BECKMAN COULTER	Access BNP Clinical Performance Evaluation Statistical Analysis Plan	Document #: NA

ACCESS BNP CLINICAL PERFORMANCE EVALUATION STATISTICAL ANALYSIS PLAN

Parent Document: Statistical Analysis Plan GLB-CA-PCD-0045

Project Name

BNP FOR DXI 9000

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My electronic signature confirms my approval of this document.

APPROVALS:	APPROVED ON (MM/DD/YYYY):
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Introduction

The IUO Access BNP assay (P/N D06227) is a paramagnetic particle, chemiluminescent immunoassay for the quantitative measurement of B-type Natriuretic Peptide (BNP) in human plasma using the DxI Access Immunoassay Analyzers. Diagnostic Accuracy of the IUO Access BNP assay will be evaluated primarily in a multi-center, prospective, study for sensitivity, specificity, Negative Predictive Value (NPV), Positive Predictive Value (PPV), and likelihood ratios (LR). Subjects will be evaluated around a rule-out cutoff.

This statistical analysis plan (SAP) is to provide details in conducting the study and analyses in order to achieve the study objectives and to ensure the quality of study in maintaining internal and external validity of the study.

The study will strictly follow the Access BNP Clinical Subject Sample Collection Enrollment Study Protocol (BNP-05-24) (EDMS title “Access BNP Clinical Enrollment Study”) and in compliance with the Good Clinical Practice (GCP) guideline. Additional relevant study background information can be found in the Access BNP on DxI 9000 Clinical Performance Evaluation: Specimen Testing Clinical Study (BNP-02-23) (EDMS title “Access BNP Clinical Performance Evaluation: Testing and Clinical Concordance Study”).

Purpose and Scope

This statistical analysis plan (SAP) covers study design, statistical analysis method, as well as data quality control for the diagnostic accuracy, where applicable, studies on Access BNP assay running on the DxI 9000 Access Immunoassay Analyzer. The SAP applies to all clinical studies required for regulatory body or peer reviewed journal submissions, directly conducted and managed by Clinical Affairs.

The results will be used to support regulatory submissions for the Access BNP assay in all geographic regions, including but not limited to the United States and European Union.

Proposed Intended Use

The intended use is documented in the “BNP 9000 User Needs” document.

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Study Objectives

Diagnostic accuracy of the Access BNP assay will be evaluated in a single prospective, multicenter, clinical study.

The objectives to evaluate the performance of the IUO Access BNP assay:

- To evaluate the clinical utility of the Access BNP assay by validating an established diagnostic rule-out cutoff and ability to assess Heart Failure (HF) severity.

Endpoints

The following measurements will be reported and evaluated for acceptable performance.

Primary Endpoints

Primary endpoints of the study will be diagnostic accuracy measured in proportions of sensitivity and specificity between Access BNP assay and final clinical HF status (determined by site diagnosis) and severity assessment measured by correlation between assay results and the New York Heart Association (NYHA) classification.

Secondary Endpoints

Secondary endpoints include:

- NPV and PPV overall
- Likelihood ratios overall
- Method concordance against Access BNP assay

There are no pass/fail criteria for secondary endpoints

Additional Endpoints

Additional endpoints include but are not limited to:

- Assay performance in different subgroups (eg: age, sex, comorbidities)
- Descriptive statistics for subjects with comorbidities
- Descriptive statistics by NYHA classification

Acceptable assay performance data are defined in respective assay design input document(s) (DIDs) and external assay performance requirements.

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Study Design and Sample Size

Study Design

This is a prospective-multicenter controlled clinical study to evaluate diagnostic accuracy and ability to assess heart failure severity of the Access BNP assay. A one-time blood sample is collected from consented and enrolled subjects, within 4 hours of presentation, representing the intended use population (adult subjects presenting with clinical suspicion of new onset heart failure or worsening symptoms suggestive of decompensated or exacerbated heart failure).

Clinical performance of the Access BNP Assay will be evaluated as an aid in the diagnosis and assessment of severity in adults suspected of having new onset heart failure or decompensated or exacerbated heart failure. Diagnostic parameters (i.e., sensitivity and specificity) will be defined by subjects' HF status as indicated by the IUO assay relative to the subjects' final clinical HF status.

