Method Evaluation & the *i-STAT TBI* Cartridge

Best practices for a successful method evaluation of the *i-STAT TBI* cartridge with the *i-STAT Alinity* system.

A method evaluation supplies evidence that the accuracy, precision and reportable range of a new method are adequate to meet the needs of the patient population and clinicians as determined by the laboratory director and/or technical consultant. Semi-quantitative tests may also benefit from a method comparison for clinical correlation.

The Laboratory Director,

- selects the laboratory staff who will take part in the method evaluation process.
- determines the processes and procedures for method evaluation, along with their approval for use.

The method evaluation is not a manufacturer's requirement and specific details, or information related to the above activities may be obtained from your accreditation or regulatory organization.

Method Evaluation of the i-STAT TBI Cartridge | NPE-5399-REV1-APOC-EN (v1.2) | Revision Date: 31-MAY-2024

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Not all products available in all regions; visit www.globalpointofcare.abbott for product intended use and system details.

INTRODUCTION

In the United States, all laboratory testing is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) law.

OVERSIGHT

CLIA creates federal standards applicable to all U.S. laboratories or sites. This oversight includes method evaluations, which supply objective evidence that a method is fit for a particular purpose, meaning the quality test performance for a specific intended use is fulfilled (CLSI EP15).[1] These standards guide laboratories on the activities needed to verify a manufacturer's test method performance claims.

It may include the following activities:

- Analytical measurement range: linearity/ calibration verification (CLSI EP06) (12)[2]
- Precision: Measurement of the variability of the new test (CLSI EP05) (11)[3]
- Reference intervals (CLSI EP 28-A3c) (10)[4]
- Trueness/Accuracy: Measurement for comparison to truth (CLSI EP09) (9,10)[5]

TEST COMPLEXITY

Laboratory requirements based on test complexity may be found in the §CLIA 493.[6] To perform moderate complexity testing, a qualified laboratory director provides management and ensures that applicable federal standards are met (§CLIA 493.1405).[6]

The *i-STAT TBI* cartridge is categorized as a "moderate complexity"[7] test.

Additional activities may be needed for tests categorized as high complexity with responsibilities related to the laboratory's designated technical supervisor (§CLIA 493.1451(b)(4))[6]. Application of standards related to high complexity testing are not applicable to the *i-STAT TBI* cartridge when used as intended with the *i-STAT Alinity* system.

MANUFACTURER'S QUALITY SYSTEM INSTRUCTIONS

The Manufacturer's Quality System Instructions (MQSI) represent activities necessary to ensure quality results (accuracy, precision, and reliability) based upon the design of the *i-STAT* system.[8]

- Perform Daily Quality Control with Electronic Simulator (Internal, External).
- Check new or replacement analyzers with the Electronic Simulator. (Internal or External).
- Check temperature strip for a new shipment of cartridges or controls.
- Ensure proper cartridge storage.
- Perform thermal probe check every six months.
- Train staff.
- Update instrument software every six months.

CONTENTS

The system-based best practices and use recommendations in this document are intended to aid customers with method evaluation activities performed for the *i-STAT TBI* cartridge using the *i-STAT Alinity* system.

Instruments and cartridges should be used by healthcare professionals trained and certified to use the system and should be used according to the facility's policies and procedures.[8]

SUPPORT & SERVICES

With website, technical support, and implementation resources, Abbott provides a number of ways to obtain *i-STAT* product support.

PRODUCT INFORMATION

To access product information, such as Instructions for Use (IFUs), Quick Reference Guides, and System Operations Manuals, visit the *i-STAT Alinity* Support section of www.globalpointofcare.abbott.

TECHNICAL SUPPORT

Abbott is prepared to assist with any questions regarding our *i-STAT* family of products.

- **For customers in the United States:**
  E-mail: techsvc@abbott.com
- **For customers Outside of the United States:**
  E-mail: oustechsvc@abbott.com

ADDITIONAL SERVICES

Abbott provides customers with additional options and services, such as implementation support and statistical analysis. Customers may work with their Abbott representative regarding the availability of field services pertaining to:

- Support of various activities that are part of a successful implementation.
- Performance Verification (PV) reports for statistical analysis using data requirements provided by Abbott.

