

Review

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Cardiac troponin assays: a review of quantitative point-of-care devices and their efficacy in the diagnosis of myocardial infarction

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Abstract: Cardiac troponin (cTn) I and T are released from myocardial cells following necrosis, i.e., cell death. An accurate measure of cTn concentrations in a patient's blood following ischemia/chest pain can enable providers to determine whether or not a myocardial infarction (MI) has occurred. Point-of-care (POC) devices that measure blood cTn concentrations in under 30 min may help to significantly reduce hospital costs by managing and triaging patients out of the emergency department as quickly as possible. The use of POC devices that measure cTnI and cTnT with a coefficient of variation (CV) $\leq 20\%$ at the 99th percentile upper reference limit (URL) limits both false positive and negative results and provides clinically acceptable findings to assist in appropriate diagnoses. This article reviews nine POC devices that measure cTn in terms of their clinical sensitivity and specificity, analytical imprecision, sample type and preparation, and each assay's principle of analysis.

Keywords: cardiac troponin; immunoassay; point-of-care.

Introduction

Cardiovascular disease (CVD) is the cause of more than 700,000 deaths in the USA each year. A total of 48% of these deaths can be attributed to coronary artery disease (CAD) [1]. Acute coronary syndrome (ACS), a subcategory of CAD, occurs when blood flow to the heart is reduced, and myocardial cells are deprived of oxygen. ACS can

be attributed to the rupture of unstable atherosclerotic plaques, which induces thrombus formation and leads to occlusion of a coronary artery. The two main types of ACS include unstable angina, in which myocardial cells are reversibly damaged, and acute myocardial infarction (AMI), when blood flow is completely blocked, resulting in myocardial cell death [2].

Previous biomarkers, such as creatine-kinase MB (CK-MB) and myoglobin, have been used to detect and determine the extent of myocardial injury [3, 4]. The current gold standard biomarker is cTn, of which both cTnI and cTnT are found in high concentrations in the myocardium. cTnI is not detectable in non-cardiac tissue [4], while several reports have recently described increased immunoreactivity to the Roche cTnT assay in patients with diseased skeletal muscle pathologies [5]. Myocardial cells that have become necrotic begin to release cTn following their death, and concentrations can be detected in the blood from as few as 2 h to several days following the onset of ACS [6]. The persistence of cTn in the bloodstream can make the diagnosis of recurrent injury potentially difficult, however, as cTn is specific to cardiac tissue, secondary increases do occur even in the clinical setting of an AMI following reinfarction [7]. With any point-of-care (POC) assay, depending on its analytical characteristics, timing between blood collections will be important to make sure changing concentrations are detectable.

In order to provide the highest quality of care to patients while keeping emergency department (ED) costs at a minimum, it is desirable that instruments used to detect cTn in the blood produce results quickly, but maintain a high degree of accuracy and precision. Contemporary instruments utilized to measure cTn in the central laboratory have acceptable clinical sensitivity and specificity, but often cannot provide the necessary turn-around-time (TAT) desired by providers for optimal care after blood draw in the ED [8]. POC devices utilize rapid, diagnostic immunoassays that are able to

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Table 1 Point-of-care cardiac troponin assay analytical characteristics.

Manufacturer	Alere	Radiometer	Radiometer	Abbott	Trinity	LSI Medicine Corporation	Response Biomedical	Siemens	bioMerieux	Roche
Device name	Triage Cardiac Panel Troponin I	AQT90 FLEX cTnI	AQT90 FLEX cTnT	i-STAT cardiac troponin I	Meritas Troponin I	PATHFAST	RAMP troponin I	Stratus CS	VIDAS	CARDIAC T Quantitative Cobas
Time, min	20	10–20	12	7	15	17	20	14	20	14
Limit of detection, µg/L	0.05	0.0095	0.01	0.02	0.019	0.019	0.03	0.03	0.01	0.05
99th percentile upper reference limit, µg/L	<0.05	0.023	0.017	0.08	0.036	0.029	<0.10	0.07	0.01	Not reported
ROC, AMI or clinical cut-off for diagnosis, µg/L	0.4 (ROC)	0.10 (AMI)	Not reported	Not reported	Not reported	0.264 (ROC)	0.3 (clinical cut-off)	0.6–1.5, determined by institution (AMI)	0.11 (clinical cut-off)	0.1 (clinical cut-off)
Coefficient of variation	12% at 0.4 µg/L	10% at 0.039 µg/L	10% at 0.03	10% at 0.10 µg/L	10% at 0.036 µg/L	5.1% at 0.029 µg/L	10% at 0.21 µg/L	10% at 0.06 µg/L	10% at 0.11 µg/L	<9% at 0.1 µg/L
Interfering substances	HAMA ^a ; Hb, TG and bilirubin at >1000 ng/mL; hematocrit with >60% PCV	No documented interferences	No documented interferences	HAMA ^a ; Hb >1400 mg/dL; hemolysis; hematocrit with >50% PCV	HAMA ^a ; Hb >1400 mg/dL; hemolysis, Hb, TG, bilirubin, and Rh factor >500 IU/mL	HAMA ^a , bilirubin >60 mg/dL, TG >1000 mg/dL, Hb >1000 mg/dL, and Rh factor >500 IU/mL	HAMA ^a , Rh factor, excessive hemolysis, Hb, TG, bilirubin, cholesterol, heparin in excessive amounts	HAMA ^a , Hb >1000 mg/dL	HAMA ^a , Hb >550 mg/dL, TG >3000 mg/dL, bilirubin >29 mg/dL	HAMA ^a , bilirubin >20 mg/dL, Hb >200 mg/dL, TG >440 mg/dL, Rh factor >300 IU/mL, biotin >10 mg/dL, lipoic acid, sTnT >1000 ng/mL, pharmaceuticals not tested
Sample type	EDTA whole blood or plasma	Lithium heparin whole blood or plasma ^b	Lithium heparin EDTA whole blood or plasma ^b	Heparinized whole blood or plasma ^b	EDTA whole blood or plasma	Lithium-heparin, or sodium-heparin, or EDTA whole blood or plasma ^b	EDTA whole blood or plasma	Heparinized whole blood	Serum or plasma, heparinized with lithium or centrifuged to eliminate fibrin	Heparinized whole blood
Volume	Transfer pipette barrel (~250 µL)	2 mL	2 mL	17 µL	200 µL	100 µL	Ramp troponin I assay tip (~250 µL)	90 µL	200 µL	150 µL
Preparation	Pipette sample onto test device and insert directly into instrument	Place collection tube directly into instrument	Place collection tube directly into instrument	Pipette sample onto test device and insert	Pipette sample onto test device and insert	Pipette sample into well 1 of reagent cartridge and insert	Dilute sample with buffer, then pipette onto test device and insert	Place collection tube directly into instrument, on board centrifugation	Centrifuge, then pipette sample into well 1 of test strip. Insert strip with solid phase receptacle (SPR)	Place test strip into instrument and apply sample

