VITROS Products Immunodiagnostic

INSTRUCTIONS FOR USE

TropI

VITROS Immunodiagnostic Products Troponin I ES Reagent Pack	REF	680 2301
VITROS Immunodiagnostic Products Troponin I ES Calibrators	REF	680 2302

Rx ONLY

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products Troponin I ES Reagent Pack

For the quantitative measurement of cardiac Troponin I (cTnI) in human serum and plasma (heparin and EDTA) using the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems to aid in the assessment of myocardial damage and risk stratification.

Cardiac Troponin I measurement aids in the diagnosis of acute myocardial infarction and in the risk stratification of patients with non-ST-segment elevation acute coronary syndromes with respect to relative risk of mortality, myocardial infarction (MI) or increased probability of ischemic events requiring urgent revascularization procedures.

VITROS Immunodiagnostic Products Troponin I ES Calibrators

For use in the calibration of the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems for the quantitative measurement of cardiac Troponin I (cTnI) in human serum and plasma (heparin and EDTA).

Summary and Explanation of the Test

Troponin I (TnI) is a protein normally found in muscle tissue that, in conjunction with Troponin T and Troponin C, regulates the calcium dependent interaction of actin and myosin.[1] Three isotypes of TnI have been identified: one associated with fast-twitch skeletal muscle, one with slow-twitch skeletal muscle and one with cardiac muscle.[2-3] The cardiac form has an additional 31 amino acid residues at the N terminus and is the only troponin isoform present in the myocardium.[4]

Clinical studies have demonstrated that cardiac Troponin I (cTnI) is detectable in the bloodstream 4–6 hours after an acute myocardial infarct (AMI) and remains elevated for several days thereafter.[5-6] Thus, cTnI elevation covers the diagnostic windows of both creatine kinase-MB (CK-MB) and lactate dehydrogenase.[3] Further studies have indicated that cTnI has a higher clinical specificity for myocardial injury than does CK-MB.[7-8]

Other conditions resulting in myocardial cell damage can contribute to elevated cTnI levels. Published studies have documented that these conditions include, but are not limited to, sepsis, congestive heart failure, hypertension with left ventricular hypertrophy, hemodynamic compromise, myocarditis, mechanical injury including cardiac surgery, defibrillation and cardiac toxins such as anthracyclines.[9-14] Factors such as these should be considered when interpreting results from any cTnI test method.

Because of its cardiac specificity and sensitivity, cTnI has been used as a reliable marker in evaluating patients with unstable angina and non-ST segment elevation acute coronary syndrome (ACS). Previous clinical studies of patients with ACS[15-16] have shown that minor increases in cTnI values provide important prognostic information about the short and long term risk of death.[10-11, 17-18] Ultimately, the assessment of the prognosis can be useful in identifying patients most likely to benefit from specific therapeutic interventions.

Principles of the Procedure

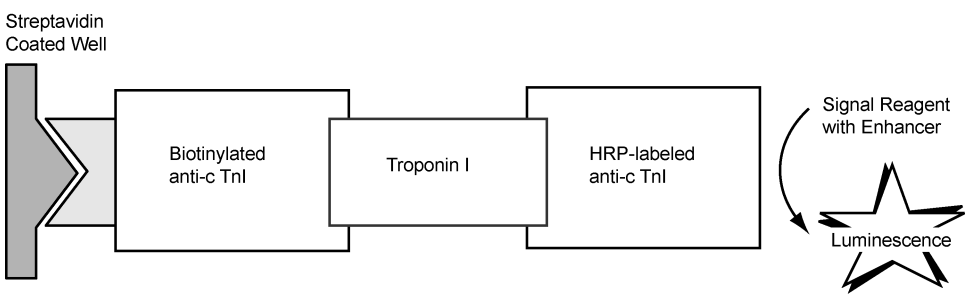
An immunometric immunoassay technique is used, which involves the simultaneous reaction of cardiac Troponin I present in the sample with a biotinylated antibody (mouse monoclonal anti-cTnI) and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-cTnI). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction.[19] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of cTnI present.

Test Type	System *	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	ECi/ECiQ, 3600, 5600, XT 7600	10 minutes	18 minutes	37 °C	80 µL

* Not all products and systems are available in all countries.

Reaction Scheme



Warnings and Precautions

WARNING: ***Potentially Infectious Material***

Human blood products provided as components of the VITROS Troponin I ES Calibrators have been obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using approved methods (enzyme immunoassays). Treat as if capable of transmitting infection.