PRODUCTS

i-STAT TBI CARTRIDGE (REF/LIST NUMBER 03S09-25)

The *i-STAT TBI* test is a panel of *in vitro* diagnostic immunoassays for the quantitative measurements of glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in whole blood and a semi-quantitative interpretation of test results derived from these measurements, using the *i-STAT Alinity* instrument.[7] A "Not Elevated" TBI test interpretation is associated with the absence of acute traumatic intracranial lesions visualized on a head CT scan.[7]

The test is to be used with venous whole blood collected with EDTA anticoagulant in point of care or clinical laboratory settings by a healthcare professional.[7]

i-STAT ALINITY SYSTEM

"The *i-STAT Alinity System* is comprised of the *i-STAT Alinity* instrument, the *i-STAT* test cartridges and accessories (*i-STAT Alinity* Base Station, Electronic Simulator and Printer)."[7]

The *i-STAT Alinity* instrument is intended for use in the *in vitro* quantification of various analytes in whole blood or plasma in point of care or clinical laboratory settings.[8] "The instrument requires i-STAT single-use cartridges containing electrodes and sensors to perform quantitative diagnostic testing on whole blood or plasma. Together, the instrument and cartridge allow the user to perform clinical testing and related administrative tasks."[8]

LIQUID QUALITY CONTROL & CALIBRATION VERIFICATION MATERIALS

Per Abbott's manufacturer's quality system instruction (MQSI), liquid quality controls are used to verify the integrity of newly received cartridges and their storage conditions.[8]

Calibration Verification (CalVer) materials are available to assist customers in verifying the accuracy of results across the reportable range.

i-STAT TBI CONTROL LEVEL 1 (REF/LIST NUMBER 06P17-25)

i-STAT TBI CONTROL LEVEL 2 (REF/LIST NUMBER 06P17-26)

The *i-STAT TBI Control Levels 1 and 2* have been formulated to produce a test interpretation of elevated. Refer to the Value Assignment Sheet (VAS) for level-specific means and ranges.[9]

i-STAT TBI CAL/VER LEVELS 1-3 (REF/LIST NUMBER 06P17-24)

The *i-STAT TBI* Cal/Ver Levels 1-3 have been formulated and designed to provide results that span the reportable range of the test.[7]

VALUE ASSIGNMENT SHEETS (VAS)

Value Assignment Sheets provide the acceptable range for each level of control and calver materials based on cartridge lot number.

Value Assignment Sheets are available on the *i-STAT Alinity* Support page of Abbott's website; visit www.globalpointofcare.abbott.

Ensure that you retain the Value Assignment Sheets used during the method evaluation with your additional performance verification records.

PREPARATION

Prior to performing a method evaluation, Abbott recommends review of the cartridge, liquid quality control material and calibration verification material Instructions for Use (IFU), the device's Quick Reference guide and System Operations Manual.

To access product information, visit the *i-STAT Alinity* Support section of www.globalpointofcare.abbott.

LABORATORY PERSONNEL

The laboratory director must ensure that the staff selected are healthcare professionals trained and competent to use the *i-STAT Alinity* system, along with any related facility policies and procedures.

EQUIPMENT

Every *i-STAT* device used with the *i-STAT TBI* cartridge for patient testing may be included in the method evaluation.

For laboratories following CLIA regulations or Accrediting Organization's standards, each instrument's performance must be verified – even if there are multiple instruments of the same make and model (§CLIA 493.1253(b)(1)).[10]

Per CAP's COM.40000 Method Validation and Verification Approval - Nonwaived Tests Phase II, if multiple identical instruments or devices are in use, there must be records showing that the method performance specifications have been separately verified for each test and instrument or device.[11]

EQUIPMENT- cont'd

As the interpretation of regulations and standards provided by accreditation organizations varies, the Laboratory director must identify and implement their laboratory accreditor's requirements.

