



METHOD EVALUATION & THE i-STAT hs-TnI CARTRIDGE

BEST PRACTICES FOR A SUCCESSFUL METHOD EVALUATION OF THE i-STAT hs-TnI CARTRIDGE WITH THE i-STAT 1 SYSTEM

A method evaluation supplies evidence that the accuracy, precision and reportable range of a new method are adequate and that the reference range meets the needs of the patient population and clinicians as determined by the laboratory director and/or technical consultant.

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 ORGANIZATIONS.



INTRODUCTION

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

OVERSIGHT

The Centers for Medicare & Medicaid Services (CMS) establish 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 §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 hs-TnI* 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 (See §CLIA 493 Subpart M Personnel for Nonwaived Testing)⁶. Application of standards related to high complexity testing are not applicable to the *i-STAT hs-TnI* cartridge when used as intended with the *i-STAT 1 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 on the design of the *i-STAT 1 System*.⁸
- Perform daily quality control with an Electronic Simulator (internal or external once each day of use).
- Check new or replacement analyzers with the Electronic Simulator (internal or external).
- Check the temperature strip for a new shipment of cartridges.
- Ensure proper cartridge storage.
- Perform a thermal probe check every six months.
- Train staff.
- Update *i-STAT 1 System* software as provided by Abbott.

CONTENTS

The document provides system-based best practices and recommendations to assist customers in evaluating methods for the *i-STAT hs-TnI* cartridge using the *i-STAT 1 System*.

It is essential that healthcare professionals, who are trained and competent to use the system, operate the instrument and cartridges in accordance with their facility's policies and procedures.⁸

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SUPPORT & SERVICES

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

PRODUCT INFORMATION

To access product information, such as Instructions for Use (IFU), Quick Reference Guides, and System Operations Manuals, visit the *i-STAT* 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
phone: 1-800-366-8020
- **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.

PREPARATION

i-STAT hs-TnI CARTRIDGE (REF/LIST NUMBER 09P81-25)

The *i-STAT hs-TnI* cartridge with the *i-STAT 1 System* is intended for use in the in vitro quantification of cardiac troponin I (cTnI) in whole blood or plasma samples in point of care or clinical laboratory settings.

The *i-STAT hs-TnI* cartridge with the *i-STAT 1 System* is intended to be used as an aid in the diagnosis of myocardial infarction (MI). The test is to be used with venous whole blood or plasma collected with lithium heparin or with venous whole blood collected without anticoagulant (do not test beyond 3 minutes from collection) at the point-of-care or clinical laboratory setting.

i-STAT 1 SYSTEM

The *i-STAT 1 System* is comprised of the *i-STAT* device, the *i-STAT* test cartridges, and accessories.

The *i-STAT* device 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 device requires *i-STAT* single-use cartridges containing electrodes and sensors to perform quantitative diagnostic testing on whole blood or plasma. Together, the device 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 instructions (MQSI), liquid quality controls can be used to verify the integrity of newly received cartridges and their storage conditions.

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

i-STAT hs-TnI CARTRIDGE (09P81-25)

Designed for measuring cardiac troponin I (cTnI) in whole blood or plasma to aid in diagnosing myocardial infarction (MI).

i-STAT hs-TnI CAL VER KIT (06P17-20)

Verifies calibration of the *i-STAT 1 System* to ensure accurate and reliable test results.

i-STAT hs-TnI CONTROL LEVEL 1 (06P17-21)

i-STAT hs-TnI CONTROL LEVEL 2 (06P17-22)

i-STAT hs-TnI CONTROL LEVEL 3 (06P17-23)

The *i-STAT hs-TnI* controls have been formulated to ensure consistent and accurate performance of the *i-STAT 1 System* when using the *i-STAT hs-TnI* cartridge. Please refer to the value assignment sheet for acceptable ranges for each level of control and Cal Ver.

VALUE ASSIGNMENT SHEETS (VAS)

Value Assignment Sheets provide the acceptable range for each level of control and Cal Ver materials based on cartridge lot number and software installed on the analyzer. Sheets are available on the *i-STAT Support* page of Abbott's website: www.globalpointofcare.abbott. Keep the Value Assignment Sheets from the method evaluation with your additional performance verification records.

