Review Article

Acute Mycordial Infarction (AMI) Diagnosis; Impact of Technology in Developing Highly Sensitive Biomarkers and Assays

ABSTRACT

Acute myocardial infarction (ACI), a cardiovascular disease have been known to be cause high morbidity and mortality rate in several countries. Hence, several serum biomarkers have evolved as a standard and bedrock for its diagnosis one of which is, cardiac troponin. This cardiac biomarker's accurate and rapid detection is critical in reducing the risk of heart attack-related complications. However, delay experienced in the determination of a patients' clinical state, coupled with time of admitting them ~~in~~ the hospital depicts ~~that~~ need for improving diagnosing ACI by developing highly sensitive biomarker. In this review we discuss, biomarkers and immunoassays employed in diagnosing acute myocardial infarction. Specifically, we reviewed and discussed cardiac troponin, a widely used biomarker. Subsequently we discuss various methods used in assessing its performance and how technology have helpe in developing more sensitive cardiac troponin to fast track its rate of diagnosis. At the end, we propose the integration of several disciplines from nanotechnology to biotechnology to develop a robust medical diagnostic system to facilitate disease diagnosis and help save lives.

KEYWORDS

Acute myocardial infarction, troponin, assays, diagnosis, sensitivity.

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1.0 INTRODUCTION

A global health crisis of major concern is cardiovascular diseases (CVD) which have been reported to be presently the leading cause of death worldwide especially in developed nations. However, its prevalence was predicted in developing nations after 2020 [1, 2]. One of the most detrimental diseases under CVD is coronary artery disease (CAD) as it is responsible for numerous deaths and hospitalizations. Statistically, CVD is responsible for 31% of global mortality rate which is equivalent to 17.7 million people [1]. Recent research indicates that 75% of CAD deaths ~~is~~ reported from low-and-middle income countries [1]. However, atherosclerosis is known to be the foremost cause of coronary heart disease with its associated deaths and morbidity globally [3]. Atherosclerosis is chronic inflammatory disease characterized by gradual deposition and accumulation of fat, necrotic debris, and immune cells into medium to large arteries resulting in the formation of atherosclerotic plaque [4]. Interestingly, every individual has atherosclerotic plaques which ~~is~~ known to develop from childhood but their variation in the accumulation rate and difficult to predict [5]. Although most of the deposited plaques do~~es~~ not manifest clinically (asymptomatic), some become stable angina (not fatal) whereas a scanty amount of plaque becomes susceptible to thrombosis [6]. Thrombosis is a major underlying causative factor for the deadly acute coronary syndrome (ACS) which encapsulates a wide range of diseases that causes regional reduction in coronary blood flow, myocardial ischaemia or infarction, and pain in the chest, neck, or arms. Hence, ACS includes unstable angina, angina, and myocardial infarction (heart attack), - STEMI or NSTEMI [1].

Acute myocardial infarction (heart attack) occurs because of the complete blockage one of the several coronary arteries due to the formation of clot when a plaque gets ruptured. This stops the flow of blood to the heart muscle (myocardium) [1]. It is worth reporting that about 38% of ~~acute coronary syndrome~~ patients have acute myocardial infarction. Myocardial infarction alone is responsible for about 100,000 hospital admissions every year, which means for every 5 minutes, hospitals in the UK experience at least 1 hospital admission [7]. In USA, the yearly new cases recorded ~~is~~ about 550,000 and 200,000 is recurrent. The success in combating AMI heavily relies on ~~the~~ accurate diagnosis. World Health Organization proposes that, for an individual to be diagnosed ~~of~~ AMI, two of the following standards ought to be met; presence of chest pain over time, alterations in diagnostic electrocardiogram (ECG), and elevation and decline of serum levels of cardiac markers [7]. Studies reveal~~s~~ that ¼ of patients with AMI suffer ~~with~~ atypical signs and symptoms but it is only ECG that depicts a highly specific tool. However, 40% of patients ~~are not able to~~ be diagnosed using ECG, hence these setbacks make diagnosis of AMI difficult. Therefore, studies of biomarkers remain the hope for deciphering AMI [8].