Patient Enrollment

Individuals presenting with clinical suspicion of heart failure will be enrolled in the study. Subjects will be considered enrolled when informed consent is obtained via a signed and dated Institutional Review Board (IRB) approved Informed Consent Form (ICF).

Participating Centers

Study enrollment and blood sample collection are conducted at a minimum of 5 and a target of approximately sixteen (16) to eighteen (18) geographically diverse U.S. clinical research sites with Emergency Departments and/or Outpatient Clinics. All clinical sites follow the same enrollment protocol Access BNP Clinical Subject Sample Collection Enrollment Study Protocol (BNP-05-24) (EDMS title "Access BNP Clinical Enrollment Study").

Sample Testing

Specimens are to be collected in K2 EDTA plasma tubes. Collected samples will be tested, within 60 days of blood collection, at external clinical sites under the testing protocol Access BNP on DxI 9000 Clinical Performance Evaluation: Specimen Testing and Clinical Concordance Study (BNP-02-23) (EDMS title "Access BNP Clinical Performance Evaluation: Testing and Clinical Concordance Study").

Sample Size

Assuming an expected performance of IUO Access BNP assay of 82% sensitivity, 400 HF positive subjects can provide > 92% power to claim sensitivity of 82% with a lower bound

confidence interval of 75%. Additionally, 600 HF negative subjects can provide > 98% power to claim specificity > 98% with a lower bound confidence interval of 95%. Thus, with an assumed 40% prevalence and 10% attrition rate the total sample size is expected to be 1,100 subjects.

The following table depicts the sample size by potential rates of prevalence:

HF Prevalence	# of Subjects HF +	# of Subjects Total
30%	400	1333
40%	400	1000
50%	400	800

Potential Bias and Measures Used to Prevent Bias

This is a typical diagnostic device evaluation study with a single arm and no active intervention. The test assay determines and reports numerical BNP measurements on fully automated instruments. HF status determined based on the IUO test result are compared to the true HF clinical status.

In addition, the following requirements are strictly followed to prevent and minimize potential biases:

- Any results deemed valid per the instruments' instruction for use cannot be excluded for any reasons other than documented human errors
- Retest only allowed on samples without valid results
- Discrepancy resolution information may be obtained on individual cases for learning purpose only. Such information is not to be used in any forms of statistical calculation.

Internal/External Validity Verification

1. For sample representativeness, demographic information on all prospectively enrolled subjects in the Diagnostic Accuracy Study will be reported.
2. The Diagnostic Accuracy Study is a prospective controlled study with detailed inclusion/exclusion criteria, major subpopulations are the prespecified age groups. Samples will be tested randomly in experienced clinical laboratories with identical reagents and automated instruments provide by the sponsor. Data poolability is assumed and poolability analysis among 3 external testing sites, will not be performed in this study
3. Sample accountability will be examined and reported. All efforts will be made to prevent missing data. Missing data is expected to be rare. Missing data analysis

will only be performed if missing data appears to be an issue affecting the study conclusion.

4. Missing data imputation will not be attempted.

Statistical Methods

Primary and Secondary Endpoints

Sensitivity, specificity, NPV, PPV, and likelihood ratios (positive and negative) of the Access BNP assay will be evaluated by comparing the Access BNP assay results to the final clinical site diagnosis.

The following are definitions of diagnostic accuracy for the IUO Access BNP assay.

IUO Access BNP assay result		Final clinical site diagnosis (HF Status)	
		HF Positive	HF Negative
	≥ Respective Cutoff	TP *(a)*	FP *(c)*
	< Respective Cutoff	FN *(b)*	TN *(d)*

TP: true positive; FP: false positive; TN: true negative; FN: false negative.