Use caution when handling material of human origin. Consider all samples potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent. Handle, use, store and dispose of solid and liquid waste from samples and test components, in accordance with procedures defined by appropriate national biohazard safety guideline or regulation (e.g. CLSI document M29). [20-21]

WARNING: ***Contains Kathon or ProClin 200 (CAS 55965-84-9)*** [22]

The VITROS Troponin I ES Reagent Pack contains 2% Kathon or ProClin 200. H317: May cause an allergic skin reaction. P280: Wear protective gloves/protective clothing/eye protection/face protection. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.

Refer to www.Orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.

WARNING

Reagents

Reagent Pack Contents

1 reagent pack containing:

- 100 coated wells (streptavidin, bacterial; binds ≥2 ng biotin/well)
- 7.0 mL conjugate reagent (HRP-mouse monoclonal anti-cTnI, binds ≥205 ng cTnI/mL) in buffer with bovine serum albumin and antimicrobial agent
- 7.0 mL biotinylated antibody reagent (biotin-mouse monoclonal anti-cTnI, binds ≥205 ng cTnI/mL) in buffer with horse serum, bovine gamma globulins, bovine serum albumin, and antimicrobial agent

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading on the system.
- As with all immunoassay protein-based solutions, inappropriate handling of the reagent pack can cause foam to occur on the surface of the reagent. Avoid agitation, which may cause foaming or the formation of bubbles.
  - If reagent packs are dropped or agitated, small levels of fine foam could be generated that may not be detected by the system.
  - Reagent packs containing fine foam that is not detected by the system, may show a negative bias.
- If you must use a dropped or agitated reagent pack before it has been allowed to settle, you should verify performance by running high and low quality control samples in duplicate after loading the pack on the system.

Reagent Pack Storage and Preparation

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	expiration date
Opened	On system	System turned on	≤8 weeks
Opened	Refrigerated	2–8 °C (36–46 °F)	≤8 weeks

- The VITROS Troponin I ES Reagent Pack is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Do not freeze unopened reagent packs.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Store opened refrigerated reagent packs in a sealed reagent pack storage box that contains dry desiccant.

Calibrator Contents

- 1 set of VITROS Troponin I ES Calibrators 1, 2 and 3 (frozen, human cTnI in serum with antimicrobial agent, 2mL); nominal values 0; 0.08 and 15 ng/mL (µg/L)
- Lot calibration card
- Protocol card
- 24 calibrator bar code labels (8 for each calibrator)

Calibrator Handling

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15–30 °C (59–86 °F) before use. Each pack contains sufficient for a minimum of 6 determinations of each calibrator.
- Handle calibrators in original containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system. Return to 2–8 °C (36–46 °F) as soon as possible after use, or load only sufficient for a single determination.

Calibrator Storage and Preparation

Calibrator	Storage Condition		Stability
Unopened	Frozen	≤-20 °C (-4 °F)	expiration date
Opened	Refrigerated	2–8 °C (36–46 °F)	≤6 weeks

- VITROS Troponin I ES Calibrators are supplied frozen.
- VITROS Troponin I ES Calibrators are suitable for use until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.
- Thaw at room temperature 15–30 °C (59–86 °F) and allow to stand for a minimum of 30 minutes. Invert 3 times to ensure mixing before use. Do not shake.
- After thawing, store in the original containers. Do not sub-aliquot.

- A visual check must be performed before each use and the material discarded should visible particulates be observed.
- The VITROS Troponin I ES test uses 80 µL of calibrator for each determination. Transfer an aliquot of each calibrator into a sample container (taking account of the minimum fill volume of the container), which may be bar coded with the labels provided. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Specimen Collection, Preparation and Storage

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

- Serum
- Heparin plasma
- EDTA plasma

Specimens Not Recommended

- Do not use turbid specimens. Turbidity in specimens may affect test results.
- Do not use hemolyzed specimens as hemolysis may affect test results.

Special Precautions

IMPORTANT: *Certain collection devices have been reported to affect other analytes and tests.*[23] *Owing to the variety of specimen collection devices available, Ortho Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.*

Specimen Collection and Preparation

- Collect specimens using standard procedures.[24-25]
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.
- Thoroughly mix samples by inversion and bring to 15–30 °C (59–86 °F) before use.
- The VITROS Troponin I ES test uses 80 µL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Handling and Storage Conditions

- Handle samples in stoppered containers to avoid contamination and evaporation.
- The amount of time samples are on the system prior to analysis should be limited to avoid evaporation. Refer to the operating instructions for your system.
- Return to 2–8 °C (36–46 °F) as soon as possible after use, or load sufficient volume for a single determination.
- Serum and plasma samples may be stored for up to 7 days at 2–8 °C (36–46 °F) or 4 weeks at -20 °C (-4 °F).
- Avoid repeated freeze-thaw cycles.