NOTE: Services available from Abbott for implementation and statistical analysis require at least two *i-STAT Alinity* instruments for duplicate testing to supply the imprecision data required by the statistical analysis software.

SOFTWARE REQUIREMENTS

The following software requirements are required prior to commencing the method evaluation:

- *i-STAT Alinity* instrument software version – OSi20 or greater.

To access device software, visit the *i-STAT Alinity* Support section of www.globalpointofcare.abbott.

ANALYZER CONFIGURATION OR CUSTOMIZATION

For best results, ensure that all *i-STAT Alinity* instruments are configured with the correct date, time and the most current software version.

- Refer to the *i-STAT Alinity Quick Reference Guide* for instrument customization and setup.
- If using *AlinIQ CWi* to customize the instrument with additional features, refer to the *AlinIQ CWi* section of the *i-STAT Alinity System Operations Manual*.

CONSUMABLES

USING THE CONSUMABLE CALCULATION WORKSHEET

Customers should ensure that they have enough products to perform the method evaluation activities as defined by their laboratory director. To aid customers with ordering i-STAT consumables (i.e., cartridges, liquid quality control, etc) for the method evaluation, a worksheet is provided on the next page. See below for considerations related to using the consumable calculation worksheet.

STUDY	CALCULATION	TOTAL (Example)
PRECISION • Includes all devices. • The laboratory director has discretion regarding the number of samples and replicates used to determine precision. For customers that request PV reports for statical analysis using Abbott's field services, a minimum of 20 replicates for each level is required. • Each box of controls provides 6 control vials for testing. Additional cartridges may be tested with the remaining fluid, if used within 4 hours of bringing the vial to room temperature. • Approximately 15 cartridge tests may be run within the 4-hour stability timeframe of the liquid quality control material.	Control Level 1 : #of replicates x 2 days x #of devices	For example - two devices, 2 days, 5 times each level N = • 20 cartridges • 2 vials of control level 1
	Control Level 2 : #of replicates x 2 days x #of devices	For example - two devices, 2 days, 5 times each level N = • 20 cartridges • 2 vials of control level 2
METHOD COMPARISON (ACCURACY) • Includes all devices. • For accreditation agencies that recognize the use of liquid quality controls and Cal/Ver materials, data collected for precision and reportable range studies may be utilized. • Each box of Cal/Ver provides 6 vials , two of each level, for testing. Additional cartridges may be tested with the remaining fluid, if used within 4 hours of bringing the vial to room temperature. • Approximately 15 cartridge tests may be run within the 4-hour stability timeframe of the *calibration verification* materials.	Number of Samples x 2 (for duplicates) x # of devices	For example - two devices and 3 samples, N = • 6 cartridges • 1 box of calibration verification levels 1-3
METHOD COMPARISON (CLINICAL CORRELATION) • Includes all devices.	Number of Samples x 2 (for duplicates, if applicable) x # of devices	For example - two devices and 20 samples, N = • 40 cartridges
PERFORMANCE VERIFICATION (REPORTABLE RANGE) • Includes all devices. • At least three samples representing low, mid and high values that span the reportable range.	Calibration Verification Levels 1-3: Numbler of Levels x # of devices	For example - two devices, N = • 6 cartridges (minimum) • 1 box of Cal/Ver
REFERENCE INTERVAL: Results from the method comparison for accuracy and performance verification of the reportable range may be used to verify the reference interval.		

i-STAT TBI CARTRIDGE CONSUMABLE CALCULATION WORKSHEET

ORDERING INFORMATION

CONSUMABLE NAME	PRODUCT REF/LIST NUMBER	QUANTITY PER BOX	ORDERED
***i-STAT TBI* Cartridge**	**03S09-25**	**25 cartridge portion packs**	
i-STAT TBI Control Level 1	**06P17-25**	**6 vials**	
i-STAT TBI Control Level 2	**06P17-26**	**6 vials**	
i-STAT TBI Calibration Verification Levels 1-3	**06P17-24**	**6 vials, two of each level**	

NOTE: For controls and calibration verification materials, additional *i-STAT TBI* cartridges may be tested with the remaining fluid within the 4-hour stability time of the materials.