PREPARATION

Before conducting a method evaluation, Abbott advises reviewing the Instructions for Use (IFU) for the cartridge, liquid quality control material, and calibration verification material, as well as the *i-STAT 1 System Manual* and User Guide.

For product information, visit the *i-STAT* 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 System*, along with any related facility policies and procedures.

SOFTWARE REQUIREMENTS

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

- Most current version of the *i-STAT* device software

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

ANALYZER CONFIGURATION OR CUSTOMIZATION

For best results, ensure that all *i-STAT* devices are configured with the correct date and time, that the correct unit sets are applied/customized (if applicable), and that the most current software version is being used.

- Refer to the *i-STAT 1 System Manual* for device customization and setup.

EQUIPMENT

Every *i-STAT* device used with the *i-STAT hs-TnI* cartridge for patient testing should 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 Checklist: COM.40000 Method Validation and Verification Approval - Nonwaived Tests, 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

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

SAMPLE HANDLING

SAMPLE COLLECTION

The *i-STAT hs-TnI* cartridge requires fresh venous whole blood or plasma samples (approximately 22 uL).⁷ Refer to the *i-STAT hs-TnI* 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 venous sample collection procedures:

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

WHEN COLLECTING VENOUS SAMPLES, CONSIDER THE FOLLOWING:

DOs

- **DO** use a collection technique resulting in good blood flow. Inadequate blood flow may produce erroneous results.
- **DO** collect a venous specimen ensuring proper order of draw and fill a lithium heparin or nonanticoagulated 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.

DONT's

- **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.

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.

SAMPLE HANDLING - CONT'D

PATIENT INCLUSION CRITERIA

Patients presenting to the emergency department with acute, severe, and prolonged chest pain are eligible for the Method Comparison study. Samples should be drawn within 48 hours of presentation with no more than three samples per patient drawn at least 2 hours apart. Include troponin and serial troponin samples for at least 2-4 patients.

SAMPLE PROCESSING (FROZEN PLASMA)

To ensure an adequate number of samples are available for the Method Comparison protocol, we recommend banking plasma samples after standard of care testing has been performed. Ensure a broad range of values are selected and bank plasma samples in advance of the scheduled Method Comparison study.

Samples should be processed using your laboratory's recommended procedure. If recommendations do not exist for processing and storing of frozen plasma samples, please follow the steps below for processing and storage:

NOTES

Select the number of samples within the clinically relevant range as determined by the Laboratory Director. Ensure to select samples BELOW and ABOVE the cut-off (99th percentile upper reference limit):

- The cut-off may be the overall 99th percentile URL or the sex-specific 99th percentile URL.
- Include troponin and serial troponin samples for at least 2-4 patients.
- Do not include more than 3 samples from the same patient.

Store sufficient volume of plasma for duplicate testing on both the *i-STAT* and comparative method.

1. Centrifuge anticoagulant blood collection tube according to the manufacturer's instructions.
2. Using a pipette, carefully transfer the plasma to a clean polypropylene tube, making sure to not disturb the buffy coat.

3. Label the sample with the following:
 - Patient identifier
 - Initial troponin value
 - Sex (when available)
 - Note if samples are serial draws from the initial
4. Store samples in a manner to easily determine the number of samples collected by sex (if applicable).
5. If aliquots are not planned for testing right away, store at $\leq -20^{\circ}\text{C}$ (-4°F) for up to three months. Samples should be frozen within 2 hours of collection.

When ready to test samples that have been frozen, follow the steps below:

6. Thaw frozen samples at room temperature ($18-30^{\circ}$ or $64-86^{\circ}\text{F}$).
7. Once thawed, mix by gently inverting the sample 10 times.
8. Centrifuge the sample for 1 minute at approximately 9600 g. Transfer the plasma to a clean polypropylene tube, making sure to not disturb the pellet.
9. A sample should be tested on the *i-STAT* and on the comparative method within 30 minutes.
 - Note that some degradation cannot be avoided, and results obtained on frozen samples may differ from those obtained initially on the fresh samples.