^aHAMA includes all heterophilic antibodies; ^bDevice tests on whole blood directly without on board separation of plasma.

deliver rapid TAT results in under 30 min, and require minimal sample handling and preparation. They employ systems that waive the necessity for skilled laboratory personnel to operate the assay, and have the potential to reduce length of ED patient stays and overall hospital costs [3, 9]. The POC industry has increased considerably in depth as biotech companies worldwide compete to develop cTn assays that demonstrate high analytical quality with short TAT [10]. This review will evaluate the efficacy of POC cTn devices from nine different manufacturers in terms of their sensitivity, specificity, imprecision, clinical cut-off, sample preparation, and assay principle (Tables 1 and 2). In the context of these parameters, the capability of each device to contribute to the accurate diagnosis of AMI will also be evaluated.

Desired qualities of POC devices

One of the major challenges in the ED is correctly identifying whether or not an AMI has occurred. Premature discharge or drawn out stays in the ED are two common outcomes of the miscategorization of CVD, and can result in malpractice lawsuits or unnecessary expenses [11]. It is the hope that with improved POC testing, patients presenting with ischemia can be appropriately triaged. In order for healthcare providers to make the most well-informed decisions for their patient's care when using POC testing, there are several qualities that the ideal POC device should employ [11].

POC devices should comply with a TAT optimally of 30 min from blood collection to reporting of results [11]. This assists in the identification of patients with high probability of having AMI, who benefit from receiving appropriate therapies as soon as possible [11]. Ideally, cTn devices should meet a 10% CV at the 99th percentile value, or the upper reference limit (URL) [12, 13]. Better imprecision increases the ability to determine small differences in cTn over time. However, few devices are marketed that actually do meet this criterion. A 20% CV at the 99th percentile value is still considered clinically acceptable, as it does not significantly increase the incidence of false positive diagnoses of AMIs [14, 15].

Received operating characteristics (ROC) curve AMI cut-offs, which are derived for providing optimal clinical sensitivity and specificity, have also been determined for POC devices. This parameter has traditionally been used as the clinical cut-off prior to the 99th percentile value, optimizing clinical sensitivity and specificity of cTn for AMI as an effective diagnostic tool. cTn concentrations

above the ROC value are highly predictive of an AMI, as it is more specific but less sensitive compared to the 99th percentile value. This is generally a higher concentration than the 99th percentile value, so when using this cut-off, more false negatives may occur. Due to this, the use of an ROC or AMI cut-off has been replaced by the more clinically sensitive 99th percentile value as a cut-off for diagnosis of an AMI, though some devices may still report both. Both of the values will vary between devices, as they are assay dependent and no standardization exists between cTn assays. It is also important to keep in mind that the 99th percentile cTnI value will vary for each device, due to lack of assay standardization and variances in analytical sensitivity. For cTnT, standardization is different for the hs-cTnT assay compared the fourth generation, contemporary assay. The limit of detection (LoD) of the assay, or its analytical sensitivity, is determined by the antibodies used, and their affinity for a specific epitope. A lower LoD allows for a quantitative cTn measurement at presentation, an informative and important measurement for future risk stratification [16]. The LoDs of the devices included in this review range from 0.0095 to 0.05 $\mu\text{g/L}$. POC devices that provide quantitative results should be used instead of those limited to qualitative results. Quantitative results are preferred because the release (rise) and clearance (fall) of cTn can be serially monitored [11, 16]. According to the Third Universal Definition of MI, the detection of a rise and/or fall of cardiac biomarker values, with at least one above the 99th percentile URL, and one additional finding of ischemic symptoms, meets the criteria for diagnosis for an AMI [15]. A recent study by Palamalai et al. utilizing four POC assays in comparison to a central laboratory assay, illustrates the variable diagnostic accuracy, predicated and each assays analytical specifications and performance [17].