CONSUMABLE CALCULATION WORKSHEET

STUDY	CALCULATION	TOTAL	
		Cartridges	Vials
PRECISION	Control Level 1 : #of replicates x 2 days x #of devices		
	Control Level 2: #of replicates x 2 days x #of devices		
METHOD COMPARISON (ACCURACY)	Number of Samples x 2 (for duplicates) x # of devices		
METHOD COMPARISON (CLINICAL ASSESSMENT)	Number of Samples x 2 (for duplicates, if applicable) x # of devices		
PERFORMANCE VERIFICATION (REPORTABLE RANGE)	Calibration Verification Levels 1-3: Numbler of Levels x # of devices		
REFERENCE INTERVAL: Results from the method comparison and performance verification studies may be used to verify the reference interval.			

METHOD COMPARISON

Accuracy is verified by comparing results to a definitive or reference method, or an established comparative method.[11] Laboratory practice may require testing for accuracy which is typically accomplished by a method comparison study. Use of matrix-appropriate reference materials, patient specimens (altered or unaltered), or other commutable materials with known concentration or activities may be used to verify accuracy.[11]

TESTING CONSIDERATIONS

To ensure best results, refer to the *i-STAT TBI* cartridge IFU and the *i-STAT Alinity* System Operations Manual for pre-requisites, blood collection options and test precautions or limitations prior to performing cartridge testing with the instrument.

There are no internationally recognized standard reference materials available for either glial fibrillary acidic protein (GFAP) or ubiquitin carboxy-terminal hydrolase L1 (UCH-L1).[7]

When performing the method comparison consider the following:

DO

- **DO** obtain samples within the clinically relevant range as determined by the laboratory director.
- **DO** use one lot number of cartridges in the method comparison and ensure that cartridges and instruments used are at room temperature.
- **DO** follow instructions for use for storage and handling of the cartridges, materials or samples.

DO NOT

- **DO NOT** improperly store or handle cartridges. Improper handling and storage may result in quality check codes or unexpected test results.
- **DO NOT** inappropriately use quality control or cal/ver materials for accuracy. **CAP does not consider quality control or calibration verification materials as appropriate for use in the method comparison study.[12]**

COMPARATIVE METHOD CONSIDERATIONS

When no comparative method is available, the laboratory director has the discretion to use available regulatory compliance guidance for verifying accuracy. For example, the CLIA guidance, *Verification of Performance Verifications*, states that "the laboratory needs to compare the accuracy of the test results it obtains when using a test system with the manufacturer's accuracy claims."[13]

Per the CLIA guidance, the laboratory can test "commercially available calibrators/calibration or quality control (QC) materials with known values, proficiency testing materials that have established values, or previously tested patient specimens with established values." [13] When an accreditation agency recognizes the use of these materials to verify accuracy, the laboratory director has discretion regarding the number of samples to include in the study.

Also per the CLIA guidance, the laboratory can compare "results of tests performed by the laboratory against the results of a reference method, or comparing split sample results with results obtained from another method which have already been shown to provide accurate results."[13] **CAP does not consider quality control or calibration verification materials as appropriate for use in the method comparison study.[12]**

When proficiency testing materials are not available, the laboratory is responsible for establishing an alternative assessment procedure (AAP) for verifying the acceptability of test performance.[14] For example, the laboratory director has discretion regarding the use of an "internal split sample procedure"[14], where a patient sample is re-tested by a different operator.

COMPARATIVE METHOD CONSIDERATIONS - cont'd

CLINICAL CORRELATION CONSIDERATIONS

Correlation studies may be useful when a specific disorder can be diagnosed or strongly suggested based upon laboratory examination, or the presence of the disorder can be independently verified.[15]

In addition to a method comparison for accuracy, laboratories may also consider a clinical correlation comparing the i-STAT TBI cartridge result to the head CT scan for patients that meet the following criteria:

- Patient is 18 years or older.[7]
- GCS is within 13 to 15.[7]
- Venous whole blood sample collected into an EDTA blood collection tube with 24 hours of injury.[7]
- Venous whole blood sample tested within an hour of collection time.[7]

For the *i-STAT TBI* cartridge, test interpretation of a Not-elevated result is associated with the absence of acute traumatic intracranial lesions on head CT scan.[7]

REFERENCE INTERVAL (RI) CONSIDERATIONS

To verify the reference interval using data from the "method comparison study, samples must be collected with an appropriate distribution of values spanning the RI, as an insufficient range may underestimate and a range too large may overestimate the strength of the correlation, unexpected biases or discrepancies in results.[16] See the verification of reference interval section in this guide.