2.0 TROPONIN AS A BIOMARKER OF AMI

Assessing an individual's body physiology and health is accomplished using indicators known to be measurable and quantifiable by biological parameters called biomarkers ~~[9]~~. A good biomarker will offer the benefit of diagnosing and predicting a disease precisely and produce a meaningful result [9]. There are several new biomarkers as shown in the figure below and how they are related to pathophysiological process used in diagnosing AMI.

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Nonetheless, troponin and creatine Kinase (CK) are the two commonly known biomarker for diagnosis of AMI. However, since 2000, troponins have been the diagnostic biomarker of AMI of choice in place of CK [10]. This was due to the specificity issue scientists encountered especially in patients having muscle and hepatic disease even though it was recommended by WHO in 1976 together with Aspartate transaminase (AST) and Lactate dehydrogenase (LDH) [11, 12]. Creatine kinase (CK) is a cardiac enzyme that is released into the blood after ~~a~~ damage to the muscle tissue has occurred, leading to an elevation in serum/plasma CK activity, which can be used as an indication for myocardial infarction [13]. Moreover, the use of gel electrophoresis to develop specific iso-enzymes of CK yielded no significant outcome and still did not improve the specificity [14]. The absence of specificity of CKMB led to search for another test with higher output; troponin.

Troponin, a constituent of the muscle myofibril was discovered 1970s as a significant protein biomarker in diagnosing AMI. Troponins have three subunits: troponin C (TnC), troponin 1 (Tn1), and troponin T (TnT), and table 1 below gives their respective functions [15]. TnT and Tn1 are however referred to as cardiac troponin (cTn) because they are readily found in the heart and skeletal muscles and thus originate from the cardiac muscles [16]. Cardiac troponins are found in myocytes, cytosolic pool, and contractile apparatus at a higher percentage compared to CKMB hence the greater sensitivity and specificity observed [17]. Rogers [18] agrees with this assertion and also states that both amino acid sequences of ~~troponin T (~~TnT~~)~~ and ~~I (~~TnI~~)~~ molecules are only present in cardiac tissue. The cardiac-specific troponin is actively transported when heart muscle cells are damaged. As a result, cardiac troponins (troponin I and T) are highly sensitive and specific markers of myocardial injury.

Chaulin [7] also stated that troponin is highly specific to cardiac tissues and it is accurate in diagnosing AMI which have previous history of ischaemic pain or ECG. Bohula et al., [19] added that troponins are very sensitive and specific that even a slight elevation in levels of troponin mean that the heart has experienced some damage, and high levels are indicative of a heart attack. In patients who have had a heart attack, troponin levels will be elevated within 6 hours, and it can remain this way for a couple of weeks after a heart attack has occurred. Furthermore, cTn relies on the size of infarct [20] which means that it gives medical scientists prognostic idea after infarction. Despite this, in observing the reperfusion therapy, the precise level of troponin could be misleading because of the wash-out phenomenon [21]. Troponin usually rises to the peak after 12 hours and remain at that level for 10 days or more and another downside using troponin is the 12-hour waiting period in making clinical decision a problem [21]. This makes the establishment of damages or death of cardiomyocytes delays to a latter period [7]. Delays in identifying disease ('ruling in') impede rapid management, while inefficiencies in excluding disease ('ruling out') obstruct examination of alternative diagnosis - which both contribute to emergency room overcrowding and annual expenses in the billions of dollars [22]. However new troponin assays which are highly sensitive have been developed to solve this problem [21]. Also, cardiac troponins are special in that it is elevated in other health conditions such as acute pulmonary edema, sepsis, chronic renal failure etc., but there could be a possibility of misinterpretation of the elevation in favour for AMI as opposed to other health conditions [23]. Gupta and Alagona, [24] reported an elevation in cTn in 50% of patients with end-stage renal disease. Therefore, troponin levels ought to be interpreted within the clinical confinement of AMI. Then also observing the trend of cTn by means of serial measurement could improve sensitivity since new onset of infarction could mean increase value and decrease value could mean resolving infarction [25]. Another

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disadvantage is that there is insufficient specificity of ischemic necrosis of cardiomyocytes in AMI because of inability to determine the causative factor and mechanism involved in the muscle damage at the initial stage, which represent a significant diagnostic clue [7]. Also, the various techniques for diagnosis are not standardized and thus results generated are not reliable. This is because, they have different manufacturers, analytical properties are varied and thus generate varied results in the same sampled patients [7]. Katrukha et al., [26] further supported this by indicating that, troponins have different half-life in blood, which is as a result of the action of protease enzymes on troponin molecules or its fragments. Therefore, this means that different assays will detect different levels of troponin, which makes it impossible to compare the result of one patient using different test kits.