Note: If the test will not be completed within 2 hours, refrigerate samples at 2–8 °C (36–46 °F).

Testing Procedure

Materials Provided

- VITROS Immunodiagnostic Products Troponin I ES Reagent Pack
- VITROS Immunodiagnostic Products Troponin I ES Calibrators

Materials Required but Not Provided

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- VITROS Immunodiagnostic Products High Sample Diluent B

- Quality control materials
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

Operating Instructions

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.
For detailed information refer to the operating instructions for your system.

Note:	Do not use visibly damaged product.

Sample Dilution

Serum and plasma (heparin and EDTA) samples with values greater than the measuring range may be automatically diluted on the system up to 10-fold (1 part sample with 9 parts diluent) by the VITROS Immunodiagnostic and VITROS Integrated systems with VITROS High Sample Diluent B Reagent Pack prior to test. Refer to the VITROS High Sample Diluent B Reagent Pack instructions for use.

Default Test Name

The default test name which will appear on patient reports is Troponin I ES. The default short name that will appear on the test selection menus and laboratory reports is TropI. These defaults may be reconfigured if required. For detailed information refer to the operating instructions for your system.

Calibration

Calibration Procedure

- Calibration is lot specific; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration.
- A Master Calibration (a dose response curve covering the full calibration range) is established for each new reagent lot. Concentrations for the linked lot of calibrators are determined from the Master Calibration.
- Ensure that the Master Calibration for each new reagent lot is available on your system.
- Process calibrators in the same manner as samples. Calibration need not be programmed if bar code labels are used; load the calibrators in any order, calibration will be initiated automatically.
- When the calibrators are processed the signal expected for each calibrator is compared against the actual signal obtained. The Master Calibration is then rescaled to reflect the differences between the actual and expected signals. The validity of this calibration curve is assessed against a range of quality parameters, and if acceptable, it is stored for use with any reagent pack of that lot.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.
- Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.
- Calibration results are assessed against a range of quality parameters. Failure to meet any of the defined quality parameter ranges will be coded in the calibration report. For actions to be taken following a failed calibration refer to the operating instructions for your system.

Refer to the operating instructions for your system for detailed instructions on the calibration process.

When to Calibrate

- Calibrate when the test reagent and calibrator lot changes.
- Calibrate every 28 days.
- After specified service procedures have been performed.
- If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

Traceability of Calibration

Calibration of the VITROS Troponin I ES test is traceable to in-house reference calibrators, which have been value assigned to correlate to another commercially available test.

Calibration Model

A modified four parameter logistic curve fit function is used to construct the Master Calibration. The calibration process rescales the Master Calibration to establish a valid stored curve for the VITROS Immunodiagnostic and VITROS Integrated Systems.

Measuring (Reportable) Range

System	Measuring (Reportable) Range
3600 5600 XT 7600 ECi/ECiQ	0.012*–80.0 ng/mL (µg/L)

* Lower limit of measuring range reported by the system software is based on the Limit of Quantitation.

The lower limit reported by the system can be reconfigured if desired. For details on how to reconfigure the lower limit refer to the operating instructions for your system.

Quality Control

Quality Control Material Selection

Controls containing suitable levels of cTnI are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. The performance of commercial control fluids should be evaluated for compatibility with this test before they are used for quality control.

Control materials may show a difference when compared with other cTnI methods if they contain high concentrations of preservatives, stabilizers, or other nonphysiological additives, or otherwise depart from a true human sample matrix.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Troponin I ES test.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- Choose control levels that check the clinically relevant concentrations.
- To verify system performance, analyze control materials:
  - After calibration
  - According to local regulations or at least once each day that the test is being performed
  - After specified service procedures are performed

If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.

- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- Refer to published guidelines for general quality control recommendations. [26]

For more detailed information, refer to the operating instructions for your system.

Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Results

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

Reporting Units and Unit Conversion

Analyte results are quoted in units of ng/mL and µg/L. To configure the units, refer to the operating instructions for your system.

Conventional	Alternate
ng/mL (µg/L× 1)	µg/L (ng/mL× 1)

Limitations of the Procedure

Known Interferences

The VITROS Troponin I ES test was evaluated for interference consistent with CLSI document EP7. [27] Commonly encountered substances were tested on 3 lots of reagents. Of the compounds tested, hemoglobin may interfere with the VITROS Troponin I ES test resulting in a positive bias as shown in the table below.

Refer to "Specificity" for a list of other compounds tested that did not show interference.