POC devices should implement measures to reduce the effect of interference by heterophilic antibodies, which can lead to falsely raised or lowered measurements [18]. Hemolyzed, lipemic, or icteric samples can also interfere with the assay, and most manufacturers will recommend against using samples with excessive hemolysis, triglycerides, or bilirubin. Hemolysis presents the largest challenge, as visible hemolysis would not be evident using whole specimens. Manufacturers should also provide a list of pharmaceuticals and other substances added at a test concentration to a sample containing cTn, tested, and found to have no significant interference with the assay's cTn result. This demonstrates the analytical specificity of the assay for cTn in the presence of other analytes potentially present in a sample. Commonly tested substances

are drugs, serum proteins, and biological byproducts, such as urea.

Sample preparation and handling must be considered when evaluating the ease of use of a POC device. They are intended for use by non-laboratory personnel and samples should be tested as soon as possible after draw to minimize TAT and maintain sample integrity. Little to no preparation is ideal to eliminate the need for training before using the device, to minimize the potential for human error, and to decrease the time to results [8]. Minimal sample handling also reduces exposure to biohazards. Devices that do not require sample centrifugation prior to testing, and accept whole blood directly from the collection tube, keep exposure at an absolute minimum. Manual dilutions are also not recommended at the POC. Heparin and EDTA are the only anticoagulants currently accepted by POC devices, but each device should specify which one is appropriate for its assay.

Current FDA and non-FDA cleared cTn POC devices: guideline compliant?

The information used to evaluate each of the devices included in this review is taken directly from the manufacturer's product insert or webpage, not the peer-reviewed literature (Tables 1 and 2).

Alere Triage Cardiac Panel

The Cardiac Panel is a two-site immunoassay capable of determining the concentrations of cTnI, CK-MB, and myoglobin in EDTA whole blood or plasma specimens. Several drops of EDTA whole blood or plasma (one micropipette barrel) should be added to the sample port of the test device, which contains all of the reagents necessary for the simultaneous quantification of cTnI, CK-MB and myoglobin mass. After addition of the sample, whole blood cells are separated from plasma by a filter in the test device. cTnI in the sample plasma then binds with monoclonal antibodies with a fluorescent tag and flows through the device by capillary action. The antibody-cTnI complexes are captured at a detection zone upstream from the sample port, which contains polyclonal capture antibodies to cTnI. The test device is inserted into the Triage Meter, which quantifies cTnI in the sample after it has been bound by both sets of antibodies. The meter will detect the amount of fluorescence within the measurement zone,

and compute the concentration of cTnI in the sample. The time from sample addition to results for this assay is about 20 min [19].

Since this assay employs murine monoclonal antibodies, there is a possibility of interference if human anti-mouse antibodies (HAMAs) are present in the sample. Though precautions have been taken to minimize this interference, it should be noted that erroneous results may occur for patients who have formed heterophilic antibodies. Hemoglobin, lipids, and triglycerides do not interfere with the assay at concentrations up to 1000 mg/dL. However, samples that are severely hemolyzed (hematocrit >60%) should be avoided. Pharmaceuticals and cTnI-related proteins that could potentially be present in samples have also been tested for cross-reactivity and interference with the assay, and none interfered. The LoD of the assay is 0.05 µg/L, and the 99th percentile URL falls below the LoD at an unspecified concentration. The lowest concentration at which total imprecision was established was 0.4 µg/L, with a CV of 12%. Thus, it is unclear whether the CV for this device falls below 20% at the 99th percentile URL, and suggests caution in using the 99th percentile as an URL. If it does not, the possibility exists of an increased incidence of false positives when using this assay. Though the specificity of the cardiac panel is acceptable, its analytical sensitivity and imprecision are not ideal per Global Task Force guidelines [19].

Radiometer AQT90 FLEX

Radiometer's AQT90 FLEX is a comprehensive cardiac panel that tests for a variety of cardiac biomarkers, including cTnI, cTnT, CK-MB, D-dimer, and myoglobin. Up to five different tests can be run on a single sample, and a new sample can be tested every 2 min. Test results are available from 10 to 20 min after sample addition depending on the analyte of interest. The cTnT assay gives a result in 12 min, but an exact running time is not reported for the cTnI assay [20]. The acceptable sample types for the determination of cTnI and cTnT include EDTA or lithium-heparin whole blood or plasma. To begin the analysis, a minimum of 2 mL of sample in a capped blood collection tube should be placed directly into the inlet of the instrument. Since no pipetting is involved, the AQT90 FLEX is one of the only devices to completely eliminate any contact with blood during testing [21, 22]. The AQT90 FLEX also tests whole blood directly without performing on board filtration or centrifugation to separate plasma from whole blood cells. The two-site immunoassay utilizes an assay cup containing antibodies in a dry, stable form. Biotinylated

Table 2 Point-of-care cardiac troponin assay detection methodologies.

