

The Challenges and Concerns Companies Face Pertaining to the US Food and Drug Administration 510(k) Process for Cardiac Biomarkers

Moderators: Fred S. Apple^{1,2*} and David Morrow^{3,4}

Experts: Christian Zaugg,⁵ David Hickey,⁶ Michelle Zaharik,⁷ John J. Blackwood,⁸ and Kirsten Jakobsen⁹

Cardiac troponin I (cTnI)⁸ and T (cTnT) have been internationally recognized as the standard biomarkers for the detection of myocardial injury, the diagnosis of myocardial infarction (MI), and risk stratification of patients presenting with symptoms of acute coronary syndrome (ACS). Laboratory medicine, cardiology, and emergency medicine organizations have endorsed the use of the 99th-percentile troponin value, which is derived from a reference population, as the medical decision-making cutpoint. From the time of the first US Food and Drug Administration (FDA) clearance of cTnT in 1995 through 2010, the FDA-cleared cTnT and cTnI assays were predicated on the ROC curve–derived cutpoint optimized for diagnostic sensitivity and specificity. The PATHFAST cTnI assay introduced by Mitsubishi in 2001 is apparently the first assay cleared by the FDA on the basis of the 99th-percentile value that aids in the diagnosis of MI. With the growing literature describing multiple research high-sensitivity cTnI (hs-cTnI) assays and an hs-cTnT assay (marketed outside the US, not FDA cleared), manufacturers are facing new challenges in bringing to market these new assays that use the 99th percentile as the cutpoint value for medical decisions. Several leaders in the in vitro diagnostics industry have been asked to give their views on important issues that affect laboratory scientists and clinicians.

On April 30, 2010, the FDA issued a “Points to Consider” paper providing an overview of both analytical- and clinical-performance expectations for assays that are to be submitted for 510(k) clearance on the basis of the 99th-percentile cutoff. What

do you see as the major analytical and clinical challenges presented by this document?



David Hickey: We at Siemens realize that the utility of troponin assays has evolved, and we understand that our methods of validating the performance of new troponin assays must also evolve. Some of the analytical and clinical challenges that would need to be addressed on the basis

of this document are as follows: First, the document focuses on the “aid in the diagnosis of myocardial infarction (MI)” intended use. There are additional recognized clinical uses for troponin assays, such as risk stratification and procedure-induced cardiac damage, that are not addressed by this paper; the use of the 99th-percentile cutoff may be inappropriate for these additional clinical uses. Second, when validating assay performance for an “aid in the diagnosis of MI” claim, we at Siemens fully agree that studies being done in the emergency department should include an “all comers” population. However, there are other means of validating assay performance that may not require initiating a new prospective study. Third, as pointed out in the paper, clinicians and laboratory scientists now recognize that troponin increases indicate myocardial injury that may or may not be due to MI. To include all other potential non-MI conditions in clinical studies with sufficient numbers to make the results valid would be

¹ Hennepin County Medical Center, Minneapolis, MN; ² Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; ³ TIMI Study Group, and ⁴ Cardiovascular Division, Department of Medicine, Brigham & Women’s Hospital, Boston, MA; ⁵ Medical and Scientific Affairs, Roche Diagnostics, Rotkreuz, Switzerland; ⁶ Chemistry, Immunoassay and Automation Business Unit, Siemens Healthcare Diagnostics, Tarrytown, NY; ⁷ Product Development, Response Biomedical, Vancouver, BC, Canada; ⁸ Product Management, Beckman Coulter, Chaska, MN; ⁹ Radiometer Medical, Copenhagen, Denmark.

* Address correspondence to this author at: Hennepin County Medical Center, Clinical Laboratories P4, 701 Park Ave., S. Minneapolis, MN 55415. Fax 612-904-4229; e-mail apple004@umn.edu.

Received September 19, 2011; accepted September 22, 2011.