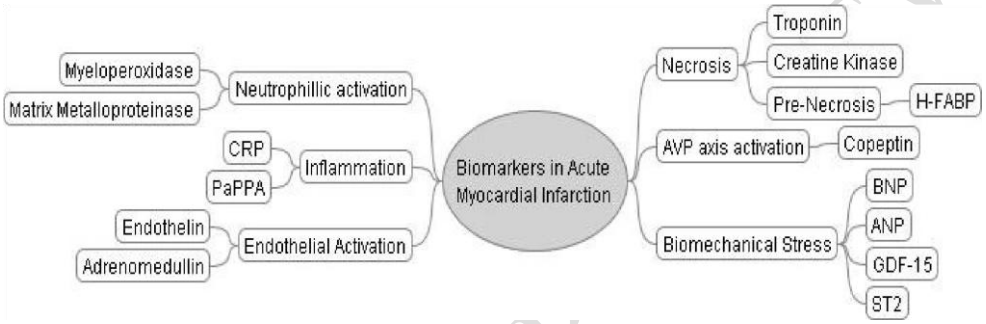

Fig. 1; Various forms of AMI biomarkers

3.0 COMPARING VARIOUS TROPONIN ASSAY PERFORMANCE

3.1 Optical Assays

3.1.1. Turbidimetry vs. Nephelometry

Turbidimetry is an optical technique that uses turbidimeter or spectrophotometer to measure the intensity of light emitted from molecules. It measures absorbance but is hindered by signal-to-noise ratio which means that it does not measure low concentrations well. Nephelometry on the other hand works on the sample principle of turbidimetry whereby it detects scattered light using nephelometer. It utilizes antigen-antibody complexes to scatter light. It is limited by the quality of matrix which means a matrix is needed to scatter inbound light.

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3.1.2. Fluorescence vs. Absorbance spectrophotometry

Spectrophotometry is a significant technique in science whereby spectrophotometer is used in measuring the amount of absorbed light or reflection by a particular compound. This method quantitatively determines the concentration of a particle either by using fluorescence or absorbance [27]. Fluorescence is when light is absorbed at one wavelength and produces light

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at another wavelength whereas with absorbance, the concentration of a particular molecule is quantified at specific wavelength. Studies have reported the benefit of absorbance spectrophotometry in determining troponin levels. Some of which include the ability to detect contaminants, ease of use and its non-invasiveness [27]. However, fluorescence spectrophotometry is highly used compared to absorbance due to its high level of sensitivity (detect more than 1000 higher than absorbance), specificity, selectivity, can detect in wide concentration range and produces valid results [27]. Fluorescence approaches are indeed preferable for smaller sampling concentrations because they are more sensitive to absorbance and offer a particular concentration for the required molecule, allowing downstream tests to be set up properly [28]. The fluorophore has strong binding characteristics, making this approach very selective for certain compounds in terms of specificity. Contaminated samples can be tested with these tests [29]. Despite this, fluorescence can be affected by bubbles, pH, and contaminants [27]. Fluorescence assays may be expensive and time-consuming, but they involve a calibration curve to compare unknown materials. They also have a concentration range that they are accurate within [30].

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3.2. Immunoassay

Diagnosis of AMI has been aided due to the discovery and advancement of immunoassays. Immunoassays works on the principle of capturing a target antigen between a “capture” antibody and a “detecting antibody” whereby each bind to a different epitope. Immunoassays use different signalling mechanisms that aid in the detection of cardiac troponins [31, 32]. When creating a sensitive test, the label and detection technique must be carefully chosen. The majority of commercial cardiac troponin tests are enzyme immunoassays, in which an immune complex produced by two or three anti-troponin antibodies is recognized ~~for~~ most cases by fluorescence or chemiluminescence [33]. Troponin immunoassays have been effectively utilized ~~for~~ a variety of research labs and point-of-care settings [34]. Handy electronic equipment and independent test kits are used in such point-of-care technologies. Troponin T and Troponin I immunoassays have now become gold standard test for determining cardiac function, both diagnostically and prognostically [35]. For its capacity to correctly detect particular antigen or antibody components in a short ~~period of~~ time, immunoassays have received a lot of attention in the last few years for clinical and scientific objectives. An immunoassay consists of an analyte, a specific antibody, and labels. Immunoassays are classified according to the kind of label used, including enzymes (ELISA), light-emitting molecules/tracers (e.g., chemiluminescence and fluorescence immunoassays), and radioactive isotopes (radioimmunoassays) [36].