Manufacturer, device, principle of analysis	Method of detection
Alere, Triage Cardiac Panel, fluorescence	cTnI in the sample binds with fluorescent monoclonal antibodies and flows through the test device by capillary action. When the antibody-cTnI complex reaches a zone specific to troponin, it is captured. The emitted fluorescence of the complex can be measured, and is proportional to the amount of cTnI in the sample.
Radiometer, AQT90 FLEX, fluorescence	Sample incubated with cTnI or cTnT specific monoclonal capture antibodies immobilized on assay cup and polyclonal tracer antibodies tagged with europium chelate, which fluoresces. Assay cup washed and dried. Signal from tracer antibody is measured by time-resolved fluorometry, and concentration of cTn is proportional to europium signal.
Abbott, i-STAT, enzyme-linked	cTnI binds to a detection antibody that carries the enzyme alkaline phosphatase. Complex migrates within cartridge and binds to a capture antibody adsorbed to the surface of an electrochemical sensor. Mixture is washed with fluid containing a substrate, which is cleaved by the enzyme. An electrochemically detectable product is released and quantified by sensor. Amount of product is proportional to amount of cTnI in the sample.
Trinity, Meritas, fluorescence	Sample added and travels through conjugate zone, where cTnI binds with fluorescently tagged monoclonal detection antibodies. cTnI-AB complexes flow through two reaction zones, and bind to capture antibodies adsorbed to the flow path. A laser induces light emission from antibody, which is measured and compared to known emission from a control zone. Amount of light detected is proportional to amount of cTnI present.
Mitsubishi, PATHFAST, enzyme-linked and Magstration®	Sample added to well containing magnetic particles coated with antibody. An enzyme-linked secondary antibody is also added. The two antibodies and cTnI form a complex, and a magnet is used to remove them from mixture. A chemiluminescent substrate is added to the new mixture, cleaved by the enzyme, an amount of light emission detected is proportional to amount of cTnI in sample.
Response Biomedical, RAMP, fluorescence	Sample incubated with monoclonal detection antibodies with a fluorescent tag and polyclonal capture antibodies, both specific to a different antigenic site on cTnI. Amount of fluorescence in a detection zone is compared to amount in a control zone, where excess detection antibodies bind. Ratio is determined, which gives cTnI concentration.
Siemens-Stratus, CS, enzyme-linked	Sample incubated with immobilized monoclonal antibodies, and a conjugate consisting of enzyme-labeled monoclonal antibodies to a different antigenic site on troponin is pipetted into reaction zone. The two antibodies form a sandwich complex with cTnI, and reaction zone is washed with solution containing substrate, initiating enzymatic activity and causing substrate fluorescence. Detected fluorescence is proportional to cTnI concentration.
bioMerieux, Vidas Ultra, enzyme-linked	Sample added to wells containing monoclonal antibodies labeled with alkaline phosphatase. cTnI binds to antibodies, and the antibody-cTnI complex mixture is cycled in and out of a solid phase receptacle (SPR) containing fixed immunoglobulins, which bind the complexes. Substrate is cycled in and out of SPR, and enzyme-catalyzed fluorescence of substrate occurs. Fluorescence intensity is proportional to cTnI concentration.
Roche, Cardiac T Quantitative, gold label signal intensity	Two monoclonal antibodies, one biotinylated and the other gold labeled, bind to different epitopes on cTnT when sample is added. Erythrocytes removed from sample, and plasma flows into detection zone. Gold-labeled complexes accumulate and form a signal line, the intensity of which is proportional to cTnT concentration. Excess gold-labeled antibodies form control line to signal validity of test.

monoclonal anti-cTn capture antibodies are preimmobilized to the streptavidin coated surface of the assay cup, and tracer antibodies tagged with europium have been added above a separating layer. When the sample is added to the assay cup and incubated, the capture and tracer antibodies bind different epitopes on cTn in the sample, forming a sandwich complex. The assay cup is washed and dried, and the europium signal given off by the tracer antibody is measured by time-resolved fluorometry. The measured signal is directly proportional to the amount of cTnI or cTnT in the sample, depending on the test being

run. The concentration of the analyte is then computed by the instrument using a built in calibration curve [21].

The AQT90 FLEX cTnI and cTnT assays have been determined to be highly specific. Both assays claim no interference by hemolytic, lipemic and icteric samples. Other potential interfering pharmaceuticals and substances were tested at concentrations approximately five times the therapeutic range, and found to have $\leq 20\%$ interference with the cTnI assay. There was also no detectable cross-reactivity of cTnI with skeletal troponin I (sTnI), cTnT or TnC [20]. Radiometer has not documented the

possibility of interference by heterophilic anti-animal antibodies for the cTnI assay. However, mouse IgG has been added as a blocker substance for interference of these antibodies with the capture or tracer antibodies employed by the assay. Potentially interfering pharmaceuticals were also tested for interference with the cTnT assay at five times the therapeutic concentration, and had <9% interference with the assay. No detectable cross-reactivity was found between cTnT and skeletal troponin T (sTnT), cTnI, and TnC [22]. Blockers have been added to the cTnT assay to minimize interference by heterophilic antibodies, and tests have shown that the blockers had the desired effect with the cTnT assay [22]. The AQT90 FLEX cTnI assay has a LoD of 0.0095 $\mu\text{g/L}$. The 99th percentile URL is 0.023 $\mu\text{g/L}$, and the concentration giving a 10% CV was 0.039 $\mu\text{g/L}$. Total imprecision at 0.024 $\mu\text{g/L}$, just above the 99th percentile URL, was found to be 17.7%, which falls below 20% and is considered clinically acceptable [20]. The AQT90 FLEX cTnT assay has a LoD of 0.01 $\mu\text{g/L}$, and a 99th percentile URL of 0.017 $\mu\text{g/L}$. The concentration that gives a CV of 10% is 0.03 $\mu\text{g/L}$. A 20% CV is not reported for this assay, and thus it is unclear whether the CV falls below 20% at the 99th percentile value. Two additional factors to consider for the AQT90 FLEX are the size of the device, and the sample volume it requires for both of the cTn assays. It is relatively large, and thus less portable than other POC devices, and also tests on a volume of 2 mL, which could be a limitation if only a small sample volume is available.