Interferent	Interferent Concentration		Units = ng/mL (µg/L)	
			Analyte Conc*	Bias**
Hemoglobin	0.062 mmol/L	100 mg/dL	0.006	0.003
Hemoglobin	0.124 mmol/L	200 mg/dL	0.006	0.030
Hemoglobin	0.186 mmol/L	300 mg/dL	0.006	0.031
Hemoglobin	0.248 mmol/L	400 mg/dL	0.006	0.033
Hemoglobin	0.310 mmol/L	500 mg/dL	0.006	0.034

* Average test concentration of replicate determinations using three different lots of reagent, concentration below the measuring range.

** Estimate of the average difference observed.

Note: These results are representative. The degree of interference at concentrations other than those listed might not be predictable from these results. Other interfering substances may be encountered in the patient population.

Other Limitations

- Interpretation of cTnI results should be done only in the context of the overall clinical picture, e.g., clinical history, ECG, and other laboratory tests indicative of cardiac damage such as CKMB, CK, or myoglobin. The triage of patients with chest pain should be based on serial samples and the typical rise and fall pattern of cTnI characteristic of cardiac damage.
- For troubleshooting purposes, if the cTnI result is inconsistent with the clinical picture and is persistently elevated, the sample should be tested for the presence of heterophilic antibodies.[28] These antibodies may be present in the blood samples from individuals regularly exposed to animals or who have been treated with animal serum products.
- Samples from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain Human Anti-Mouse Antibodies (HAMA). Such samples may show either falsely elevated or falsely depressed values when tested with this method.[29]
- Streptokinase may interfere negatively with the predicted concentration of cTnI. Care should be taken when interpreting cTnI results after administration of streptokinase.
- A high dose hook effect was not observed in samples up to 14,000 ng/mL (µg/L).
- Biotin levels in serum remain elevated for up to 24 hours after oral or intravenous biotin administration.[30]

Expected Values and Interpretation of Results

It is recommended that each laboratory establish its own expected values for the population it serves. The decision to rule out AMI should not be made based on the data from a single blood collection.

As a guide, the following decision limits were determined in the VITROS Troponin I ES test:

Upper Reference Limit (URL)

The 99th Percentile URL is 0.034 ng/mL (µg/L).

This value was based on 21 estimates of the URL using >10,000 serum, heparin and EDTA plasma samples from individual donors and included variation from raw materials, analyzer, operator, manufacturing processes and reagent age.

Interpretation of Results

The National Academy of Clinical Biochemistry Standards of Laboratory Practices (NACB) and the International Federation of Clinical Chemistry (IFCC) recommend[31-33] a minimum of three serial blood samples within 24 hours of admission. **Serial sampling is recommended to detect the temporal rise and fall of cTnI levels characteristic of AMI.**

The Joint European Society of Cardiology/American College of Cardiology (ESC/ACC) and the National Academy of Clinical Biochemistry Standards of Laboratory Practices (NACB) recommends that the diagnosis of AMI includes the presence of clinical history suggestive of Acute Coronary Syndrome (ACS) and a maximum concentration of cardiac troponin exceeding the 99th percentile of a normal reference population [upper reference limit (URL)] on at least one occasion during the first 24 hours after the clinical event.[31, 34]

The World Health Organization (WHO)[35] requires two of the following criteria for the diagnosis of AMI: elevated cardiac markers results (cTnI, myoglobin, and/or CKMB), evolutionary changes in ECG, history of chest pain.

Increased cTnI concentrations can be found in conditions other than AMI that can result in myocardial damage. Published studies have documented that these conditions include, but are not limited to, sepsis, congestive heart failure, hypertension with left ventricular hypertrophy, hemodynamic compromise, myocarditis, mechanical injury including cardiac surgery, defibrillation and cardiac toxins such as anthracyclines.[9-14] Factors such as these should be considered when interpreting results from any cTnI test method.

AMI Cutoff

The VITROS Troponin I ES test AMI diagnostic cutoff is 0.120 ng/mL (µg/L). A clinical study was performed consistent with CLSI document GP10.[36] The study was conducted at two external clinical sites using prospectively collected, serially drawn specimens. A total of 506 patients with symptoms of acute coronary syndrome (ACS) were diagnosed as AMI or non-AMI according to the ESC/ACC/AHA criteria [34, 37-38], 74 patients were diagnosed as AMI and 432 patients were diagnosed as non-AMI. The peak sample from each patient's serial draw was obtained and used in the analysis. The peak cTnI result is defined as the highest cTnI concentration observed in the serial draw obtained from each patient.