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3.2.1. Homogenous vs. Heterogenous immunoassay

Homogeneous immunoassays have a simply liquid phase and don't need to be washed. It allows measurement of an assay by a simple mix and read procedure without the necessity to process samples by separation or washing steps. In comparison to heterogeneous immunoassays, homogeneous immunoassays are quicker and easier to automate. Furthermore, homogenous immunoassays have forms that are competitive [37].

3.2.2. Fluorescence immunoassays (FI)

Fluorescence immunoassay (FI) is a widely known optical technique that is involved in signal transduction and quantification of molecules in homogenous and heterogeneous assays. It is capable of quantifying cardiac troponins via immunofluorescent labelling with antibodies [38]. FI is preferred because of its high sensitivity, accuracy, ~~quick~~ and ease in modification of molecules with fluorescence tag. However, its operation requires highly trained operators and it is very bulky and expensive [38].

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3.2.3. Chemiluminescence immunoassays

Chemiluminescence (CL) refers to light emission (photons) which are usually emitted by molecules in an excited state and relaxed in the ground state as induced by chemical reaction. The attachment of immunoreaction with CL makes it possible to find the concentration of troponin depending on the intensity the light the chemical reaction emits, and this is also called Chemiluminescence immunoassays. It is highly sensitive, has a wider linear range, and is easy to operate and automate [39].

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Table 1: Advantages of several cardiac troponin assays [40].

Type of Immunoassay	**Sensitivity - Lowest detection limit of troponin (lug/L)**	**Advantages**
Enzyme-linked immunosorbent assay	0.1	It is highly sensitive and cardiac-specific
Chemiluminescence	0.027	Detection is faster, very sensitive, rapid, and quantitative
Fluorescence immunoassay	0.1	Highly sensitive
Electrical detection	0.000001	Ultrasensitive, label free, real time detection
Surface plasmon resonance	0.25	Fast, improved sensitivity, specific, reproducible

Colorimetric detection	0.01	Low cost and fast detection
Aptamer	1.1938	Fast detection

4.0 MEASUREMENT OF ASSAY PERFORMANCE

In patients suspected of having ~~acute coronary syndrome (~~ACS~~)~~, notably those with non-ST elevation myocardial infarction, quantitative measurement for cardiac biomarkers, specifically cardiac troponins have become the source of clinical decision making [41]. However, several key analytical parameters are assessed when measuring biomarkers including cardiac troponin immunoassays. These parameters reveal the diagnostic value of cardiac troponins. Limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) are referred to as analytical sensitivity as they examine the low concentration of troponins [42]. Other parameters include 99th percentile and Coefficient of Variation (CV%), precision (RCV II) and accuracy (Bias). Limit of blank refers to the highest concentration of an assay that can be detected in a sample that do not contain the analyte being studied [7]. This means LoB is estimated by way of measuring replicates (usually 20) of the blank sample, finding its mean and standard deviation. The mean signal is employed to estimate the zero concentration of the biomarker. However, there is a bias when the mean of the blank equates to a non-zero concentration. Also, LoB ought to be employed as the lowest detectable quantity of any biomarker since it has minimal effect in measuring but rather very effective in providing support to determine other indicators like LoD [42]. ~~Limit of detection (~~LoD~~)~~ also denotes the lowest concentration of troponin that be detected using the test in use. Usually, LoD value is higher than LoB. LoD can be determined by measuring 2060 replicates of several samples with a low measure of troponin with values close to LoB. The mean value of each low biomarker sample and standard deviation is found [42]. The sensitivity and precision of a cTn assay determine its detection limits. Increased sensitivity and accuracy allow for early diagnosis of increased or changing cTn levels, and hence of acute myocardial damage and MI [43]. Research indicates that LoD is highly significant in diagnosing AMI in that LoD of current highly sensitive assays could be just a few ng/L or less 1 ng/L which is 100 times more sensitive and helps to detect myocardial injuries in every individual cell and about 50%-100% of healthy individuals with measurable level highly sensitive cardial troponin (hs-cTn) [44]. Limit of quantification (LoQ) is also known as functional sensitivity and refers to the minimum concentration level at which troponin in a test sample can be assessed with acceptable repeatability and accuracy within a particular standard [7]. Unfortunately, the non-existence of gold standard reference range which could allow the establishment of a true cardiac troponin value in a sample makes the determination of bias impossible. The idea of total analytical error is however highly considered when explaining an assay's LoQ [58]. Dynamic range reveals the actual measurements of the assays and ~~its~~ significance to the measurements at the physiological concentration range [7]. Also, the dynamic range was reported to be directly associated to the limits of an assay at low concentration [7]. The 99th percentile is utilized as the reference limit for every measure having an imprecision limit of 10% [45]. However, the 99th percentile value could vary in