Abbott i-STAT

The Abbott i-STAT uses a two-site enzyme-linked immunosorbent assay (ELISA) method to detect cTnI in heparinized whole blood or plasma. Like Radiometer's AQT90 FLEX, no separation of whole blood cells from plasma occurs within the device; whole blood can be tested directly. The incubation period for this assay is 7 min, and a minimum of 17 μL of sample is required. When a specimen is injected into the sample inlet of the i-STAT cTnI test cartridge, it is brought into contact with an electrochemical sensor, where detection antibodies tagged with alkaline phosphatase enzymes are deposited. The antibodies dissolve into the sample and bind cTnI. Capture antibodies specific to another epitope of cTnI are also adsorbed to the surface of the sensor, and bind the antibody-cTnI complexes. Excess sample and reagent is washed off the sensor with a wash fluid containing a substrate for alkaline phosphatase. The enzyme cleaves the substrate, and an electrochemically detectable product is released. The

product can be quantified by the sensor, and its concentration is directly proportional the amount of cTnI in the sample [23].

Samples that may contain HAMA or other heterophilic antibodies have the potential to interfere with this assay, and though the device contains reagents intended to minimize the effect of these antibodies, results that appear inconsistent with clinical information should be evaluated for the possibility of HAMA interference. Visibly hemolyzed samples should also be avoided, as excessive hemolysis can cause a decrease in alkaline phosphatase activity. Hematocrits with >65% PCV may also increase test imprecision. No other interferences with drugs or cTnI related proteins potentially present in blood samples have been noted. This assay demonstrates a LoD of 0.02 $\mu\text{g/L}$, and the 99th percentile URL occurs at 0.08 $\mu\text{g/L}$. A 10% CV is reached at a cTnI concentration of 0.10 $\mu\text{g/L}$, and a 20% CV is reached at 0.07 $\mu\text{g/L}$ [23]. Though the i-STAT does not demonstrate ideal imprecision with <10% CV at the 99th percentile URL, the precision of the device is satisfactory with <20% CV at 0.08 $\mu\text{g/L}$. The i-STAT is also a handheld device, and its portability and rapid TAT makes it convenient for use in critical care settings [23].

Trinity Meritas

Trinity Biotech's Meritas POC analyzer is a new device that was approved for use in Europe (CE Mark) as of January 2014, and is currently undergoing clinical trials in the US to obtain FDA clearance. The analyzer is used in conjunction with the test cartridge, a lateral flow immunoassay that contains a plastic chip with a defined structure unique to the analyte of interest. The chips are produced by injection molding, creating a defined micropillar structure on each chip that does not vary from batch to batch. Clinical studies will be essential to validate lot to lot consistency. The use of plastic instead of the commonly used nitrocellulose membrane is thought to improve consistency in structure of the test cartridges. The unique micropillar structure also allows for an even flow of plasma over the chip surface. To begin testing, approximately 200 μL of EDTA whole blood or plasma is pipetted into the assay sample port directly from the blood collection tube, and whole blood cells are separated from plasma in the separation membrane of the assay port. The plasma then travels through the assay's conjugate zone, and cTnI in the sample binds with fluorescent monoclonal detection antibodies. cTnI-antibody complexes flow through two detection zones where they bind to capture antibodies adsorbed to the flow path. The unbound detection antibodies are

captured in a control zone just past the detection zones. A laser diode is then used to induce fluorescence emission from the tagged antibodies. The intensity of the measured fluorescence in the detection zone is quantified as compared to fluorescence in the control zone, and this ratio is used to calculate the concentration of cTnI in the sample via a built in calibration curve. The time from sample addition to result for the Meritas assay is about 15 min [24].

Information about the specificity and possible interferences with this assay is currently available in a CE mark package insert. There is a possibility of interference by heterophilic antibodies at extremely high titers, however, this assay has been designed to minimize the effect of these antibodies. Results are unaffected by hematocrits in the range of 30%–50%, and hemolysis up to 8%. No significant interference was noted for commonly used pharmaceuticals and other possible interfering compounds. The Meritas demonstrates a LoD of 0.019 $\mu\text{g/L}$ for whole blood, and 0.012 $\mu\text{g/L}$ for plasma. The 99th percentile URL occurs at 0.036 $\mu\text{g/L}$ for whole blood [25]. Clinical performance of the analyzer was evaluated at this value, and a CV of 10% was achieved [24]. The Meritas is about the size of a small desk telephone, facilitating ease of transport for use in the ED.

LSI Medience Corporation PATHFAST

The PATHFAST cTnI utilizes a chemiluminescent enzyme immunoassay (CLEIA) and MAGTRATION® technology to quantitatively measure the cTnI concentration in Li-heparin, Na-heparin, or EDTA-K₂ whole blood or plasma. If using whole blood, the sample should be mixed gently in the blood collection tube before testing. A vortex mixer should not be used. To begin testing, the reagent cartridge is placed in the cartridge rack of the PATHFAST, and approximately 100 μL of specimen is dispensed into well 1. The other 15 wells of the cartridge either contain reagent to be used in the assay or are empty. The cartridge is then loaded, and the sample is mixed with alkaline phosphatase labeled anti-cTnI monoclonal antibodies and anti-cTnI monoclonal antibodies coated with magnetic particles. The two antibodies are solubilized in the sample, and bind with different molecular epitopes on cTnI, thus forming a sandwich immunocomplex. A magnet is used to remove the complexes from the mixture, and unbound enzyme labeled antibody is left behind. A chemiluminescent substrate is then added to the mixture of immunocomplexes, and, after a short incubation, undergoes reaction with the enzyme. The intensity of the luminescence generated by the product of this reaction is

measured, and the concentration of cTnI in the sample is calculated using a calibration curve. The time from sample addition to result for this assay is 17 min [26].