⁸ Nonstandard abbreviations: cTnI, cardiac troponin I; cTnT, cardiac troponin T; MI, myocardial infarction; ACS, acute coronary syndrome; FDA, US Food and Drug Administration; hs-cTnI, high-sensitivity cTnI; POC, point of care.

difficult and possibly labor- and cost-prohibitive. Fourth, the major analytical challenge in validating the “diagnosis of MI” intended use is the question of what the diagnosis of MI will be based on during the clinical study. Clinically, the diagnosis of MI is largely based on the rise and fall in troponin values, with at least 1 value exceeding the 99th-percentile cutpoint. With the increased analytical sensitivity of the new troponin assays, it is possible that the “experimental” assay will result in values exceeding the 99th percentile and will demonstrate the rise and fall of troponin concentrations while the “routine assay” will not. Additionally, the increase in concentration might occur more quickly because of the high sensitivity of the assay. If the diagnosis of MI must be based on the current generation of troponin assays, this will present a dilemma. Finally, the clinical trials suggested in the FDA document have a higher level of complexity and require more resources than trials done to establish substantial equivalence to a predicate device that is achieved through method comparisons.



challenge for manufacturers.

John Blackwood: The challenge is to demonstrate an adequate level of diagnostic specificity at the 99th-percentile upper reference limit. At this low cutpoint, the diagnostic specificity will be dramatically lower than what laboratory scientists expect. This will create an educational



second analytical challenge is to provide an assay with an acceptable low-end imprecision. From a clinical point of view, the challenge is that these high-sensitivity assays are detecting an increasing number of patients with non-ACS troponin increases. Although these increases are meaningful prognostically, clinicians often struggle with the diagnosis and manage-

Kirsten Jakobsen: The first major analytical challenge in assay development is the lack of standardization/harmonization of troponin I assays. Additionally, since the guidelines are pushing for high-sensitivity assays able to detect troponin in the majority of normal individuals, the

ment of these patients. We can make analyte-specific assays but not definitive diagnosis-specific assays.

Finally, this document requires the use of an independent central adjudication panel to determine clinical truth, rather than an analytical comparison to a predicate device or concordance with the hospital diagnosis. It is a logistical challenge to implement such a process.



Michelle Zaharik: The largest clinical challenges presented by the document are related to the increased size and scope of clinical studies, changes to the requirements for predicate testing, and the new requirement for the use of an adjudication panel. The document appropriately

identifies that the size of a clinical study should be based on power calculations that use a prevalence of MI of 10%–20%; however, the example provided uses a prevalence of 20%–25%, which is not likely to be found in an “all comers” emergency department population (prevalence, approximately 10%–15%), thereby underestimating the size of the required clinical study. If the other new requirements are added (serial testing, use of a 5-member adjudication panel), the true scope and cost of the desired clinical study would be burdensome and cost-prohibitive.

The FDA has also changed its approach to the use of predicate devices and thus substantial equivalence, such that cTnI 510(k) submissions are now being treated more like Premarket Approval submissions. The document states that neither a method comparison to a predicate device nor testing of clinical study samples on a predicate device is required, and that the only use for a predicate is for comparison of the new device to the *cleared* clinical performance of the predicate. Further complicating matters is that no cTnI assay, with the recent exception of the Mitsubishi PATHFAST cTnI assay, has been cleared with a diagnostic sensitivity and specificity based on the use of the 99th-percentile value as the medical-decision point.

Also of note is that elimination of the method comparison to a predicate device makes the issue of troponin assay harmonization even more obscure, since the cleared labeling is often the only place where a slope comparison between assays can be found. By no longer requiring this information as part of new-device labeling, this lack of requirement of predicate testing will serve only to further obscure the differences between the cTnI assays until manufacturers make a con-

certed effort to publish method-comparison data in accepted journals. The importance of correlation studies for cTnI assays may be underestimated in terms of educating end users about the differences between nonharmonized cTnI assays that can impact the safety and effectiveness of the assay, if incorrect reference intervals/cutpoint values are used.

