ADVIA Centaur and ACS:180 Troponin (cTnI) Clinical Applications and Assay Troubleshooting

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Purpose

- To clarify the ADVIA Centaur and ACS:180 cTnI clinical applications and performance expectations with chest pain patients.
- To explain the different cutpoints that laboratories may elect to use for the ADVIA Centaur and ACS:180 cTnI assay.
- To provide instruction on ADVIA Centaur hardware troubleshooting for instruments with an occurrence of cTnI elevated, non-reproducible discrepant sample events.

Background Information

The use of troponin is rapidly evolving. Historically, it was used to rule-in or rule-out patients with very typical signs and symptoms of Acute Myocardial Infarction (AMI). More recently, Troponin testing has expanded into other areas for risk stratification and prognosis of patients termed as “atypical MI” or “low-grade MI”. Additionally, cardiac researchers and other experts have changed the definition of what defines a “positive troponin”. The new guidelines issued by a Joint Committee of the European Society of Cardiology (ESC) and the American College of Cardiologists (ACC) have redefined MI as “any troponin level in the blood above the 99th percentile of the normal or control population”.

The changes in thinking and terminology amongst cardiologists, laboratorians and researchers devoted to Cardiovascular Disease testing has left many Clinical Laboratories quite confused. This document was written to help address the following questions:
1. What are the different philosophies and cutpoints?
2. How does the analytical performance of this assay affect results at the different cutpoints?
3. What cutpoint should I recommend my laboratory to use?
4. Situations where the cTnI can be positive (0.10ng/mL) but the patient does not have an MI and/or the Cardiac Catheterization is negative.

A description of the three applications for cTnI testing and the performance expectations of each are listed below. It is important to understand the cutpoint(s) your customer is using and for what reasons so that you can explain what the expectations for precision should be. This information is also necessary when investigating complaints regarding cTnI assay performance.

**Clinical Applications – Past and Present**

The first troponin assays were FDA cleared for diagnosis of MI in the early 1990s. These assays were developed to maximize clinical sensitivity and specificity in order to reduce the greatest number of false positives and false negatives. The ACS:180/ADVIA Centaur cTnI assay used 112 confirmed MI and 166 confirmed non-AMI individuals with at least two of the following findings to establish clinical diagnosis:
- chest discomfort of ≥ 20 minutes
- abnormal electrocardiogram (ECG), i.e. ST-segment elevation
- elevated cardiac enzymes

The MI patients in this study had very clear signs and symptoms of heart attack. Using the appropriate statistical methods, it was determined that 1.5 ng/mL was the best cutpoint to differentiate between MI and non-MI in patients who were experiencing chest pain and had a typical ST-segment elevated ECG.

In the late 1990s, the National Academy of Clinical Biochemistry (NACB) issued their “Standards of Laboratory Practice: Recommendations for the Use of Cardiac Markers” consensus statement. These recommendations stated that detection of any myocardial injury with a specific cardiac marker such as cTnI is clinically important and warrants the incorporation of a low abnormal decision limit for the optimum use of such markers in acute coronary syndromes patients. This was the first time that Troponin testing had been recommended for the wider group of Acute Coronary Syndrome (ACS) patients and not just for MI patients. The NACB guidelines further recommended that a lower decision limit or cutpoint for cardiac assays be guided by the 97.5th percentile among healthy individuals.

A study was conducted by an international group of physicians called the TIMI (Thrombolytics in Myocardial Infarction) group which expanded upon the NACB guideline recommendation of using the 97.5th percentile of normal. In the TIMI Ilb study, the outcomes of patients with unstable angina (UA) and Non-ST Elevated MI (NSTEMI), as defined by electrocardiographic (EKG) profiles, were assessed for negative clinical events in the first 43 days after admission into the study with symptoms of chest pain.
The data from this study provided Bayer with one of the first FDA cleared studies for a risk stratification claim of 0.10ng/mL. The authors concluded that UA/NSTEMI patients with cTnI $\geq 0.10$ ng/mL had a 3 to 4 fold increased risk (odds ratio) of a coronary event occurring compared to those patients with values $<$0.10 ng/mL. This additional indication for use of cTnI at cutpoints approximately 10 fold lower than previously used was initially called “Risk Stratification of Acute Coronary Syndrome”.