CONSUMABLES

USING THE CONSUMABLE CALCULATION WORKSHEET

Customers should verify they have sufficient products to carry out the method evaluation activities as specified by their laboratory director. To assist 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. Refer to the considerations below for using the consumable calculation worksheet.

STUDY	CALCULATION	TOTAL (EXAMPLE)
PRECISION <ul style="list-style-type: none"> Includes all devices. The laboratory director has the discretion to decide the number of samples and replicates needed to determine precision. For customers requesting PV reports for statistical analysis using Abbott's field services, a minimum of 20 replicates per level is required. To meet the requirement of verifying each device, it is recommended to test duplicates of two levels of control on each device on both day 1 and day 2. If fewer than five devices are included in the method evaluation, increase the number of tests per device on days 1 and 2 so that the total number of results reaches at least 20 for each level of control. Each box of controls includes 6 control vials for testing. Additional cartridges can be tested with the remaining fluid if used within 4 hours of bringing the vial to room temperature. 	Control Level 1 : #of replicates x 2 days x #of devices	5 replicates x 2 days x 2 devices N = <ul style="list-style-type: none"> 20 cartridges 2 vials of control level 1
	Control Levels 2 or 3: #of replicates x 2 days x #of devices	5 replicates x 2 days x 2 devices N = <ul style="list-style-type: none"> 20 cartridges 2 vials of control level 2
METHOD COMPARISON (ACCURACY) <ul style="list-style-type: none"> Includes all devices. For accreditation agencies that recognize the use of liquid quality controls and/or Cal Ver materials, data collected for precision and reportable range studies may be used for accuracy. Test a minimum of one patient sample on each device (in duplicate) and on a comparative device (in duplicate). Once a patient sample has been tested on each device, the remainder of the patient samples for the Method Comparison (for Accuracy Verification) study could be evenly distributed across all devices, or they could be tested on a representative number of devices. 	Number of Samples x 2 (for duplicates) x # of devices	2 patients x 2 (duplicates) x 2 devices N=8 cartridges minimum Lab director discretion for how many samples to use for method comparison.
PERFORMANCE VERIFICATION (REPORTABLE RANGE) <ul style="list-style-type: none"> Includes all devices. At least three samples representing low, mid and high values that span the reportable range. 	Calibration Verification Levels 1-3: Number of Cal Ver levels x # of devices	2 devices N = <ul style="list-style-type: none"> 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.		

CONSUMABLES - CONT'D

i-STAT hs-TnI CONSUMABLE CALCULATION WORKSHEET

ORDERING INFORMATION

CONSUMABLE NAME	PRODUCT CODE/LIST NUMBER	QUANTITY PER BOX	ORDERED
i-STAT hs-TnI Cartridge	09P81-25	25 cartridge portion packs	
i-STAT hs-TnI Control Level 1	06P17-21	6 vials x 1.0 mL	
i-STAT hs-TnI Control Level 2	06P17-22	6 vials x 1.0 mL	
i-STAT hs-TnI Control Level 3	06P17-23	6 vials x 1.0 mL	
i-STAT hs-TnI Cal Ver Levels 1-3	06P17-20	6 vials, 2 of each level (3 levels) x 1.0 mL	

NOTE: For controls and calibration verification materials, additional *i-STAT hs-TnI* 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 or 3: #of replicates x 2 days x #of devices		
METHOD COMPARISON (ACCURACY)	Number of Samples x 2 (for duplicates) x # of devices		
PERFORMANCE VERIFICATION (REPORTABLE RANGE)	Calibration Verification Levels 1-3: Number 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

TESTING CONSIDERATIONS

Accuracy is verified by comparing results to a definitive or reference method, or an established comparative method. In laboratory practice, accuracy testing is often conducted through a method comparison study. This process may involve using matrix-appropriate reference materials, patient specimens (altered or unaltered), or other commutable materials with known concentrations or activities to verify accuracy. To ensure best results, refer to the *i-STAT hs-TnI* cartridge IFU and the *i-STAT 1 System Manual* for pre-requisites, blood collection options and test precautions or limitations prior to performing cartridge testing with the device.

Refer to the *i-STAT hs-TnI* IFU for information on blood collection options and test limitations and interferences. Specimens should be collected according to the facility's policies and procedures.