OFF-SITE LABORATORY CONSIDERATIONS

When considering use of an off-site laboratory, the following information may be assessed to decide if this approach will yield expected results:

- Samples must be processed exactly as instructed by the off-site laboratory.
- Samples for the off-site laboratory and the *i-STAT* System must be collected at the same time.
- Samples must be tested per the test timing criteria provided in the *i-STAT TBI* cartridge IFU.

Delays in testing caused by transport to the off-site laboratory may cause unexpected biases or discrepant results.

CONSIDERATIONS RELATED TO MANUFACTURER ASSISTANCE

Regarding assistance from the manufacturer, the Laboratory Director must verify the standards or regulations from their laboratory accreditor before obtaining the implementation and statistical analysis service options available from Abbott. The laboratory accreditor may or may not allow full or partial assistance from the manufacturer.

For example, the College of American Pathologist (CAP) checklist, COM.40300 "Verification of Test Performance Specifications—FDA-Cleared/ Approved Tests"[12], discusses conditions for when a manufacturer aids a laboratory in setting up a new FDA-approved or -cleared test. It states "the lab must make sure that the personnel who will perform the test participate in the verification or validation study"[17] and "if the personnel don't participate, there must be some way to confirm that performance is consistent with in-house studies performed by lab personnel."[17]

While COLA says that the manufacturer can "assist by providing materials, procedures and statistical analysis"[18]; the manufacturer "may not perform the actual testing of samples used in the verification process..."[18]

METHOD COMPARISON CONTINUED

SAMPLE COLLECTION

The *i-STAT TBI* cartridge requires fresh whole blood (approximately 20 µL) from venous samples.[7] Refer to the *i-STAT TBI* IFU for information on blood collection options and test limitations and interferences. Specimens should be collected according to the facility's policies and procedures.

Laboratories may refer to the following CLSI standard for proper sample collection procedures:

- GP41 - *Collection of Diagnostic Venous Blood Specimens* provides procedures for diagnostic venous blood collection.[19]

Follow the facility's policies or procedures for the collection of venous samples. When collecting venous samples, consider the following:

DO

- **DO** use a collection technique resulting in good blood flow. Inadequate blood flow may produce erroneous results.
- **DO** collect a specimen, ensuring proper order of draw, and then fill an EDTA blood collection tube to capacity, as indicated by the tube manufacturer.
- **DO** fill the cartridge to the fill line immediately. Delay in filling the cartridge may produce erroneous results. Quality check failures will occur when the sample does not reach the fill line indicated on the cartridge.

DO NOT

- **DO NOT** collect a sample from an arm with an IV line. "Collecting blood from an arm that is being infused with IV fluids carries potential risk for erroneous and misleading test results."[19]
- **DO NOT** underfill a blood collection tube. Follow tube manufacturer instructions for proper filling.
- **DO NOT** incorrectly handle or incorrectly fill the cartridge as this will generate a quality check failure.

DATA COLLECTION

To aid customers with documenting results for the method comparison study, this section provides considerations for the data collected as part of the study. The Laboratory Director defines the processes and procedures used to perform the method comparison activities and the related data collection. The worksheet available from Abbott is an optional aid and does not replace data collection instructions from the facility's procedures nor data requirements from any statistical software being used.

PATIENT HEALTH INFORMATION AND SAMPLE CONSIDERATIONS

"It is incumbent on all covered entities and their business associates to thoroughly comprehend and apply the HIPAA Privacy Rule requirements, ensuring the protection of health information, and thus, fulfilling their roles as guardians of their patients' sensitive data."[20] Follow your facility's policy and procedures for handling patient health information and properly de-identifying information when applicable.