In 2000, a Joint Committee of the European Society of Cardiology (ESC) and the American College of Cardiology (ACC) redefined MI as any amount of myocardial necrosis (heart cell death) caused by ischemia (lack of oxygen). They indicated that the preferred biomarker for myocardial damage is cardiac troponin as it has nearly absolute myocardial tissue specificity, as well as high sensitivity, and identifies even microscopic zones of myocardial necrosis. An increased value for cardiac troponin should be defined as a measurement exceeding the 99th percentile of a reference control group of “normal” or non-diseased individuals. This meant that any elevation of cTnI above the upper limit of the reference population would now be defined as an MI.

**Analytical Applications and Performance Expectations:**

1) WHO Recommendation (Classical definition):

The first or “classical” recommendation of MI as defined by the World Health Organization requires that patients meet 2 of 3 criteria:

- chest discomfort of significant duration ($\geq 20$ minutes)
- ECG changes consisting of ST-segment elevation
- an increase in cardiac marker levels

The diagnostic cutpoint as published in the ADVIA Centaur and ACS:180 cTnI IFU is 1.5 ng/mL. This cutpoint is consistent with the WHO recommendation for confirmation of an MI as it maximizes clinical sensitivity and specificity with “classically” diagnosed MI and non-MI patients.

2) Risk Stratification Cutpoint:

cTnI risk stratification is applicable to patients experiencing chest pain that are diagnosed with unstable angina or NSTEMI. Risk stratification is applicable only to patients that are having chest pain and is based on serial testing over the first 24 hours after the onset of chest pain or admission to the hospital. It is NOT to be used as a screening test for MI. The risk stratification concept is used to estimate the probability of the patient having a major cardiac event in the next 30 –60 days. Risk stratification is only applied to unstable angina and NSTEMI patients because chest pain patients showing ST elevation on ECG are by definition having an MI and a biochemical marker is not necessary to make this diagnosis.
The ADVIA Centaur and ACS:180 cTnI risk stratification cutpoint of 0.10 ng/mL, was determined from the TIMI 11B study\(^1\). Studies have shown that at the risk stratification cutpoint, a 20-25% total CV is expected. Because the assay evidences a higher than average total CV at the risk stratification cutpoint, a patient sample may yield a replicate value on either side of the cutpoint with a greater frequency of occurrence compared to a lower CV assay. This observation does not represent a discrepant sample.

ADVIA Centaur and ACS:180 cTnI customers using risk stratification are encouraged to perform a precision study to understand performance expectations within their laboratory at risk stratification levels.

To ensure optimal reliability of cTnI determinations at the very lower range of the assay (ie. 0.10 ng/mL), it is recommended that serial sampling be performed on all “low positive” results. The cTnI value should always be assessed in combination with the other clinical signs and symptoms of the patient. Laboratories may also consider instituting a range at which all very low samples are repeated. Utilizing serial sampling, good clinical judgement and repeat testing of extremely low results will ensure the most accurate assay results possible.

3) ESC/ACC Redefinition of Myocardial Infarction

An MI as redefined by the European Society of Cardiology (ESC) and the American College of Cardiology (ACC) requires an increased cTnI level which exceeds the 99\(^{th}\) percentile of the reference control population and at an acceptable imprecision (CV) of <= 10%.

The ADVIA Centaur and ACS:180 cTnI assay IFU states that the 99\(^{th}\) percentile of “apparently healthy” samples is 0.07 ng/mL. The total precision at this level is 20-25%. Because of the level of imprecision associated with a result below 0.10 ng/mL, Bayer Diagnostics does not recommend reporting of results below 0.10 ng/mL.

Higher sensitivity cTnI assays are required by all manufacturers, to meet the new criteria of an MI as defined by the ESC/ACC. Bayer Diagnostics is in the process of developing a high sensitivity assay for the ACS:180, ADVIA Centaur and IMS systems.

**Goals of the High Sensitivity assay:**

1. Improve the ability to identify troponin I patients as early as possible
2. Increase the predictive value of adverse outcomes (i.e. risk stratification)
3. Reduce the level of imprecision as much as technically possible, at the lowest part of the curve. This will increase the accuracy of the result and the clinical assessment of the patient.