Finally, the requirement for use of a 5-member adjudication panel is burdensome and costly, and to our knowledge at Response Biomedical, the benefit in terms of increased safety and efficacy vs comparison to the clinical performance of a predicate device has not been clearly demonstrated.



Christian Zaugg: In general, the FDA document provides many useful aspects but ends where the scientific consensus ends and the debate on high-sensitivity troponin assays begins. As a consequence, manufacturers of high-sensitivity troponin assays face major uncertainties

and development risk in addressing analytical and clinical challenges. To the diagnostics industry, however, the major challenge does not arise from specific analytical and clinical challenges, but predominantly from the lack of expert consensus and, consequently, the lack of FDA guidance on a few controversial key points. As a consequence, manufacturers have to make (potentially premature) decisions on subject selection and study design for reference-value studies as well as for costly accuracy and validation studies in the diagnosis of acute MI. Furthermore, the lack of guidance for troponin assays in terms of both risk stratification and therapy guidance in ACS patients may put these important intended uses out of reach for high-sensitivity troponin assays in the US for some time. This will place these assays at a substantial disadvantage to less-sensitive but previously cleared assays. Thus, the lack of expert consensus and consequent FDA guidance may delay both approval and access to the high-sensitivity troponin assays.

How do you view the guidance regarding the enrollment of subjects for determination of the 99th-percentile normal reference value?

David Hickey: Our understanding at Siemens is that the document is to be used by manufacturers in preparing for pre-Investigational Device Exemption discussions with the FDA. While there are several sugges-

tions in the document that are appropriate, the final agreement on clinical trial plans for each new or revised troponin assay will come from the pre-Investigational Device Exemption discussions with the FDA. Industry, clinicians, and the FDA should provide input as to the subjects included in the determination of the 99th-percentile cutpoint.

John Blackwood: Since there are currently competing schools of thought on the topic, it would be helpful to clarify exactly how to define a cardiac “normal.” Specific guidelines on inclusion and exclusion criteria for subjects to be included in 99th-percentile cutpoint determinations are needed.

Kirsten Jakobsen: The guidance stipulates that the method for prequalifying normal individuals be described but does not provide direction on how to do it. Without such guidance, manufacturers are not likely to define the same method (e.g., inclusion of imaging and natriuretic peptide testing in addition to a questionnaire and a physical examination), which may lead to 99th-percentile values that are not comparable and are confusing to the end users.

Michelle Zaharik: The choice of the “normal” reference population is becoming more crucial in light of the movement toward hs-cTnI assays, and the lack of clarity provided in this document is concerning. There is no firm guidance on a minimum number of “normal” study subjects, and the manufacturer merely needs to specify how they defined “apparently healthy.” The complexity of cTnI assay harmonization will now be compounded by confusion over what different manufacturers consider to be “normal.” For consistency, a clear definition is required for the population used in a “normal” study, with identification of mandatory exclusion criteria, required covariates, and the number of subjects required in each covariate population.

Christian Zaugg: The FDA recommendation has provided helpful aspects on the determination of the 99th-percentile normal reference cutpoint value. These aspects include basic concepts on reference intervals and, to some extent, sample size. Moreover, specifications on the gender and age of the reference population are requested, as these factors potentially influence the 99th-percentile value. It is still not clear how to consider and balance these influencing factors among the subjects enrolled for reference studies. Additionally, it is not clear what efforts are required to ascertain the health of the enrolled subjects and particularly the absence of identifiable structural heart disease. The lack of expert consensus and consequent FDA guidance on

these points translates into a substantial and costly development risk on the side of troponin assay manufacturers, because for any reference study, the resulting 99th percentile and the subsequent pivotal diagnostic accuracy and validation studies can be questioned. As a second consequence, differences in reference studies and varying definitions of “high-sensitivity troponin assays” will render comparability across these assays even more difficult than it already has been for conventional troponin assays (predominantly due to lack of standardization). An expert consensus on the term “high-sensitivity troponin assay” and characterization of the reference population is greatly needed as a basis for further FDA recommendations and successful clinical development.