The PATHFAST has been designed to minimize interference by heterophilic antibodies that may be present in patient samples. However, complete elimination of their effect cannot be guaranteed. Test results that are inconsistent with the patient history or clinical evaluation should be evaluated for interference by heterophilic antibodies. An interference with the assay of <10% was noted for samples containing free and conjugated bilirubin (60 mg/dL), triglycerides (1000 mg/dL), hemoglobin (1000 mg/dL) and rheumatoid factor (500 IU/mL). No drug was found that significantly interfered with the assay, and there was no significant cross-reactivity with cTnT or sTnI. Since this assay accepts specimens using Li-heparin, Na-heparin, or EDTA-K₂ as anticoagulants, each of the three types of samples were tested to see they produced significantly different results in cTnI concentration. It was found that the choice of anticoagulant had no significant effect on the assays results. The limit of quantitation (LoQ) for the PATHFAST is 0.019 $\mu\text{g/L}$, and the 99th percentile URL is 0.029 $\mu\text{g/L}$. The PATHFAST surpasses a CV of <10% at the URL, achieving a CV of 5.1% at 0.029 $\mu\text{g/L}$. Thus, the PATHFAST is the POC device with the best total imprecision [26].

Response Biomedical RAMP

The RAMP Troponin I Assay is an immunochromatographic test that quantitatively measures the amount of cTnI in well-mixed EDTA whole blood. This device accepts whole blood only, which is not problematic at the POC if blood goes straight from the patient's bedside to the collection tube for immediate testing. Only if plasma samples are preserved to be tested at a later time would this prove to be a limitation of the device. The RAMP also requires the sample to be diluted with buffer before it can be tested. The buffer must be removed from refrigeration to equilibrate to room temperature for at least 15 min prior to testing. A transfer device with a tip of approximately 250 μL in volume is provided, and used for transfer of sample to the buffer vial. The sample and buffer must then be slowly mixed in the vial by pressing and releasing the plunger of the transfer device 10 times. After these steps are taken, the mixture can be injected into the sample well of the test cartridge and loaded into the instrument. Upon injection, red blood cells are separated from plasma and retained in the sample pad. The plasma migrates along the testing strip and encounters monoclonal anti-cTnI

antibodies tagged with fluorescent particles, which bind to cTnI. Further along the strip in the detection zone, the cTn-antibody complexes are bound and captured. Excess fluorescently tagged antibodies are captured in the internal standard zone. The RAMP measures the amount of fluorescence emitted in the detection zone as compared to the internal standard zone, and from this ratio, quantifies the amount of cTnI in the sample [27].

Blood samples that are excessively hemolyzed may interfere with the RAMP assay and produce erroneous results. sTnI, cTnT, and cTnC were all tested for cross-reactivity with the assay at concentrations of 1000 µg/L, and were found to have no effect on results. HAMA, human anti-goat antibodies (HAGA), human anti-rabbit antibodies (HARA) and Rh factor were also tested and have minimal cross-reactivity with the assay. However, results from patients known to possess these antibodies should be interpreted with caution. Care should be taken to ensure that their results are consistent with the clinical picture. Hemoglobin, triglycerides, bilirubin, cholesterol, and heparin were also evaluated for cross-reactivity, and had no significant effect on assay results at concentrations up to 1500 mg/dL, 3000 mg/dL, 80 mg/dL, 500 mg/dL, and 66 IU/mL, respectively. The LoD for the RAMP is 0.03 µg/L, and the 99th percentile URL was 0.10 µg/L. A 20% CV occurs at 0.10 µg/L, and a 10% CV occurs at 0.21 µg/L. Thus, the RAMP achieves an adequate level of precision at the 99th percentile URL with a CV of 20%. The main limitation of this assay is that it requires excessive sample handling, which leads to more preparation time and more room for user error. Though this device is able to comply with a TAT of <1 h, it likely takes more than 30 min from blood draw to produce a result. The assay itself takes 20 min to run from the time the test cartridge is inserted, and time for buffer equilibration to room temperature, sample dilution, and sample mixing must also be accounted for [27].

Siemens Stratus CS

The Stratus CS Acute Care Troponin I is a POC fluorometric analyzer that utilizes a two-site sandwich immunoassay to quantitatively measure cTnI in lithium or sodium heparin whole blood only. Whole blood must be collected in a tube qualified for use with the Stratus analyzer. The collection tube is placed directly into the instrument cannula after inversion to ensure uniform suspension of red blood cells. Two milliliters of blood from the tube is then transferred to a rotor within the analyzer, and centrifuged to isolate plasma. The plasma sample is then incubated with

dendrimer-linked immobilized monoclonal antibodies, which react with cTnI in the sample. An enzyme-labeled antibody, which is specific to a different epitope on cTnI, is added next, and an antibody-cTnI-labeled antibody sandwich is formed. A wash solution containing substrate is then added to the incubation site, eluting the unbound antibodies, and reacting with the enzyme on the bound, labeled antibodies. This reaction initiates fluorescence of the bound substrate, which is quantified by an optical system within the instrument. The amount of fluorescence is directly proportional to the amount of cTnI in the sample, and the concentration is calculated by the analyzer [28]. The result is displayed on the analyzer approximately 14 min from sample insertion [29].