The Receiver Operator Curve (ROC) compares clinical sensitivity and specificity at various decision thresholds. The optimum decision threshold maximizes the area under the curve (AUC) and represents the highest sensitivity and specificity for the test. The AUC for the VITROS Troponin I ES test was 0.964. Using the cutoff of 0.120 ng/mL cTnI, the sensitivity of the VITROS Troponin I ES test was 95% (95% CI of 87–99%) and the specificity was 93% (95% CI of 90–95%).

Clinical Sensitivity and Specificity

Samples were collected and analyzed at two independent clinical sites for sensitivity and specificity. Samples were prospectively collected and serially drawn, at the time intervals indicated below, determined from the time of admission. The data are presented in the table below:

		Hours Post Admission		
		0–6 hrs	6–12 hrs	12–24 hrs
VITROS Troponin I ES Test (AMI cutoff = 0.120 ng/mL)	% Sensitivity	70 (86/123)	89 (78/88)	90 (43/48)
	% Specificity	96 (683/711)	94 (420/447)	94 (206/220)

Note: The results above are representative data and may vary from study to study. Each laboratory should confirm the validity of the diagnostic cutoff for the population it serves.

Risk Stratification

In a prospective study, 395 patients with myocardial ischemia symptoms suggestive of Acute Coronary Syndrome (ACS) were hospitalized in one medical center to rule in or rule out myocardial infarction (MI). Serial blood samples were obtained from each patient and tested using the VITROS Troponin I ES test. These patients were followed up after baseline cTnI testing and monitored for short-term adverse cardiac events, which include re-hospitalization due to recurring ischemia, MI and all cause death.

The results were analyzed using the 99th percentile upper reference limit [0.034 ng/mL (µg/L)], as recommended by the ESC/ACC/AHA [34, 37-38] consensus document for the redefinition of MI.

The findings from this study verified previous clinical studies [15-16, 39] that showed patients with minor increases in cTnI values provide important prognostic information about the long and short-term risk of death for patients with ACS.[17-18, 40] The data collected from this study indicate that patients with baseline cardiac troponin I values above the 99th percentile upper reference limit had a significantly higher short-term risk of death or recurrent ischemic events after presentation.

Performance Characteristics

Limit of Detection

The limit of detection (LoD) for the VITROS Troponin I ES test using human serum pools is 0.012 ng/mL (µg/L), determined consistent with NCCLS document EP17[41] and with proportions of false positives (α) less than 5% and false negatives (β) less than 1%; based on 600 determinations, with 150 blank and 450 low-level samples. The Limit of Blank (LoB) = 0.007 ng/mL (µg/L). The limit of quantitation (LoQ) is 0.012 ng/mL (µg/L) as determined by the lowest concentration at which precision and accuracy design requirements are still met and within the linear range of the test.

Limit of Blank, Limit of Detection and Limit of Quantitation

LoB*		LoD**		LoQ	
ng/mL	µg/L	ng/mL	µg/L	ng/mL	µg/L
0.007	0.007	0.012	0.012	0.012	0.012

* Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term “analytical sensitivity.”

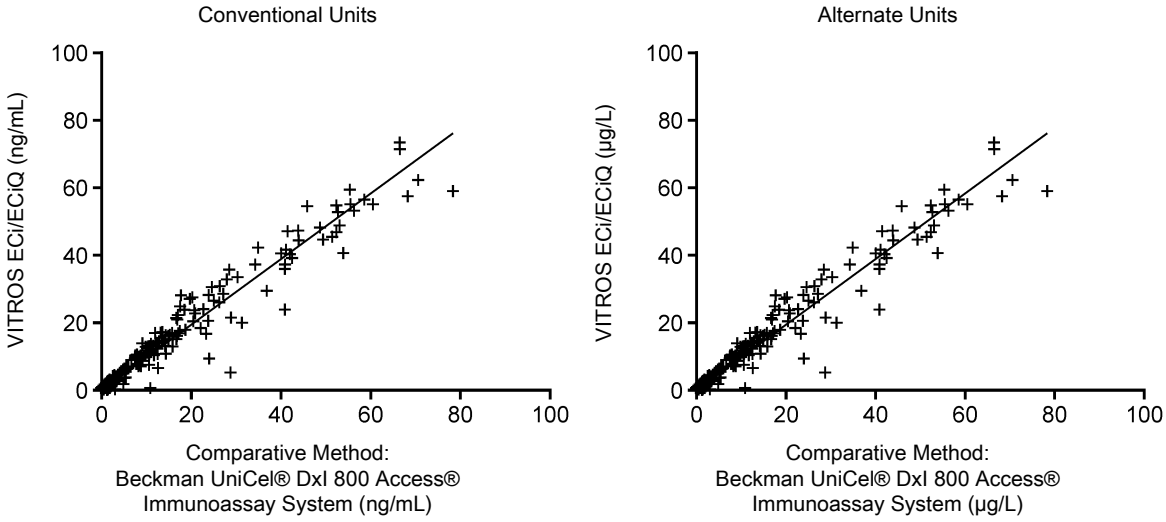
** Proportions of false positives (α) and false negatives (β) were less than 5% and 1% respectively; based on 600 determinations, with 150 blank and 450 low-level samples.