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age, race or gender [45]. The 99th percentile URL for a particular test is generated using data from a healthy control group. A population of at least 300 healthy persons with an adequate age, ethnic, and gender mix is necessary for competent estimation of the 99th percentile URL for a current cTn test, according to recommendations. Because the 99th percentiles in high-sensitivity tests are gender-specific, at least 300 healthy females and 300 healthy men should be examined [46].

Coefficient of variation (CV%) is an indicator of imprecision of the assay as it reveals the variation of the assay when compared to the concentration of cardiac troponin. CV becomes higher when concentration is low (ideal 10%) or less ~~of~~ the diagnostic cut-off value. CV is calculated by CV= SD/mean. CV% is a very significant parameter in determining the accuracy of a highly sensitive assay. An assay is deemed very accurate and sensitive when CV% does not go beyond 10% [47]. cTn tests with a cumulative CV at the 99th percentile URL of 10% to 20% are also acceptable in clinical practice, making them "clinically useful" because the risk of classifying patients using these assays is minimal [41].

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Table 2: cTn testing analytical fundamentals that are commonly agreed upon [48].

Analytical Quality Specification	Description
LoB	The lowest signal is generated in a fluid (i.e., typically the buffer or diluent of the assay) with zero cTn concentration.
LoD	The value generated in a biological sample with the lowest measurable cTn concentration.
LoQ	The minimal concentration of cTn can be measured with ≤ 10% imprecision.
99th Percentile	Value of cTn corresponding to the 99th percentile of a reference population of ostensibly healthy subjects.
Percentage of measurable values in healthy subjects	Percentage of cTn values < 99th percentile that can be obtained in a reference population of ostensibly healthy subjects.

Table 3: Analytical Characteristics for determining accuracy and sensitivity of assays [7].

Coefficient of Variation (Assay Inaccuracy in %) of High-Sensitivity Immunoassays (hs-cTnI and hs-cTnT)	
CV% value	**Brief Description, Comment**
CV% ≤ 10	High-precision (most preferred for clinical use)
10 ≤ CV% ≤ 20	Non-high accuracy, but acceptable for clinical use

CV% ≥ 20	Inaccurate, and unacceptable for clinical use

Percentile (%) of measurable values < 99th percentile in healthy subjects	
<50	Moderately sensitive immunoassays
50–75	1st generation high-sensitivity immunoassays
75–95	2nd generation high-sensitivity immunoassays
>95	3rd generation high-sensitivity immunoassays
99–100	4th generation high-sensitivity immunoassays

Ratio between the 99th percentile and LoD	
<1 Highly sensitive	(clinically acceptable) immunoassays

5.0. HOW IMPROVED ASSAYS THROUGH TECHNOLOGY HAVE HELPED DIAGNOSIS OF AMI

The management of myocardial infarction ~~have~~ improved in recent years due to the improvement of troponin assays. The development of high-sensitivity troponin tests, which can measure troponin levels in close to 95% of the general population, is expected to enhance clinical treatment for patients with myocardial infarction [49]. The use of high-sensitivity troponin assays may be beneficial because they allow for the early ruling out of myocardial infarction based on very reduced troponin concentration levels; precise and efficient diagnosis of myocardial infarction based on troponin-based techniques followed by early treatment initiation; that are only discernible by high-sensitivity assays [49]. The advantage of more sensitive assays, particularly in individuals with subsequent heart problems, makes it easier to identify patients with AMI more quickly, boosting the efficacy of evidence-based AMI therapy [50].