The Siemens Stratus CS is designed to minimize interference from heterophilic antibodies, but it is still possible that human anti-animal antibodies present in patient samples could cause falsely elevated or depressed results. Samples with excessive hemolysis (Hb >1000 mg/dL) should not be used, as cellular debris resulting from these samples, not the hemoglobin itself, has been found to elevate cTnI results up to 0.13 µg/L in apparently healthy individuals. No other interferences by pharmaceuticals, proteins, or other elements potentially present in human blood samples were noted for this assay. cTnC, cTnT, sTnT, and sTnI were included in this testing and found to have no significant cross-reactivity with cTnI. The LoD for the Stratus CS is 0.03 µg/L, and a CV of 20% is achieved at this value. At 0.06 µg/L, this device achieves a CV of 10%. The 99th percentile URL occurs at 0.07 µg/L, thus, the Stratus reaches a CV <10% at the 99th percentile URL. A benefit of using this device is that it, like Radiometer's AQT90 FLEX, accepts whole blood directly from the collection tube and automatically centrifuges and pipettes sample into test cartridges. A downside is that a collection tube qualified for use on the analyzer must be used, which could be problematic if the hospital laboratory does not normally use the necessary brand of tube. Additionally, the 2 mL sample volume required by this device is relatively large by POC standards, and may prove problematic when limited sample is available. The large size of the Stratus CS, in comparison to more modern, space-saving devices, is another limitation of its use in critical care settings [28].

bioMerieux Vidas Troponin I Ultra

bioMerieux's Vidas Troponin I Ultra (TNIU) utilizes a one-step immunoassay sandwich method with fluorescence detection. This assay accepts only plasma or serum, as the device employs no on board method of separating

whole blood into its components. Without the ability to use whole blood, some would argue that this assay is not POC. Samples must be collected in lithium heparin tubes, or tubes with no additive. When using the latter, samples must be centrifuged following coagulation to eliminate fibrin prior to testing, as the role of Li-heparin is to prevent formation of fibrin. Regardless of the tube used, an initial centrifugation must be performed to separate red blood cells from plasma. Following centrifugation, the sample should be well mixed using a vortex. A volume of 200 μL of sample can then be added to well 1 of 10 in the TNIU test strip. Most of the wells contain reagent necessary for the assay, and some are empty. The solid phase receptacle (SPR), which serves as the pipetting device for this assay, is inserted along with the test strip. The device then transfers the sample into wells containing anti-cTnI antibodies labeled with alkaline phosphatase, and cTnI-antibody complexes form. The SPR is also coated with immobilized anti-cTnI antibodies, which are specific to a different molecular epitope on cTnI. The mixture is cycled in and out of the SPR several times, allowing the second anti-cTnI antibody to bind the cTnI-antibody enzyme conjugates, forming a sandwich complex. Unbound antibodies are removed during washing, which is followed by two identical detection steps. A substrate specific to alkaline phosphatase is cycled in and out of the SPR, and the enzyme catalyzes its hydrolysis into a fluorescently detectable product. The fluorescence is quantified and the concentration of cTnI is calculated using relationships to two built in calibration curves [30].

It is recommended against using hemolyzed, lipemic, or icteric samples with the Vidas when possible. However, no significant interference was noted for samples with up to 550 mg/dL of Hb, 3000 mg/dL of triglycerides, and 29 mg/dL of bilirubin. Given these values, this device appears to have a higher sensitivity to interference with Hb, triglycerides and bilirubin as compared to other POC assays. Since this assay utilizes immunoglobulins from mice, interference may also occur with HAMA or other heterophilic antibodies contained in the sample. No significant drug interference or cross-reactivity with other forms of cTn or sTn was found. The LoD for this device is 0.01 $\mu\text{g/L}$, with a 99th percentile URL of 0.1 $\mu\text{g/L}$. The Vidas does not achieve a CV of 10% until 0.11 $\mu\text{g/L}$, and at 0.013 $\mu\text{g/L}$, the CV is approximately 28%. Hence, this device does not comply with the recommendation of a 20% CV at the 99th percentile URL, and its use in POC testing could cause a higher incidence of false positive or negative diagnoses [14]. The amount of sample preparation necessary when using the VIDAS is not appropriate for POC testing. The assay itself takes 20 min to run, but time it takes to

transport, centrifuge, vortex and transfer the sample to the test strip, in addition to running the test, could be longer than 30 min. The sample handling required in this procedure also allows for more steps at which user error could occur [30].

Roche Cobas CARDIAC T quantitative

Roche's Cobas is one of two POC device currently marketed that quantitatively measures cTnT in patient samples. It is also a handheld device, and thus is convenient in critical care settings where space is limited [30, 31]. To begin testing, a cTnT sensitive test strip is inserted into the device, and a 150 μL sample of heparinized whole blood is applied to the strip. No sample preparation is necessary. The device separates the erythrocytes in the sample from plasma, which migrates into the detection zone of the assay. There are two types of monoclonal antibodies to cTnT in the detection zone; one biotinylated, and the other gold labeled. These antibodies form sandwich complexes with cTnT in the sample, and accumulate in the detection zone, displayed as a reddish signal line. Excess gold-labeled antibodies accumulate and form a control line, which signals that the test was valid. The intensity of the signal line is proportional to the concentration of cTnT in the sample. The optical system of the Cobas measures the intensity of the signal and control lines, and converts the signal intensity to a quantitative cTnT concentration. This concentration is displayed on the device screen approximately 14 min from sample addition [32].