How do you view the guidance regarding the enrollment of subjects for diagnostic-accuracy studies and validation studies of diagnostic-sensitivity and -specificity claims?

David Hickey: For the “aid in diagnosis of MI” intended use, the subjects included in the study should be the reference interval subjects and the all comers with chest pain in the emergency department population.

John Blackwood: The appropriate subjects to be enrolled in a troponin clinical trial are all patients who would have a troponin measurement ordered to help rule in or rule out acute MI under the current standard of care. The requirement for serial blood samples limits the investigators’ ability to address this entire population, since a large number of patients presenting with chest pain are currently discharged after a single blood draw, provided that acute MI has been ruled out.

This requirement results in a high proportion of the chest pain patients, who are in fact managed with troponin, being excluded from the study. Consequently, there is potentially an underestimation of the true clinical specificity of troponin.

Kirsten Jakobsen: The guidance defines the patient population as all comers to the emergency room. The recommendation to use primary-care facilities and to include 3 or more sites to ensure that the total population being tested reflects the demographic and geographic differences does allow the opportunity to achieve performance specifications meaningful to the end user. We at Radiometer think that positive and negative predictive values should be reported in addition to diagnostic sensitivity and specificity. The challenge here is that compared to older generations of assays, high-sensitivity assays will detect more non-ACS cardiac troponin increases, leading to a reduced positive predictive value for MI. This will result in a chal-

lenge in educating clinicians as to why high-sensitivity cardiac troponin assays, with their improved analytical characteristics, do not improve the positive predictive value for MI.

Michelle Zaharik: The document requires a prospective “all comers” study and lists certain covariates and comorbidities that should be evaluated. Unfortunately, there is no guidance given as to what would be considered a minimum number of study subjects to be assessed in each category (e.g., gender, age, smoking status, use of cardiotoxic drugs, renal failure, etc.) to generate sufficient statistical significance, or if the association with covariates and comorbidities is intended only to be observational.

Christian Zaugg: Similarly to reference-value studies, the FDA has provided useful recommendations on subjects to enroll for diagnostic-accuracy and validation studies. In agreement with the intended-use population, these subjects should be all comers presenting to the emergency room and suspected of having myocardial ischemia, i.e., the patients for which troponin testing is indeed ordered. It remains unclear, however, whether patients with ST elevations should be included in the study and the statistical analysis of diagnostic sensitivity and specificity. Most, but not all, patients presenting with ST elevations will be immediately triaged for reperfusion therapy. Clear-cut ST-elevation MI patients will not provide a second blood sample and consequently not appear in the analysis. Still, patients with unclear ST elevations not prompting immediate reperfusion therapy could be included in the study and the statistical analysis. The decision regarding whether or not to include these patients will affect the diagnostic sensitivity and specificity of the troponin assay. Similarly to the reference population above, guidance on inclusion and analysis of ACS patients with unclear ST elevations would be very helpful.

How do you view the guidance regarding the recommendations on how risk-stratification outcomes analysis should be approached for both short- and long-term adverse effects?

David Hickey: The document focuses on the diagnosis of MI; it does not cover risk stratification.

John Blackwood: The guidelines do not specifically address risk stratification of ACS with troponin. It appears that the FDA intends to issue further guidelines regarding their expectations for risk-stratification studies. One problem that complicates risk-stratification studies is that the 30-day and 1-year incidences of adverse cardiac events are so low in ACS pa-

tients that the number of patients required to produce statistically significant results would probably need to be very large. If ACS trials become too difficult to manage, many, if not all, risk-stratification claims may disappear.