WHEN PERFORMING THE METHOD COMPARISON, CONSIDER THE FOLLOWING:

DOs

- **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** ensure units of measure are correctly set.

DONT's

- **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 accuracy study.¹²

COMPARATIVE METHOD CONSIDERATIONS

If 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 Specifications*, 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. This can be done by testing commercially available calibration verification or quality control (QC) materials with known values, proficiency testing materials that have established values, or previously tested patient specimens with established values. This can also be done by comparing 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 has already been shown to provide accurate results.”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.

CLIA requires comparison studies if a laboratory performs the same test using different methodologies or instruments. The lab must define the relationship between test results using the different methodologies, instruments, or testing sites.

METHOD COMPARISON - CONT'D

CLINICAL CORRELATION CONSIDERATIONS

CONCORDANCE

Concordance is defined as the percentage of agreement between results from two methods. This is determined by converting a numerical result to either “elevated” or “non-elevated” based on a given decision level for a specific set of data.

Concordance will be calculated at the 99th percentile upper reference limit as stated in the i-STAT hs-TnI Instruction for Use and the package insert for the comparative instrument.

Always refer to the manufacturer’s package insert at the site for information regarding the 99th percentile upper reference limit.

Any results that are greater than the 99th percentile upper reference limit are elevated. Results that are at or below the decision level are not elevated.

When duplicates are tested, the results of both replicates are used in the concordance calculations.

Differences in calibration and antibody specificity between methods often cause regression slopes to be different. Concordance data can be used to assess the diagnostic equivalence of two different methods.

REFERENCE INTERVAL (RI)

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.

MANUFACTURER’S 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. The note 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

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

- Difficulty collecting sample via venipuncture.
- Under-filled blood collection tube.
- Sample drawn from an arm with an intravenous (IV) line.

METHOD COMPARISON - CONT'D

DEVICE & CONSUMABLE INFORMATION

At a minimum, the model and serial numbers for the devices involved in the method evaluation should be documented. *The i-STAT 1 System* requires a software update periodically. 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 & RETENTIONS

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.

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.

THE REPORTABLE RANGE FOR THE i-STAT hs-TnI CARTRIDGE IS:

2.9 – 1000.0 ng/L or pg/mL stored.



METHOD COMPARISON - CONT'D

CALIBRATION VERIFICATION

The values at the low and high end of the device's reportable range can be confirmed with calibration verification material or patient samples with known values.

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.

Proficiency testing samples with known results, or reference samples may be used to expand the verified range.

IMPORTANT NOTE: PROFICIENCY SAMPLES FOR USE WITH THE *i-STAT* HS-TnI CARTRIDGE ARE AVAILABLE FROM 2 PROFICIENCY PROVIDERS: API AND WSLH.

The *i-STAT* *hs-TnI* cartridge is a factory calibrated test.⁷ Calibration verification materials are available from Abbott. *i-STAT* *hs-TnI* Cal Ver Levels 1 -3 span the reportable range.⁷

TESTING CONSIDERATIONS

For best results, refer to the *i-STAT* *hs-TnI* cartridge IFU for testing pre-requisites, limitations, and precautions.

WHEN PERFORMING THE PERFORMANCE VERIFICATION ACROSS THE REPORTABLE RANGE, CONSIDER THE FOLLOWING:

DOs

- **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** include patients presenting to the emergency department with acute, severe, and prolonged chest pain, chest discomfort, or equivalent ischemic symptoms consistent with Acute Coronary Syndrome (ACS).

DONT's

- **DO NOT** improperly store cartridges. Using an *i-STAT* *hs-TnI* 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 may generate a quality check failure.
- **DO NOT** draw more than 3 samples from the same patient drawn at least 2 hours apart.

DATA COLLECTION

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 **Data Collection Worksheet**, available online from Abbott's [i-STAT hs-TnI Resource Center](#), is an optional aid, does not replace data collection instructions from the facility's procedures nor data requirements from any statistical software being used.



▶ SCAN THE CODE TO VISIT
THE i-STAT^{hs-TnI}
RESOURCE CENTER

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

Precision testing could be performed using the *i-STAT hs-TnI* controls or in-house developed sample/control set as directed by the laboratory director.