Accuracy (Method Comparison)

Accuracy was evaluated consistent with NCCLS document EP9.[42] The plot and table show the results of a method comparison study using patient samples analyzed on the VITROS ECi/ECiQ Immunodiagnostic System compared with

those analyzed using the Beckman Unicel® DxI 800 Access® Immunoassay System. The relationship between the 2 methods was determined by Deming regression. [43]

The table also shows the results of method comparison studies [42] using patient serum samples analyzed on the VITROS ECi/ECiQ Immunodiagnostic System compared with those analyzed using the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System. The relationship between the 2 methods was determined by Passing and Bablok regression. [44]



System	n	Slope	Correlation Coefficient	Conventional Units (ng/mL) Range of Samples	Conventional Units (ng/mL) Intercept	Alternate Units (µg/L) Range of Samples	Alternate Units (µg/L) Intercept
ECi/ECiQ vs. Comparative Method	260	0.9790	0.9764	0.015–73.5	0.2191	0.015–73.5	0.2191
3600 vs. ECi/ECiQ	99	0.9551	0.9996	0.033–66.7	-0.0045	0.033–66.7	-0.0045
5600* vs. ECi/ECiQ	99	0.9713	0.9997	0.033–66.7	-0.0045	0.033–66.7	-0.0045

* Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Precision

VITROS ECi/ECiQ Immunodiagnostic System

Precision was evaluated consistent with NCCLS document EP5. [45] Two replicates each of 2 frozen control sera and 4 frozen human sample pools were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 3 reagent lots on 3 different systems. The data presented are a representation of the product performance.

VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System

Precision was evaluated consistent with NCCLS document EP5. [45] Two replicates of each of 3 control samples and 2 frozen patient pools were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 1 reagent lot on each system. The data presented are a representation of the product performance.

System	Units = ng/mL (µg/L) Mean Troponin I Conc	Within-run* SD	Within-run* CV (%)	Within-calibration** SD	Within-calibration** CV (%)	Within-lab*** SD	Within-lab*** CV (%)	No. Observ	No. Days
ECi/ ECiQ system 1	0.024	0.001	4.2	0.003	12.5	0.002	8.3	84	29
	0.063	0.001	1.6	0.003	4.8	0.003	4.7	84	29
	0.091	0.001	1.1	0.003	3.3	0.003	3.3	84	29
	0.413	0.016	4.0	0.017	4.2	0.018	4.3	84	29
	5.23	0.050	1.0	0.112	2.2	0.106	2.0	84	29
	56.3	0.810	1.5	1.75	3.2	1.49	2.6	84	29

System	Units = ng/mL (µg/L)								
	Mean Troponin I Conc	Within-run*		Within-calibration**		Within-lab***		No. Observ	No. Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ ECiQ system 2	0.028	0.001	3.7	0.003	11.1	0.003	10.7	84	29
	0.064	0.001	1.6	0.004	6.3	0.004	6.2	84	29
	0.092	0.001	1.1	0.004	4.4	0.004	4.3	84	29
	0.433	0.005	1.2	0.011	2.6	0.015	3.4	84	29
	5.58	0.047	0.9	0.117	2.1	0.139	2.5	84	29
	61.4	0.618	1.0	1.82	3.0	1.76	2.8	84	29
ECi/ ECiQ system 3	0.027	0.001	3.7	0.002	7.4	0.003	11.1	84	29
	0.059	0.002	3.4	0.004	6.8	0.005	8.5	84	29
	0.086	0.001	1.2	0.005	5.9	0.006	7.0	84	29
	0.393	0.005	1.3	0.011	2.8	0.016	4.0	84	29
	5.05	0.065	1.3	0.101	2.0	0.183	3.6	84	29
	62.8	0.823	1.3	1.75	2.8	2.96	4.7	84	29
3600	0.030	0.002	6.7	0.003	10.0	0.004	12.5	92	23
	0.068	0.001	1.5	0.003	4.4	0.003	4.3	96	24
	0.301	0.005	1.7	0.011	3.7	0.011	3.6	96	24
	5.55	0.064	1.2	0.163	2.9	0.172	3.0	96	24
	62.9	0.610	1.0	2.12	3.4	2.78	4.4	92	23
5600 ****	0.027	0.001	3.7	0.003	11.1	0.003	9.7	92	23
	0.070	0.001	1.4	0.003	4.3	0.003	4.3	100	25
	0.297	0.005	1.7	0.009	3.0	0.008	2.6	100	25
	5.40	0.055	1.0	0.123	2.3	0.127	2.2	100	25
	62.0	0.550	0.9	2.48	4.0	1.83	2.9	92	23