Kirsten Jakobsen: There is a lack of guidance on this issue that is preventing manufacturers from providing the risk-stratification information for clinicians. While there seems to be a general agreement in the literature that short-term follow-up is 30 days, guidance on the definition and prevalence of adverse events during follow-up (hence sample size) is needed.

Michelle Zaharik: There was no guidance provided in this document on specific requirements for risk-stratification studies.

Christian Zaugg: So far, the FDA recommendations have been limited to the intended use, “aid in the diagnosis of MI.” Consequently, the FDA has not provided any recommendation on the use of troponin assays for risk stratification. In clinical practice, however, admission troponin values contribute to the integrated risk assessment of non-ST-elevation ACS patients, particularly for early risk assessment that translates into guidance for therapy. Specifically, non-ST-elevation ACS patients with increased troponin values have been demonstrated to benefit from early invasive treatment strategy and platelet glycoprotein IIb/IIIa inhibitors. Because of the lack of guidance, high-sensitivity troponin assays will probably not be cleared for risk stratification in the US for some time. Accordingly, the intended-use statement of the recently FDA-cleared PATHFAST cTnI-II assay explicitly excludes risk stratification. This exclusion will undermine the clinical usefulness of any cleared high-sensitivity troponin assay because of the awkward situation that a high-sensitivity troponin assay may be used only to aid the diagnosis of MI. However, a second, conventional, and less-sensitive troponin assay (previously cleared for risk stratification) will have to be used in parallel to assess risk and guide therapy. This situation may undermine the true medical value of high-sensitivity troponin assays and the clinical management of patients. This is because the benefit of high-sensitivity troponin assays in the rapid diagnosis of acute MI may be partially offset by applying less-sensitive troponin assays for risk stratification. At the same time, safety aspects of high-sensitivity troponin assays with regard to patients undergoing (unjustified) invasive treatment strategies must be considered and weighed against the potential clinical benefits of the assay. Thus, guidance and data on how to establish cutpoints for high-sensitivity troponin assays for guiding therapy in non-ST-elevation

ACS patients are urgently needed to unlock the full medical value of these assays. In the meantime, these cutpoints may be derived from sample data banks taken from intervention studies of ACS patients.

How do you view the guidance regarding the difference in the types of studies needed to be performed for submission for clearance of a point-of-care (POC) assay, compared with a central-laboratory assay?

David Hickey: All quantitative troponin assays should be developed, verified, and validated as robustly as possible. The FDA generally requires assays intended for use at the POC to be tested in that setting. It could be a substantial challenge to find POC settings in which the studies contemplated by this document could be carried out.

Kirsten Jakobsen: There are no specific POC recommendations regarding performance besides the general consensus that these devices should strive to have an analytical and clinical performance as close as possible to the central-laboratory instruments. The big difference between POC and central-laboratory instrument studies is that in the former the testing must be performed by the targeted end users, including emergency room nurses.

Michelle Zaharik: Historically, the only specific requirements for a POC submission were to include POC sites in the clinical trial to validate the environment of use and the different end users, and to carry out imprecision studies with both whole blood and a relevant controls/standards matrix. However, the FDA has not addressed the challenges of a submission for whole-blood POC assays and specifically those with no approved alternative sample matrix claim. This became relevant during discussions with the FDA on the requirement of having to run a preliminary clinical study to determine a whole-blood POC assay’s 99th-percentile value, instead of testing characterized banked frozen samples, as may be done with other platforms that use serum or plasma as their primary sample type.

Of further relevance to POC manufacturers, although not formally documented, is the fact that it has been an accepted reality that POC assays may not have the same performance in terms of analytical and diagnostic sensitivity and specificity as central-laboratory assays. On the basis of communications with the FDA and the lack of discussion of POC requirements in the document, it is implied that POC and central-laboratory analyzers now must demonstrate the same level of performance. Although this is a desirable goal,

it may not be immediately achievable, especially with the transition toward hs-cTnI assays and the associated evolution of performance expectations. If the intention of the FDA is to improve the quality of cleared POC assays to improve safety and efficacy, the guidance provided in its document has generated substantial challenges that may stall or inhibit submissions for improved POC assays to come to the market. In the end, this means that current POC assays will remain on the market, resulting in neither the fostering of innovation nor the improvement of safety and efficacy of POC assays.