Estimates of sensitivity and specificity are calculated as:

$$Sensitivity = 100 \times \frac{a}{a + b}$$

$$Specificity = 100 \times \frac{d}{c + d}$$

$$PPV = 100 \times \frac{a}{a + c}; \quad NPV = 100 \times \frac{d}{b + d}$$

$$LR^{+} = \frac{sen}{1 - spc} = \frac{a/(a + b)}{c/(c + d)}$$

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$$LR^{-} = \frac{1 - sen}{spc} = \frac{b/(a+b)}{d/(c+d)}$$

All enrolled subjects with valid Access BNP (P/N D06227) assay test result and final clinical site diagnosis will be included in the diagnostic accuracy endpoint analysis, *i.e.,* per protocol analysis. Point estimate, event counts, and a 2-sided 95% Score confidence interval will be reported.

Severity Assessment Study

Statistical significance of positive correlation between medians of the Access BNP assay results and NYHA class will be tested using the Jonckheere-Terpstra test. Medians of BNP values, measured by Access BNP assay, of subjects in NYHA HF class I, II, III, and IV will be reported.

Method Concordance Study

Method concordance between Access BNP on the DxI 9000 Immunoassay Analyzer (P/N D06227) and Access BNP on Access 2 (P/N 98200) (predicate) will be calculated based on the 100 pg/mL cutpoint. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) will be evaluated independently. Point estimates and confidence intervals will be reported for all estimates.

$$PPA = 100 \times \frac{\#\ of samples\ above\ cutpoint\ for\ both\ test\ and\ comparator\ assays *}{\#\ of\ samples\ above\ cutpoint\ by\ comparator\ assay *}$$

$$NPA = 100 \times \frac{\#\ of samples\ below\ cutpoint\ for\ both\ test\ and\ comparator\ assays *}{\#\ of\ samples\ below\ cutpoint\ by\ comparator\ assay *}$$

Additional Analyses

Event counts and point estimates will be reported on all informational endpoints. There will be no explicit pass/fail criterion or statistically supported claims made for any informational endpoints. Additional descriptive statistics will be reported when appropriate including mean, median, SD, minimum, maximum, Inter Quartile Range (IQR), 5th and 95th percentile.

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Informational data will also be provided and includes (but not limited to):

- Assay performance in different subgroups (ex: age, sex, comorbidities)
- Descriptive statistics for subjects with chronic kidney disease and other comorbidities
- Descriptive statistics by NYHA classification

Statistical Programs/Coding

All computation will be performed by SAS (version 9.4 or higher) using existing and validated code. Validated programs and the validation form will be stored in the appropriate Device history record.

Proposed Data Output

Standard demographic tables, including but not limited to age, gender, ethnicity, health history, region of residence will be reported in descriptive statistics. Additional descriptive statistics will be reported including but not limited to the following (stratified by gender and overall); mean, standard deviation, N, minimum, maximum, 5th and 95th percentiles, and percent greater than 100 pg/mL. Descriptive statistics of subjects with NYHA class I will be reported, if available.

Approvals

The SAP is reviewed and approved by the appropriate functions listed in GLB-QS-PCD-0046 in the control system e.g., EDMS.

Role	Name
Development (R&D)	Maureen Quin
Quality	Dominique Davidow
Regulatory	Neha Desai
Clinical Affairs	Polly Robar and Yolanda Sanchez
Other(s)	Nicole Winden (author – Biostats)

References

- CLSI EP12-A2 2008 User Protocol for Evaluation of Qualitative Test Performance

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- CLSI EP24-A2 2011 Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Draft Guideline-Second Edition
- ISO 20916: In vitro diagnostic medical devices- Clinical Performance studies using specimens from human subjects- Good Study Practice
- GLB-CA-PCD-0033 Analysis Validation
- GLB-QS-PCD-0046 Design Controls Approvals

Change Control

SUMMARY OF CHANGE: *Document creation*	
SECTION #	**REASON FOR CHANGE / OBSOLESCENCE**
N/A	*Document creation*

TRAINING REQUIREMENT

☐ Training is required for this revision.
- ☐ Initial release
- ☐ Remediation training
- ☐ Change affects process

☐ Training is not required for this revision.
- ☐ Change does not affect process
- ☐ Obsolescence

☒ Training is not required for this document type.

IMPACT ASSESSMENT

Evaluate any interrelated QMS and/or Business processes to determine if changes are required. If yes, list affected documents. If no, indicate no impact.	**IMPLEMENTATION PROCESS IMPACT**
	None
	IMPLEMENTATION PLAN
	None