With the advent of highly sensitive assays via technology, scientists ~~are able to~~ diagnose AMI at a higher precision with less than 10% CoV at the 99th percentile. Also, the LoD is lower than the upper reference limit which helps to detect cardiac troponin isoforms in relatively high proportion (at least 50%) of the healthy population with an accurate calculation [51]. With improvement in assays, the limit of detection of highly sensitive troponin (hs-cTn) is as low as 10 times compared to normal cardiac troponin and it is faster as well as cost-effective in diagnosing AMI [52]. Moreover, ~~prior to~~ the improvement of troponin assays, validation of results for AMI took 6-9 hours but with high sensitivity troponin assays, it only takes 2-3 hours to validate results [53]. In addition to that, technological advancement in developing highly sensitive assays has paved the way to distinguish AMI relative to gender and age. Men have twice as much ~~of~~ highly sensitive troponin (hs-cTn) than in women particularly due to the higher size of ventricular mass in men [54]. With respect to age, the elderly is now reported to have higher concentration of troponin as compared to younger ones [55]. Previously, Ziebig et al., [56] argued that, using normal sensitive assays could not detect any cardiac troponins in urine. However recent reports indicates that, highly sensitive cardiac

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troponin have opened other diagnostic avenue using non-invasive techniques that utilizes urine and oral fluid though more research is needed to fully comprehend its practical significance. In the report, the use of highly sensitive immunoassay led to the detection of cardiac troponin in all subjects and was even high in hypertensive patients than normal individuals [57].

6.0. CONCLUSION

This brief literature review highlights the impact technology has had on the diagnosis and management of AMI. A specific focus was on cardiac troponins which have become a significant biomarker in detecting AMI. This cardiac biomarker's accurate and rapid detection is critical in reducing the risk of heart attack-related complications. Technology have helped develop highly sensitive troponin assays that can detect lower concentration of troponins and hence improved diagnosis of AMI. In ~~acute myocardial infarctions (~~AMI~~)~~ and ~~acute coronary syndromes (~~ACS~~)~~ the use of technology eliminates delays in detection and monitoring and saves ample ~~of~~ time on delivery of service. However, there should be a guideline and standard set in for assessment of the efficiency of the result provided. It will help to know which technology will of the greatest value to patients. Also, any diagnostic approach has benefits and limitations; nevertheless, ~~in order~~ to optimize, the future of medical diagnostic systems design will be accompanied by definitive integrating of existing disciplines with other disciplines such as nanotechnology, biotechnology, and genetics.

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Table 4: Table of high sensitive high sensitive assays (hs-cTn) performance7

Company/Platform/Method	LoB (ng/l)	LoD (ng/l)	CV%	99th-Percentile (General and by Gender), ng/l
Abbott/Alinity i systems/Alinity I STAT High Sensitive Troponin-I; commercial OUS	1.0	1.6	4.0	General-26.2 F-15.6 M-34.2
Abbott/ARCHITECT i systems/ ARCHITECT STAT High Sensitive Troponin-I; commercial	0.7-1.3	1.1	4.0	General −26.2 F-15.6 M-34.2
Beckman Coulter/Access 2, DxI/Access hsTnI; commercial – OUS	0.0-1.7	1.0-2.3	3.7	General −17.5 F-11.6 M-19.8
Beckman Coulter/Access 2, /Access hsTnI; commercial – US: Serum	0.0-0.8	1.0-2.0	6.0	General −18.2 F-11.8 M-19.7
Roche/cobas e801/cTnT-hs 18-min and STAT; commercial	2.5	3	<10	General-14.0 F-9.0 M-16.0
Siemens ADVIA Centaur XP/ XPT High Sensitivity TnI (TNIH), US & OUS; commercial	0.50	1.6	<4.9	General-46.5 F-39.6 M-58.0

Singulex Clarity cTnI; commercial	0.02	0.08	2.39	General – 8.67 F – 8.76 M – 9.23

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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