VALUE ASSIGNMENT SHEETS

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 Support* section of www.globalpointofcare.abbott. The sheet may be printed and stored with the records of the method evaluation

TESTING CONSIDERATIONS

For best results, refer to the *i-STAT hs-TnI* cartridge and *i-STAT hs-TnI* controls IFU, for testing prerequisites, material handling, limitations, and precautions. .

WHEN PERFORMING A PRECISION STUDY, CONSIDER THE FOLLOWING:

DOs

- **DO** use one lot number of controls and cartridges for the study.
- **DO** test two levels of controls (Level 1 plus Level 2 or Level 3). 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 handling the controls to ensure accurate results.
- **DO** perform the control test using the quality control pathway on the *i-STAT 1 System*.

DONT’s

- **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* device.
- **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 hs-TnI* controls IFU.⁹

PRECISION STUDY - CONT'D

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 additional 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 hs-TnI pre-market notification* (K240984), a 20-day precision study, was performed. The precision of the *i-STAT hs-TnI* cartridge with the *i-STAT 1 System* was evaluated using venous whole blood and plasma samples collected in lithium heparinized tubes.

ACCEPTABILITY OF RESULTS

The precision data provided in the *i-STAT hs-TnI pre-market notification* (K240984) is representative of the data submitted to the FDA. It is not intended to be used as part of assessing the acceptability of the precision study.

i-STAT hs-TnI Control Levels 1, 2 and 3 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 the precision study.

The laboratory director has the authority to determine the acceptability of results, considering the number of days and replicates outlined in their precision study procedure or as specified by the statistical analysis software.

PRECISION STUDY DATA COLLECTION WORKSHEET

As an optional resource for customers, the **Data Collection Worksheet**, is available online from Abbott's [i-STAT hs-TnI Resource Center](#) to assist in collecting data for the precision study.

This worksheet ensures thorough documentation of device information, control lot details, and replicate results.

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. Guidance provided in CLSI EP05-A3.⁷



SCAN THE CODE TO VISIT
THE *i-STAT hs-TnI*
RESOURCE CENTER

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

Based on the test results from venous whole blood specimen testing, the 99th percentile upper reference limit (URL) of an apparently healthy population for the *i-STAT hs-TnI* cartridge was determined to be as follows:

GENDER	N	99TH PERCENTILE (ng/L, pg/mL)	90% CI (ng/L, pg/mL)
FEMALE	490	13	(10, 17)
MALE	404	28	(19, 58)
OVERALL	895	21	(14, 30)

Gender-specific cut-off values are recommended for high-sensitivity assays.⁷ Representative data are provided in the ‘Expected Values’ section of the IFU. Results obtained in individual laboratories may vary. Each facility should establish its own reference range using the *i-STAT hs-TnI* cartridge.²²

The customization of the device will allow for gender-specific cut-off values.

VERIFYING REFERENCE INTERVALS

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 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(s) to determine how the laboratory will report results that are greater than the highest verified level or less than the lowest verified level.¹³

VERIFICATION OF ADDITIONAL OR REPLACEMENT INSTRUMENTS

Abbott does not have specific manufacturer requirements for testing liquid quality controls. However, Abbott provides the following suggestions for the laboratory director to consider when verifying additional or replacement devices:

PRECISION STUDY

Test at least two levels of control samples for each test conducted on a new or replacement device.

- Ensure the results fall within the acceptable range(s) on the Value Assignment Sheet(s).
- Keep the Value Assignment Sheet(s) with the data as proof that the results were within acceptable limits.

REPORTABLE RANGE

Test the low, mid, and high levels of calibration verification samples for each cartridge that will be performed on a new or replacement device.

- Ensure the results fall within the acceptable range(s) specified 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 calibration verification 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 reportable range study to assess accuracy. Additionally, test one or more patient samples on the new or replacement device and the comparative method or a previously verified *i-STAT 1 System*.

The differences between the new or replacement device 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.