Christian Zaugg: No specific FDA recommendation has been provided for POC troponin assays. The troponin criteria of the Universal Definition of MI are assay and platform independent, and there is no obvious reason why quantitative POC troponin assays should have different performance requirements than central-laboratory assays.

With the ongoing development of high-sensitivity troponin assays, what new analytical challenges arise for implementing these assays onto your current instruments/platforms?

David Hickey: We at Siemens do not anticipate any unusual challenges in developing the next generation of troponin assays.

John Blackwood: An assay exhibiting a dynamic range spanning many orders of magnitude that maintains imprecision and accuracy at low concentrations is an analytical challenge. As new information regarding isoforms and complexes of troponin emerges, low concentrations of troponin may be difficult to measure accurately.

Kirsten Jakobsen: It is always a challenge to improve imprecision and thus lower the limit of quantification for an assay.

Michelle Zaharik: The ability to reliably detect cTnI at low nanogram-per-liter concentrations in a “normal” population with $\leq 10\%$ CV at the 99th percentile is a substantial challenge, not only for the current Response Biomedical RAMP lateral-flow platform but to all prospective manufacturers of a lateral-flow type of cTnI assay. Thus, we at Response Biomedical believe the success of lateral-flow cTnI assays in the emergency department could be seriously jeopardized.

Christian Zaugg: In contrast to many other biomarkers, the clinical decision range for troponins is near the lower analytical limits of the assays. Apart from the

signal-to-noise ratio and potential interferences, a major challenge has therefore been to reach a high sensitivity and a low imprecision at the lower concentration range across all assay versions and platforms. Thus, assay manufacturers need to provide consistent 99th-percentile values and imprecision across assay versions and platforms. Moreover, the assays need to provide a low imprecision at the lowest concentration range, i.e., below the 99th-percentile cutpoint down to the limit of detection ($<10\%$ CV at the 99th percentile as required and a clinically acceptable CV of $<20\%$ at the limit of detection). According to the Universal Definition of MI, the concentration range below the 99th percentile is relevant for the diagnosis of acute MI, too. Specifically, one value above the 99th percentile is required, together with a rise and/or fall of troponin. As a logical consequence, one of 2 serial troponin values may be below the 99th percentile. Still, the value below cutpoints provides useful information with regard to the important rise and/or fall criterion and therefore needs to be as precise as possible. Minimal-change values for the rise and/or fall criterion of troponin are still a matter of debate. Any such criterion will critically depend on assay imprecision around the cutpoint value and therefore be assay dependent. Validated, assay-specific algorithms will therefore likely be a necessity to assist clinicians in the routine use of high-sensitivity troponin assays.

What do you think is the greatest potential barrier facing diagnostics manufacturers aiming to introduce new troponin assays?

David Hickey: For the successful introduction and adoption of new troponin assays or assays for other cardiac markers with greater sensitivity, it is of the utmost importance that the global healthcare community (physicians and laboratory scientists) be well educated on how best to use, interpret, and act on the information the assays provide. Without this level of rigor, the appropriate utilization of the assay will not be realized.

John Blackwood: The next generation of troponin assays will require an improved level of reproducibility and sensitivity at the very low end of the analytical measuring range. Recent guidance requires more rigorous and complex clinical and analytical testing to support troponin assay submissions.

Kirsten Jakobsen: The greatest barrier is that opinion leaders are pushing for high-sensitivity assays but a majority of clinicians have difficulties managing the results provided by these new methods. There will be a need for useful δ values optimally able to differentiate

between “no elevation,” “acute elevation,” and “acute elevation due to MI.” This will require more data on the biological variation, both in healthy subjects and in patients presenting with chest pain.