* Within-run (repeatability). Between Duplicate precision averaged over all runs

** Within-calibration. Total precision with weighted components of within-run, between–run and between-day variation.

*** Within-lab. A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from at least 4 calibrations

**** Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Low Concentration Precision Profile

To assess the low-end precision on the VITROS ECi/ECiQ Immunodiagnostic System, eight low troponin I serum sample pools were tested in singleton, twice a day on 20 occasions over 28 days using 2 lots of reagent. From the resulting imprecision profiles, the coefficient of variation (CV) at the 99th percentile URL of 0.034 ng/mL (µg/L) was 10%. The low-end precision was verified on the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System across 30 days, using a stabilized human sample pool. This demonstrates that the VITROS Troponin I ES test meets the recommendations as described by the ESC/ACC [34] for acceptable imprecision in a high sensitivity troponin I test.

Specificity

Substances that do not Interfere

The VITROS Troponin I ES test was evaluated for interference consistent with CLSI document EP7. [27] Of the compounds tested, none was found to cause a bias of >10% with the test at the concentrations indicated at clinically relevant cTnI concentrations of 0.400 ng/mL (µg/L).

Compound	Concentration	
Acetaminophen	1324 µmol/L	200 µg/mL
Albumin	na	8 g/dL
Allopurinol	2740 µmol/L	400 µg/mL
Ambroxol	1050 µmol/L	400 µg/mL
Ampicillin	144 µmol/L	50 µg/mL
Ascorbic acid	342 µmol/L	60 µg/mL
Aspirin	3.62 mmol/L	650 µg/mL
Atenolol	38 µmol/L	10 µg/mL

Compound	Concentration	
Furosemide	1210 µmol/L	400 µg/mL
Ibuprofen	2425 µmol/L	500 µg/mL
Low MW Heparin	na	5 U/mL
Methyldopa	105 µmol/L	25 µg/mL
Nifedipine	173 µmol/L	60 µg/mL
Nitrofurantoin	269 µmol/L	64 µg/mL
Nystatin	na	7.0 µg/mL
Oxytetracycline	10.9 µmol/L	5.0 µg/mL

Compound	Concentration		Compound	Concentration	
Bilirubin	342 µmol/L	20 mg/dL	Phenytoin	365 µmol/L	100 µg/mL
Biotin	10.2 nmol/L	0.25 µg/dL	Propranolol	24 µmol/L	5.0 µg/mL
Caffeine	515 µmol/L	100 µg/mL	Quinidine	62 µmol/L	20 µg/mL
Captopril	230 µmol/L	50 µg/mL	Sodium Azide	3.08 mmol/L	200 µg/mL
Cinnarizine	1090 µmol/L	400 µg/mL	Sodium Heparin	na	8 U/mL
Cocaine	33 µmol/L	10 µg/mL	Streptokinase	na	1.96U/mL
Diclofenac	169 µmol/L	50 µg/mL	Theophylline	1380 µmol/L	250 µg/mL
Digoxin	7.8 nmol/L	0.61 µg/mL	t-PA	na	2.3 µg/mL
Dipyrone	30 mmol/L	1000 mg/dL	Trimethoprim	258 µmol/L	75 µg/mL
Dopamine	4250 µmol/L	650 µg/mL	Triolein	33.9 mmol/L	3000 mg/dL
Erythromycin	273 µmol/L	200 µg/mL	Verapamil	353 µmol/L	160 µg/mL
Fibrinogen	na	1000 mg/dL	Warfarin	32.4 µmol/L	10 µg/mL

n/a = not applicable

Cross-Reactivity

The cross-reactivity of the VITROS Troponin I ES test was evaluated by adding the following substances to samples containing no analyte.

Cross-reactant Tested	Concentration	Mean cTnI Result of Cross-reactant Pool Units=ng/mL	% Cross-reactivity
Skeletal Troponin I	1000 ng/mL	0.082	0.008
Cardiac Troponin C (Recombinant)	1000 ng/mL	0.017	0.002
Cardiac Troponin T (Recombinant)	1000 ng/mL	ND*	ND*
Actin (from Rabbit Muscle)	1000 ng/mL	ND*	ND*
Myosin (Recombinant)	1000 ng/mL	ND*	ND*
Tropomyosin (from porcine muscle)	1000 ng/mL	ND*	ND*
CK-MB (Recombinant)	1000 ng/mL	ND*	ND*
Myoglobin (Recombinant)	1000 ng/mL	ND*	ND*

* ND = Not Detectable. Concentration was below the measuring range of the test.