Be sure to document:

- the ID number used for the patient samples tested as part of the method comparison.
- other considerations related to the collection of the sample and any medications that may assist with the troubleshooting of results or when reviewing data in statistical analysis reports.
- the collection date and the time of the samples as objective evidence of meeting procedural test timing requirements.

The following information may be helpful in determining the cause of discrepant results:

- Difficulty collecting sample via venipuncture.
- Under-filled EDTA blood collection tube.
- Sample drawn from an arm with an intravenous (IV) fluid.
- Patient receiving unfractionated or low molecular weight heparin.
- Patient receiving anti-platelet medications.

METHOD COMPARISON CONTINUED

DEVICE & CONSUMABLE INFORMATION

At a minium, the model and serial numbers for the devices involved in the method evaluation should be documented. The *i-STAT Alinity* system requires a software update every 6 months. It is important to document the software version of the instruments at the time the activities are performed for the method comparison.

For best results, Abbott recommends the use of one lot number of *i-STAT* cartridges in the method comparison study. The cartridge lot number details should be captured with the data collection.

If applicable, obtain the following information for the laboratory instrument used as the method comparator:

- Reagent lot number(s)
- Reagent calibration date(s)

RECORDS AND RETENTION

Once the method comparison study has been completed, it is the responsibility of the Laboratory Director to review, approve and store all records associated with the study. These records are part of the evidence to support completion of the method evaluation activities. The laboratory accreditor may have additional guidelines regarding the length of time the records are required to be stored.

ACCEPTABILITY OF METHOD COMPARISON STUDY

The criteria for acceptability of results varies by regulatory agency and publication.

The Laboratory Director has discretion regarding the definition of criteria and the acceptability of the results of the study.

VERIFICATION OF REFERENCE INTERVALS

Regulatory standards may require the verification of the reference intervals or reference range for the test method.

REFERENCE INTERVALS

Reference intervals "are fundamental tools used by healthcare and laboratory professionals to interpret patient laboratory test results, ideally enabling differentiation of healthy and unhealthy individuals."[16] The reference intervals for the panel of tests on the *i-STAT TBI* cartridge using venous specimens are:

GFAP: <47–53 pg/mL and UCH-L1: <87-251 pg/mL.[7]

Based upon Abbott's reference interval study, "test results with the i-STAT TBI cartridge with the i-STAT Alinity system, 0.7% (1/150) of the individuals...had a test interpretation of "elevated" for biomarkers".[7] See the *i-STAT TBI* IFU for more details.

VERIFYING REFERENCE INTERVALS

The standard approach to verify RIs recommended by the Clinical Laboratory Standards Institute (CLSI) EP28-A3c guideline for routine clinical laboratories is to collect and analyze a minimum of 20 samples from healthy subjects from the local population.[4] Results for healthy patient samples from the method comparison study may be used towards satisfying this recommendation.

Refer to CLSI EP28-A3c – *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory*; Approved Guideline for information about the use of method comparison data to verify reference range, found at www.clsi.org.

REPORTING REFERENCE INTERVALS

Reference intervals (RIs) are most commonly defined as the central 95% of laboratory test results expected in a healthy reference population.[16]

The Laboratory Director and/or the Technical Consultant/Technical Supervisor need to determine how the laboratory will report results that are greater than the highest verified level or less than the lowest verified level.[13]

PERFORMANCE VERIFICATION OF THE REPORTABLE RANGE

Regulatory standards may require performance verification across the reportable range.[2] Reportable range verification may be met by using matrix appropriate materials, which include low, mid, and high concentrations with recovery of results that fall within a defined range of target values.

REPORTABLE RANGE

The reportable range or reporting interval for the TBI cartridge panel of tests are GFAP: 47 - 10,000 pg/mL and UCH-L1: 87 - 3,200 pg/mL. [7]

PERFORMANCE VERIFICATION CONSIDERATIONS

Performance verification of values outside of the therapeutic range and on the lower/higher end of the device's reportable range may not be possible given the lack of commercially available materials or samples that span the full reportable range.