Michelle Zaharik: The cost required for larger clinical studies with serial testing and the adjudication panel review requirements is a major barrier, especially for smaller companies that do not have the deep pockets of industry leaders. Add to this the uncertainties that remain in defining various study requirements, and this results in the submission of a 510(k) becoming a high-risk situation, resulting in a feeling of great unease that may impact the number of manufacturers willing or able to pursue a new troponin submission.

Christian Zaugg: The 3 major barriers to introduce new troponin assays are related to a large extent to new regulatory expectations and a lack of guidance. The first potential barrier consists of sharply rising development timelines, costs, and risk. This barrier is predominantly due to new regulatory expectations (stated in the above-mentioned FDA document), as well as a lack of expert consensus and FDA guidance on critical aspects, which were discussed earlier. Increasing costs and risks, as well as continuing pressure to reduce the prices of diagnostic tests, will eventually undermine returns on investment and cause manufacturers to rethink development of new troponin assays.

A second potential barrier to new troponin assays is the position of the FDA to limit assay displays to the concentration interval above the 99th percentile when the assay is used for aid in diagnosis (and not for risk stratification). This position blunts the high-sensitivity properties of high-sensitivity troponin assays. Furthermore, it may undermine the ability of these assays to be used according to the Universal Definition of MI that allows 1 out of 2 values below the 99th percentile (see above).

A third barrier is the lack of guidance on the intended use of troponin for risk stratification and therapy guidance in ACS patients. This will presumably cause major delays or even prevent clearance of high-sensitivity troponin assays for these important intended uses. This is exemplified by the recently cleared Mitsubishi PATHFAST assay, which must not be used for risk stratification. Consequently, even when cleared for aid in the diagnosis of acute MI in the US, these assays will be at a substantial commercial disadvantage to less-sensitive assays that have previously been cleared for risk stratification. This may undermine manufacturer decisions to develop high-sensitivity troponin assays in the US.

What is the major issue you hear your customers raising regarding current commercial assays for troponin?

David Hickey: Customers are pleased with the performance of Siemens' commercial assays. The primary questions customers raise revolve around the diagnostic sensitivity of these assays and the appropriate interpretation of results. If customers are unaware that they might observe increased troponin results in non-MI ACS, they may incorrectly perceive some increased troponin results as “false positive.” The majority of these concerns are resolved with additional education and consultation.

John Blackwood: False-positive results from currently available commercial assays are the biggest issue. In addition, there is considerable frustration in the marketplace that a satisfactory international standard for troponin does not exist, which can lead to commercial assays not all being aligned analytically.

Kirsten Jakobsen: As mentioned earlier, the chief complaint is the increasing number of troponin increases due to non-ACS causes. The second is the inability to rule out MI in <2 h.

Michelle Zaharik: The majority of Response Biomedical's customers' concerns relate to the lack of assay harmonization, where they are unable to use the same cutpoints and reference ranges for multiple platforms. This issue becomes critical when a site has decided that the addition of a POC method is needed to meet the turnaround time recommended by the current American College of Cardiology/American Heart Association guidelines for acute MI.

Christian Zaugg: Customers have not been reporting major concerns related to the contemporary (fourth-generation) cTnT assay. The hs-cTnT (fifth-generation) assay was developed to meet the requirements of the Universal Definition of MI. As a result of improved analytical sensitivity, the clinical diagnostic specificity of all high-sensitivity troponin assays is decreased. Accordingly, reduced diagnostic specificity and increased numbers of positive troponin test results have been the major concern with customers, particularly in the phase right after conversion from the conventional assay to the hs-cTnT assay. However, this concern can be adequately addressed by preparation and education of clinicians and laboratory physicians about non-ACS etiologies of troponin increases, the importance of a rise and/or fall in serial troponin values, and that troponin testing (like other biomarkers) should not be used without the clinical context.