The efficacy of the Cobas assay is unaffected by bilirubin ≤ 20 mg/dL, Hb ≤ 200 mg/dL, triglycerides ≤ 440 mg/dL, Rh factor < 300 IU/mL and biotin ≤ 10 mg/dL. High concentrations of lipoic acid can also lead to falsely depressed measurement values. The increase in measured cTnT values caused by sTnT at a concentration of 1000 $\mu\text{g/L}$ can be up to 30% for this assay. For each other assay included in this review, the interference by other forms of troponin was insignificant when tested. Heterophilic antibodies present in patient samples may also react to give falsely depressed or elevated values. Though this test has been formulated to reduce interference from these antibodies, complete elimination of their effect cannot be guaranteed. The Cobas is the only POC device included in this review that has not been tested for interference caused by pharmaceuticals at therapeutic concentrations. The LoD of the Cobas is 0.05 $\mu\text{g/L}$, and a result above 0.1 $\mu\text{g/L}$ constitutes a high risk of AMI. Though a 99th percentile URL is not reported for this assay, the majority of variation coefficients were below 9% over the entire measuring

range of the device (0.1–2 µg/L). Thus, the precision of this device is below 20% across the range of the device, and is appropriate for POC testing; and the 99th percentile therefore should not be used. The main issue with the use of the Cobas in POC testing is potential interference with the assay. The user must be more careful than is necessary with other assays that patient samples are not hemolyzed, icteric, or lipemic, and must also be more aware of potential interferences by pharmaceuticals or other forms of troponin [32].

Limitations

First, each assays' analytical characteristics are presented based on manufacturers package inserts. We recognize that there is minimal discussion of peer-reviewed literature, which either validates these parameters or perhaps shows different findings. Frequently, the information in the package insert does not match what is noted in real life or validated by the clinical laboratory in practice, often because manufacturers use specimens which are not patient specimens and may have a different matrix. Second, we have not addressed the clinical role of POC assays regarding risk assessment and outcomes. To the best of our knowledge, not POC assay is cleared by any regulatory agency for risk stratification. Third, we know of no accurate data that addresses use and or integration of POC assays into global clinical practice,

Conclusions

cTnI and cTnT are the biomarkers of choice for diagnosis of AMI because of their high specificity to myocardial tissue [33]. Devices that are sensitive to cTn at very low concentrations, maximize analytical specificity to cTn by minimizing interference, and minimize the CV at the 99th percentile URL, all while keeping TAT under 30 min, are ideal for POC testing. Minimal sample preparation and handling by personnel operating the device is desirable in order to reduce the possibility of user error, and decrease TAT. Devices that comply with these guidelines will decrease the number of false positive and negative results when diagnosing an AMI in the ED. POC testing also has the potential to reduce cost and patient's length of ED stay.

All POC devices included in this review are quantitative, diagnostic immunoassays that utilize antibodies specific to cTn. The antibodies differ by assay in that they are

engineered to bind to a particular molecular epitope. This gives each device a different sensitivity to cTn; those with a high sensitivity being preferable. Devices by Trinity, Radiometer, Alere, and Response Biomedical all utilize fluorescently tagged detection antibodies to quantify cTnI. Abbott, Mitsubishi, Siemens, and bioMerieux have produced assays that use enzyme-labeled antibodies to bind cTnI, and then deliver a substrate that is quantifiable after reaction with the enzyme. Mitsubishi's design is unique because the second antibody the PATHFAST employs is tagged with a magnetic particle, which facilitates removal of cTnI complexes to be quantified. Roche's quantitative assay is distinct from the others because it measures cTnT instead of cTnI, and uses gold-labeled antibodies to detect cTnI. When interpreting the sensitivities of each device in the context of the technology, it is not likely that fluorescence versus substrate detection causes a difference in sensitivity.

The RAMP and VIDAS are at a disadvantage for cTn POC testing because of the sample preparation required. The additional steps of centrifugation and manual sample dilution are unnecessary for most assays, and increase TAT and the possibility of user error. Portable devices like the Meritas, Cobas, and i-STAT that can test on a drop of whole blood directly from the collection tube enhance simplicity of use and are optimal for the future of cTn POC testing. Devices that accept whole blood straight from the collection tube, including the AQT90 FLEX and the Stratus CS, keep sample handling at an absolute minimum, but are heavy and difficult to transport, which may be undesirable in some EDs.

Seven of the 10 assays included in this review comply with the recommendation of a CV $\leq 20\%$ at the 99th percentile URL, rendering them acceptable for clinical use at the POC. Alere's Triage, Radiometer's cTnT assay, and bioMerieux's VIDAS have not achieved or not documented a CV $\leq 20\%$ at this value. Four devices, the Trinity Meritas, Mitsubishi PATHFAST, Siemens Stratus, and Roche Cobas have achieved the ideal recommendation of a CV $\leq 10\%$ at the 99th percentile URL. This high imprecision allows for measurement of small changes in cTn concentration over time in order to determine a rise or fall of cTn concentrations and accurately diagnose AMI. Devices that can achieve this level of imprecision represent the future of POC testing as cTn concentrations continue to be a valuable tool for assessment of ACS patients. The benefit will extend past patient diagnosis to a reduction of hospital costs by limiting the length of patient stays in the ED [32]. It is clear that POC testing will be fundamental for the future of emergency cardiac care, and will improve quality of care, limit costs, and decrease triage time in hospitals worldwide. However, no high sensitivity POC assays are currently available for

clinical practice that would complement the growing, global use of high sensitivity central laboratory cTnT and cTnI assays used globally outside the USA.

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