It is the responsibility of the Laboratory Director to:

- determine the appropriate samples to be used for the verification, and the closeness of the sample concentrations.
- define the criteria for accepting or rejecting the verification of the reportable range.

In the absence of any suitable commercially available control/calibration verification material, patient samples with known values, proficiency testing samples with known results, or reference samples can be used to expand the verified range.

IMPORTANT NOTE: Proficiency samples for use with the i-STAT TBI cartridge are not currently available from proficiency providers.

The *i-STAT TBI* cartridge is a factory calibrated test.[7] Calibration verification materials are available from Abbott. *i-STAT TBI CALVER Levels 1 -3* span the reportable range.[7]

TESTING CONSIDERATIONS

For best results, refer to the *i-STAT TBI* cartridge IFU for testing pre-requisites, limitations and precautions. When performing the performance verification across the reportable range, consider the following:

DO

- **DO** include samples within the clinically relevant range as determined by the Laboratory Director.
- **DO** use one lot number of cartridges in the performance verification and ensure that cartridges and instruments are at room temperature prior to testing.
- **DO** follow instructions for use for the storage and handling of cartridges, materials or samples to ensure accurate results. Improper preparation and use of the cartridge and samples may cause discrepant results or quality check failures.

DO NOT

- **DO NOT** improperly store cartridges. Using an *i-STAT TBI* cartridge that has not come to room temperature or is outside the room temperature expiration date may result in generation of quality check failures or unexpected test results.
- **DO NOT** incorrectly handle and fill the cartridge, as this will generate a quality check failure.

RECORDS AND RETENTION

It is the responsibility of the Laboratory Director to review, approve and store all records associated with the study.

NOTE: The laboratory accreditor may have additional guidelines pertaining to the length of time the records are required to be stored.

PRECISION STUDY

"Precision" is defined in two different ways: (1) the degree to which the same method produces the same results on repeated measurements, and (2) the degree to which values cluster around the mean of the distribution of values.[21] Imprecision (standard deviation (SD), % coefficient variation (CV)) is the statistical expression of the differences between these measurements. The precision study should be performed over at least two days to satisfy CLIA 493.1253 (b)(1)(i) requirements.[10]

PRECISION STUDY CONSIDERATIONS

The laboratory is responsible for verifying that it can repeatedly test the same samples under different conditions and get the same or comparable results (reproducible), regardless of which member of the laboratory's testing personnel performs the test (operator variance).[13]

i-STAT TBI CONTROLS

The *i-STAT TBI Control* Level 1 and Level 2 are intended for use with the *i-STAT TBI* cartridge on the *i-STAT* Alinity System, and values assigned to these controls may not be commutable with other commercial methods.[7]

VALUE ASSIGNMENT SHEETS (VAS)

Abbott recommends documenting the Value Assignment Sheet information for the control level and cartridge lot used in the study. Value Assignment Sheets are available in the *i-STAT Alinity* Support section of www.globalpointofcare.abbott. The sheet may be printed and stored with the records of your method evaluation.

TESTING CONSIDERATIONS

For best results, refer to the *i-STAT TBI* cartridge and *i-STAT TBI Control* Level 1 and Level 2 IFUs, for testing pre-requisites, material handling, limitations and precautions. When performing the precision study consider the following:

DO

- **DO** use one lot number of controls and cartridges for the study.
- **DO** test both levels of controls. A minimum of 20 results for each level is recommended for proper statistical analysis using Abbott's PV report service.
- **DO** follow instructions exactly for handing the controls to ensure accurate results.
- **DO** perform the control test using the quality control pathway on the *i-STAT Alinity* instrument.

DO NOT

- **DO NOT** test less than the number of samples required by the software used for statistical analysis. A minimum of 20 results for each level is recommended for proper statistical analysis using Abbott's PV report service.
- **DO NOT** test control material in the patient test or Cal/Ver test pathway on the *i-STAT Alinity* instrument.
- **DO NOT** place controls that have been thawed and brought to room temperature back in the freezer.
- **DO NOT** test control material past the 4 hour stability as directed by the *i-STAT TBI* controls IFUs.[9]

PRECISION STUDY CONTINUED

ACCEPTABILITY OF RESULTS FOR PRECISION STUDY

When reviewing results, the control result is considered acceptable when it is within the range specified in the Value Assignment Sheet.