However, a large clinical study using the current Bayer assays demonstrated conclusively that the 0.1 ng/mL cut off, in spite of the 20% CV, identified an additional 15% of patients at risk of an adverse event than a higher cut off with a lower (10%) CV.\(^3\)
**Note: cTnI Sensitivity and Assay Range**

The ADVIA Centaur and ACS:180 cTnI IFU states the analytical sensitivity is below 0.10 ng/mL but then further defines this as 0.03 ng/mL with a 95% upper confidence limit of 0.07 ng/mL. To be clear, our analytical sensitivity is 0.03 ng/mL but **because of the level of imprecision associated with a result below 0.10 ng/mL, Bayer Diagnostics does not recommend reporting of results below 0.10 ng/mL.** This is notated in the ASSAY SUMMARY section of the ADVIA Centaur package insert.
What Cutpoint(s) Should I Recommend my Lab to Use?

This is a decision that should be made by the Laboratory Director or Pathologist in conjunction with their Emergency Room Physicians and Cardiologists. Your role as a Bayer Diagnostics representative is to present the information on the various recommendations (ie. WHO, ESC/ACC, Risk Stratification) but to let the laboratory determine how they will report their cTnI values. Laboratories around the world are using cutpoints between 0.10 and 1.5 ng/mL. The cutpoint(s) used is the decision of each individual laboratory.

Situations where the cTnI is positive (0.10ng/mL) but there is no MI and/or the Cardiac Catheterization is negative.

There are clinical situations in which the patient may have a positive cTnI due to reasons other than MI. In these situations, there is cardiac necrosis leading to positive cTnI levels in the blood, but the cell death is not in the pericardium. In these cases, the laboratory and physician may falsely label the result as a cTnI false positive. To prevent this, please provide a copy of publications # 4 and 5 to your laboratory. Discuss the information detailed and strongly recommend that they distribute these publications to their physicians.

TnI Sample Integrity

Plasma collection tubes are frequently used for TnI sample testing. Laboratories should follow the sample handling guidelines as outlined by the collection tube manufacturer to ensure samples are handled and centrifuged properly, and free of fibrin prior to analysis. The presence of fibrin or other particulate matter can lead to spurious results

Hardware Troubleshooting:

To troubleshoot cTnI assay performance, please use the information provided above to first determine which application the laboratory is using for cTnI.

If the laboratory is using risk stratification, it is important to assess the customer’s TnI assay performance based on the low end precision expectations of the assay as shown by the precision profile above.

When investigating TnI assay performance, please follow the instructions below to troubleshoot the instrument.

1) Wash Manifold Inspection
   • Remove and inspect the aspirate probes, ensure the probes are washed correctly.
• Check the aspirate probe guides for wear and tear, and replace if necessary.
• Check the home position of the aspirate probe using the tool and on-line procedure.
• Check the bottom position of the aspirate probe using the on-line procedure.
• Using the ‘aspprwsh.cds’ test in controller diagnostics, check the flow of water through the wash manifold. Lower the aspirate probes home position if necessary to ensure an efficient wash.
• Check the front to back position of the aspirate probe into cuvette. Ensure the probe is entering the middle of the cuvette.
• Inspect the wash manifold for any signs of leakage, remove and syringe through if required.
• Check the quality of the 16 o-rings at the junction block into the left-hand side of the wash manifold and replace if necessary.

2) Reagent Probe Inspection
• Check the calibration of the reagent compartment mixer.
• Check the calibration of reagent probes 1 and 2 (all positions, including the cuvette bottom).
• Check the calibration of the sample probe.

3) Other System Checks
• Check acid and base dispensing
• Perform a V66/V67 test in controller diagnostics. Check the water reservoir and dispense ports for bleach contamination using pH paper (Support Bulletin 078N0180-01).
• Check to ensure the luminometer is clean.
• Check for bacterial contamination of the instrument.

4) Cleaning Solution Valves
• Please reference Support Bulletin 078N0180-01 within e-BRITE for hardware information related to the cleaning solution valves.
Action

Please ensure your local technical support personnel are advised of the ADVIA Centaur and ACS:180 cTnI clinical applications, performance expectations, and assay troubleshooting information. This information should be used to manage customer inquiries regarding cTnI assay performance and discrepant sample reports.

For further information, please contact Lauren Foohey of Global Strategic Marketing or your branch Cardiac Marketing manager.

References

1 Alpert J. for the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction. JACC 2000;36:959-69.


4 Jaffe A. Elevations in cardiac troponin measurements: false false positives, Cardiovascular Toxicology 2001;01:87-92.


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This document issued by Stacey Neighbor, Service & Support. (508-660-4625).