REFERENCES

1. CLSI. User Verification of Precision and Estimation of Bias; Approved Guideline – Third Edition. CLSI document EP15-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2014.
2. CLSI. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd ed. CLSI guideline EP06. Clinical Laboratory Standards Institute; 2020.
3. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05- A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
4. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI document EP28-A3c. Wayne, PA. Clinical and Laboratory Standards Institute; 2008.
5. CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd. Ed. CLSI guideline EP09c. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
6. 42 CFR Part 493. Laboratory Requirements. 42 CFR 493.1405 Laboratory director qualifications; 42 CFR 493.1451 STANDARD Laboratories Performing High Complexity Testing, Technical Supervisor Responsibilities. Centers for Medicare & Medicaid Services, Department of Health and Human Services. Available at <https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493> [Accessed 03-December-2024].
7. 510(k) Pre-Market Notification i-STAT hs-TnI (K240984). Available at <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm>
8. Abbott. *i-STAT 1 System Manual*. Rev April 2024. Available at <https://www.globalpointofcare.abbott/us/en/support/istat-system-customers/documentation.html>
9. Abbott. *i-STAT hs-TnI Control Levels 1, 2 and 3 Instructions for Use*. Rev Nov. 2024. Available at <https://www.globalpointofcare.abbott/us/en/support/istat-system-customers/cartridge-instructions-for-use.html>
10. 42 CFR Part 493. Laboratory Requirements. 42 CFR 493.1253 STANDARD Establishment and Verification of Performance Specifications. Centers for Medicare & Medicaid Services, Department of Health and Human Services. Available at <https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493/subpart-K/subject-group-ECFRc96daead380fed/section-493.1253>. [Accessed 03-December-2024].
11. CAP CHECKLIST (ALL COMMON). COM.40000 Method Validation and Verification Approval - Nonwaived Tests College of American Pathologists. Available at <https://www.cap.org/laboratory-improvement/accreditation/accreditation-checklists>.
12. CAP CHECKLIST (ALL COMMON). COM.40300 Verification of Test Performance Specifications - FDA-cleared/approved Tests College of American Pathologists. Available at <https://www.cap.org/laboratory-improvement/accreditation/accreditation-checklists>.
13. CLIA. Clinical Laboratory Amendments (CLIA) Verification of Performance Specifications. Centers for Medicare & Medicaid Services. Available at <https://www.cms.gov/regulations-and-guidance/legislation/clia/downloads/6064bk.pdf>
14. CLSI. Using Proficiency Testing and Alternative Assessment to Improve Medical Laboratory Quality. 3rd ed. CLSI guideline QMS24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
15. Maxim LD, Niebo R, Utell MJ. Screening tests: a review with examples. *Inhal Toxicol*. 2014 Nov;26(13):811-28. doi: 10.3109/08958378.2014.955932. Epub 2014 Sep 29. Erratum in: *Inhal Toxicol*. 2019 Jun;31(7):298. PMID: 25264934; PMCID: PMC4389712
16. Ozarda Y, Higgins V, Adeli K. Verification of reference intervals in routine clinical laboratories: practical challenges and recommendations. *Clin Chem Lab Med*. 2018 Dec 19;57(1):30-37. doi: 10.1515/cclm-2018-0059. PMID: 29729142. Available at: <https://pubmed.ncbi.nlm.nih.gov/29729142/> [Accessed 24-May-2024]
17. CAP Today. New checklist hones lab's verification and validation requirements. July 2020. Available at <https://www.captodayonline.com/new-checklist-hones-labs-verification-and-validation-requirements/> [Accessed 24-May-2024].
18. COLA. LabGuide 13 How to Verify Performance Verifications. Columbia, MD: Commission on Office Laboratory Accreditation; 2012.
19. CLSI. Collection of Diagnostic Venous Blood Specimens. 7th ed. CLSI standard GP41. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
20. HIPAA Privacy Rule available at <https://www.hipaajournal.com/hipaa-privacy-rule> [Accessed 29-May-2024]
21. Hanneman SK. Design, analysis, and interpretation of method-comparison studies. *AACN Adv Crit Care*. 2008 Apr-Jun;19(2):223-34. doi: 10.1097/01.AACN.0000318125.41512.a3. PMID: 18560291; PMCID: PMC2944826.
22. Abbott. *i-STAT hs-TnI Cartridge Instructions for Use*. Rev November 2024.

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