If an out-of-range result is obtained and it can be confirmed that the cause was operator error, the result can be discarded and replaced with a result from a new cartridge. If more than one out-of-range result is obtained, the operator should review and practice the procedure prior to testing additonal cartridges.

The Laboratory Director has discretion on whether to discard additional out-of-range results and the continuation or restart of the precision study. It is best practice to ensure that decisions about out-of-range results in the precision study are documented in the method evaluation record.

PRECISION STUDY COMPARISON

Per the *i-STAT TBI* pre-market notification (K234143), a Semi-quantitative 20-day precision study was performed. The precision of the GFAP and UCH-L1 assays in the i-STAT TBI cartridge with the *i-STAT Alinity System* was evaluated using plasma samples spiked with native or recombinant GFAP and UCH-L1 antigens at various levels across the reportable range of the GFAP and UCH-L1 assays, and two (2) controls (*i- STAT TBI* Control L1 and Control L2) and was based upon guidance provided in *CLSI EP05-A3*.[7]

The study was executed over 20 non-consecutive days, two (2) runs per day that were separated by a minimum of two (2) hours, by at least two (2) operators using three (3) lots of the *i-STAT TBI* cartridges. Due to the inability to store or freeze whole blood samples to maintain sample stability over multiple days, plasma samples were used for this study. The *i-STAT TBI* pre-market notification (K234143) provides the study results for the *i-STAT TBI* controls in the performance characteristics section, Table 3.

PRECISION STUDY COMPARISON - cont'd

The averaged statistics for total (within laboratory) precision (SD, standard deviation) are represented in the table within the pre-market notification. SD and %CV are typical of current performance; however, results in individual laboratories may vary from this data.

ACCEPTABILITY OF RESULTS

The precision data provided in the *i-STAT TBI* pre-market notification (K234143) is representative of the data submitted to the FDA. It is **not** intended to be used as part of assessing the acceptability of your precision study.

i-STAT TBI Control Level 1 and Level 2 Value Assignment Sheets only provide the mean and the range for the control materials. They are also **not** intended to be used as part of assessing the acceptability of your precision study.

The laboratory director has discretion regarding the acceptability of results based upon the number of days and replicates defined in their precision study procedure or as specified by the statistical analysis software used.

PRECISION STUDY DATA COLLECTION WORKSHEET

As an optional aid for customers, a worksheet is available to collect data related to the precision study.

This worksheet helps ensure that all the device information is captured, along with control lot information and replicate results.

VERIFICATION OF ADDITIONAL OR REPLACEMENT INSTRUMENTS

Abbott does not have manufacturer's requirements for testing liquid quality controls. Abbott offers the following suggestions for the Laboratory Director's consideration when verifying additonal or replacement instruments:

PRECISION STUDY

Test two levels of control samples for each test that will be performed on a new or replacement instrument.

Results must be within the acceptable range(s) on the Value Assignment Sheet(s). Store the Value Assignment Sheet(s) with the data as evidence that results were within acceptable limits.

In cases where available control materials do not span the reportable range, patient samples with known values can be used to expand the verified range.

ACCURACY

Use the data from the method comparison (for accuracy) study to assess accuracy. In addition, test one or more patient samples on the new or replacement instrument and a comparative method or on a previously verified *i-STAT* device.

The difference(s) between the new or replacement instrument and the comparative method or previously verified device should not exceed the laboratory's required level of agreement between systems.

REFERENCE INTERVALS

Use the reference intervals established at the time of the initial verification. The reference ranges programmed into the instrument and found in the IFU are intended only as guides for interpreting results. Since reference ranges can vary depending on demographics such as age, sex, race and ethnicity, it is recommended that reference ranges be determined by the facility and approved by the Laboratory Director.

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