction for use cannot be excluded for any reasons other than documented human errors. • Access 2 valid result can only be excluded from analysis in the case a retest is needed on DxI 9000 and outside of the 2-hour window of sample removal from the -70C (or colder) freezer. In this case a new sample is thawed and tested on both DxI 9000 and Access 2. The first Access 2 valid result would be excluded from analysis. • Updated section 17 Study Materials to include Access 2 materials. • Updated Appendix A PI Protocol signature page with the correct protocol title. • Updated Sr Manager approver name

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Approvals

The Project Specific TOC defines the approvers for this document (minimum approvers) and is based on GLB-QS-PCD-0046.

Role	Name	Title
Development (R&D)	Mo Quin	Staff Development Scientist
Development (R&D)	Ryan Saffert	Director, Development Science
Quality	Dominique Davidow	Staff Quality Assurance Scientist
Regulatory	Stephanie Garth	Principal Regulatory Affairs
Regulatory	Neha Desai	Staff Quality and Regulatory Affairs
Selling and Marketing	Julie Medina	Product Manager, CCIA Reagents
Clinical Biostatistics	Nicole Winden	Staff Biostatistician
Medical & Scientific Affairs	Lindsay (Xuan) Sun	Medical Director
Clinical Affairs Author	Polly Robar	Clinical Study Manager
Clinical Affairs Manager	Yolanda Sanchez	Senior Manager, Clinical Affairs

My signature confirms my review and approval of this document for its intended use.

APPROVALS:	APPROVED ON: *(mm-dd-yyyy)*
Robar, Polly	09/11/2024 19:22:15 (UTC)
Davidow, Dominique	09/11/2024 19:27:15 (UTC)
Garth, Stephanie	09/11/2024 19:47:25 (UTC)
Medina, Sompong J	09/11/2024 20:33:32 (UTC)
Quin, Maureen	09/11/2024 20:56:48 (UTC)
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Bettes, Nicole	09/12/2024 14:02:42 (UTC)
Saffert, Ryan	09/12/2024 16:17:34 (UTC)