What do you see as the future of additional development for troponin or other biomarkers?

David Hickey: As the analytical sensitivity of troponin assays continues to improve, the potential for expanding the clinical utility of troponin testing exists. It is within the realm of possibility that the utility of troponin could expand such that the assay is no longer used primarily in the acute-care setting for patients suspected of MI. The future for new cardiac biomarkers is still materializing. With the burden of cardiovascular disease expanding as the global population continues to age, researchers and manufacturers will need to focus on identifying new markers—and new uses for existing biomarkers—that will allow clinicians to make distinct changes to clinical practice, thus improving outcomes and reducing the cost of care for the patients they treat.

John Blackwood: Laboratories are always pressured to get their troponin results out faster. New technologies may allow faster assay times combined with improved low-end analytical sensitivity. I do see a need for laboratory scientist and physician education and guidance from the in vitro diagnostics manufacturers on how to best use troponin assays. Additionally, new markers might further fill gaps along the cardiovascular disease continuum.

Kirsten Jakobsen: We at Radiometer certainly need to see the efforts of the IFCC Working Group on cTnI standardization/harmonization succeed. With the discrepancies observed between the fourth- and fifth-generation troponin T assays, one might also consider similar work on cTnT. In both cases, a “clinical” standardization might also prove useful.

Michelle Zaharik: The near future appears uncertain for the development and clearance of new troponin tests until several of the issues discussed in this article are addressed, either by the FDA through public disclosure or follow-up guidance documents, or through the indirect evidence gleaned from subsequent cleared troponin assays. Manufacturers may choose to stall the submission of novel cTnI 510(k) documents to the FDA and focus instead on markets outside of the US that are viewed as more accepting of innovations and improvement. It is hoped that this will occur only in the short term and that discussions between the FDA and manufacturers will soon result in clarity and consensus.

Christian Zaugg: A future helpful tool in the daily clinical routine will likely be algorithms for rapid and optimized differential diagnostic performance in ruling in or out acute MI in chest pain patients in the emergency department. These algorithms may consider various cutpoints, rise/fall criteria, and the clinical context for providing rapid rule in/out and improving diagnostic specificity. Such algorithms will likely be assay specific and must each be supported by solid clinical evidence. An algorithm for rapid rule in/out of acute MI within 1–2 h after admission to the emergency department has been shown for the hs-cTnT assay. Furthermore, the introduction of high-sensitivity troponin assays has left very limited room for other biomarkers in the diagnosis of acute MI or recurrent infarction. There is clearly more room for additional prognostic biomarkers, given that they provide incremental information that is useful to guide therapy in mid- and high-risk non-ST-elevation ACS patients.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: C. Zaugg, Roche Diagnostics; D. Hickey, Siemens Healthcare Diagnostics; M. Zaharik, Response Biomedical Corporation; J.J. Blackwood, Beckman Coulter; F.S. Apple, *Clinical Chemistry*, AACC; D.A. Morrow, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: F.S. Apple, Instrumentation Laboratories, Abbott Diagnostics, and Ortho Clinical Diagnostics; D. Morrow, Beckman Coulter, Roche, Siemens, Instrumentation Laboratories, and Ortho Clinical Diagnostics.

Stock Ownership: None declared.

Honoraria: F.S. Apple, Abbott Diagnostics, Instrumentation Laboratories, and Alere; D. Morrow, Roche.

Research Funding: F.S. Apple, Abbott Diagnostics, Roche Diagnostics, Radiometer, Siemens, Response Biomedical, Instrumentation Laboratories, Bio-Rad, Ortho Clinical Diagnostics, BRAHMS, and Genetech.

Expert Testimony: None declared.

Note: In an attempt to present all points of view on this topic the FDA was invited to participate in this Q&A but declined.

Previously published online at DOI: 10.1373/clinchem.2011.165860