Cross-reactivity was expressed as the mean result obtained for the cross-reactant pool divided by the cross-reactant concentration in percentage term.

$$\% \text{ Cross-reactivity} = \frac{\text{Mean Result for the Cross-Reactant Pool (ng/mL)}}{\text{Concentration of Cross-Reactant (ng/mL)}} \times 100$$

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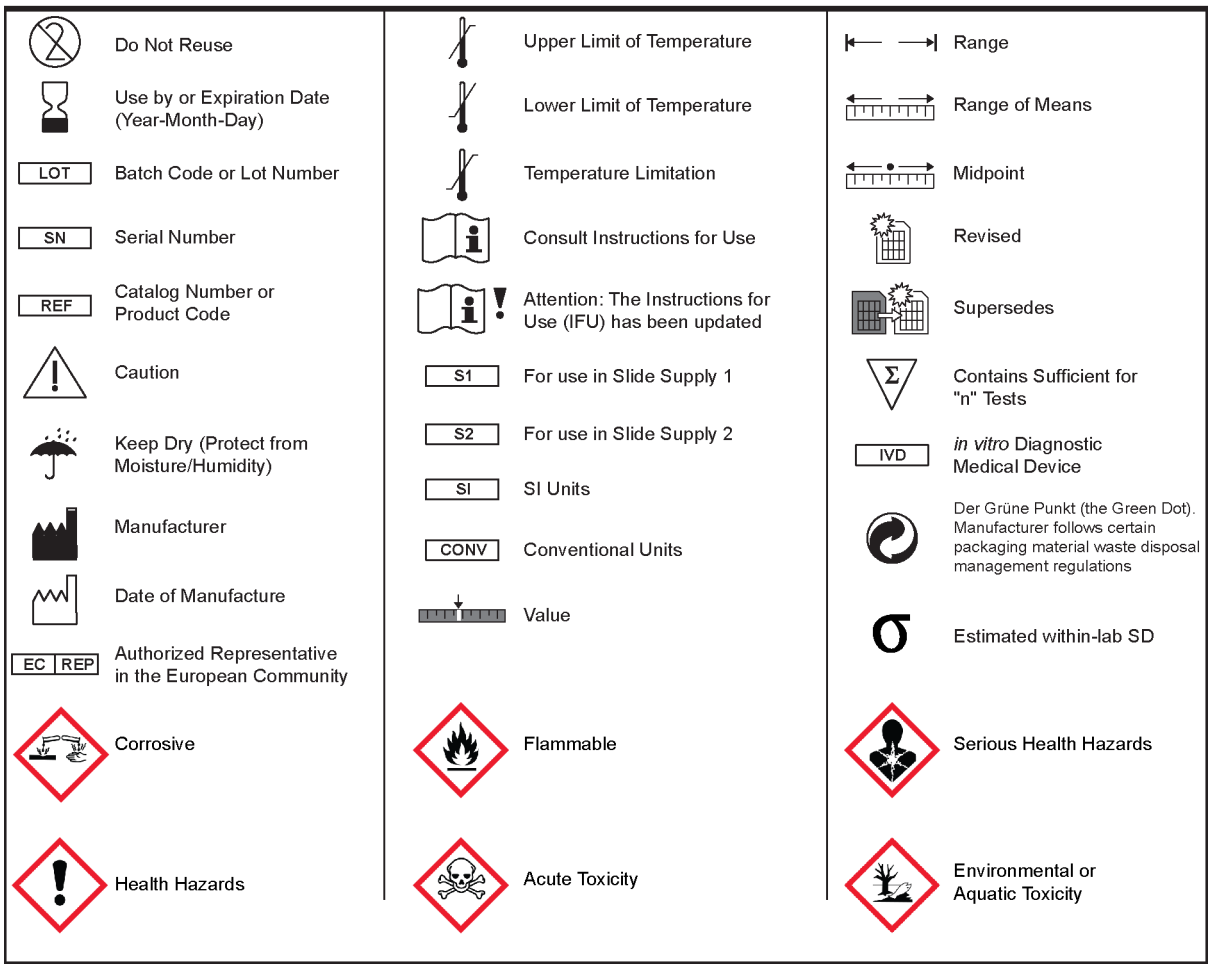

Revision History

Date of Revision	Version	Description of Technical Changes*
2020-04-22	9.0	Calibrator Storage and Preparation: changed -20 °C to ≤-20 °C

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

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