

Earlier Detection of Myocardial Injury in a Preliminary Evaluation Using a New Troponin I Assay With Improved Sensitivity

Stacy E.F. Melanson, MD, PhD,¹ David A. Morrow, MD,² and Petr Jarolim, MD, PhD¹

Key Words: Acute coronary syndrome; Myocardial injury; Troponin; Early detection; Sensitive assay; Early presenters

DOI: 10.1309/Q9W5HJTT24GQCXXX

Abstract

Cardiac troponins are important biochemical markers for defining the presence of myocardial injury. However, limitations in troponin testing exist, including the relatively late increase in troponin after onset of ischemia. We therefore evaluated a more sensitive troponin assay for detection of myocardial injury in “early presenters.” Discarded serial specimens were obtained from 103 patients who had a negative cardiac troponin I (cTnI) result followed by a positive cTnI result. Results were obtained using our current cTnI method and a new more sensitive assay, TnI-Ultra (Siemens Medical Solutions, Diagnostics Division, Tarrytown, NY). Medical records were reviewed to determine the clinical diagnosis. Precision studies yielded a 10% coefficient of variation at the diagnostic cut points for cTnI (0.10 ng/mL [0.10 µg/L]) and TnI-Ultra (0.04 ng/mL [0.04 µg/L]). TnI-Ultra was positive before cTnI in 66 (64.1%) of 103 cases. We conclude that the more sensitive assay, TnI-Ultra, has better analytic performance and has the potential to detect myocardial injury earlier than the current cTnI assay.

Cardiac troponins I and T have a central role in the diagnosis and management of patients with suspected acute coronary syndrome (ACS).¹⁻⁴ The biochemical diagnosis of myocardial infarction (MI) is defined by troponin status. In addition, troponin provides valuable information for assessing the risk of recurrent ischemic events and for guiding therapeutic decisions. The analytic performance of troponin assays, in particular their sensitivity, has improved significantly since their initial clinical implementation.⁵⁻⁷ The European Society of Cardiology (ESC) and the American College of Cardiology (ACC) jointly recommend using a diagnostic cut point at the 99th percentile of the reference population with an optimal coefficient of variation (CV) of less than or equal to 10%.¹ Thus, the threshold for myocardial injury has been continually redefined as assay performance has improved, in conjunction with data demonstrating important clinical implications of increased concentrations of troponin in patients with suspected ACS.^{1,2,8-10}

Current troponin testing has limitations, including antibody specificity, assay imprecision, lack of standardization, and the relatively late increase in the circulating troponin level after the onset of ischemia. Studies of first- and second-generation assays have shown lower diagnostic sensitivity of troponin when measured early (<6 hours) after symptom onset.¹¹⁻¹⁴ As such, patients seeking care early in the course of the myocardial injury, so called early presenters, will often have undetectable levels of troponin on initial evaluation, but will later have a positive troponin result. Early presenters carry a similar or worse prognosis compared with patients who have detectable troponin levels at initial examination.¹⁵ Given the central clinical goal of rapid triage for patients with chest pain syndromes and the potential benefits of early intervention,¹⁶ this delay in a detectable rise in the troponin level has led to

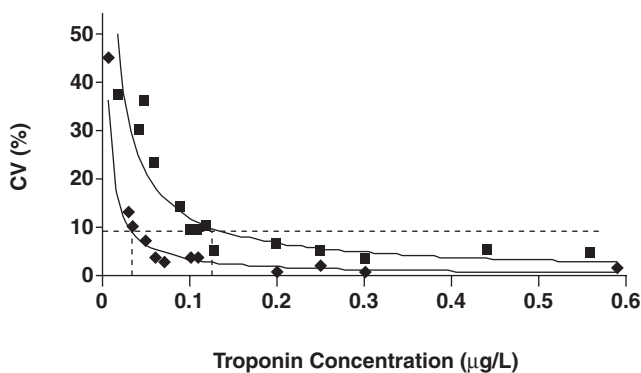
interest in its combined use with “early markers” of necrosis such as myoglobin or fatty acid binding protein. However, it is possible that improvements in the early sensitivity of troponin would obviate the need for additional, potentially duplicative, biomarkers of necrosis.¹⁷

We thus evaluated the potential of a newly available more sensitive assay for troponin I (TnI-Ultra, Siemens Medical Solutions, Diagnostics Division, Tarrytown, NY) to improve early detection of myocardial injury in patients whose serial cardiac TnI (cTnI) testing revealed an initially negative cTnI result followed by a positive cTnI result in at least 1 of the subsequently collected specimens.

Materials and Methods

Approval for this study was acquired from Brigham and Women’s Hospital (Boston, MA) Human Research Committee. By using our laboratory information system, we identified patients who initially had 1 or more negative cTnI (<0.10 ng/mL [0.10 µg/L]) specimens followed by 1 or more positive cTnI (≥0.10 ng/mL [0.10 µg/L]) results within 24 hours. We collected discarded plasma specimens from this cohort of patients, including the initially cTnI– and all subsequently cTnI+ and/or cTnI– samples drawn within 72 hours from the first cTnI test. Specimens were collected between November 2005 and May 2006 and stored at –70°C.

We currently perform the cTnI assay on the ADVIA Centaur analyzer (Siemens Medical Solutions). This assay for cTnI is a 3-site sandwich immunoassay using direct chemiluminescent technology with polyclonal and monoclonal TnI antibodies. The manufacturer states an assay range of 0.10 to 50 ng/mL (0.10–50 µg/L). The CV at 0.10 ng/mL (0.10 µg/L) ranges from 10% to 12% in our quarterly evaluations ■ **Figure 1**.



■ **Figure 1** Troponin assay precision. The coefficient of variation (CV) at decreasing concentrations of cardiac troponin I (cTnI; squares) and TnI-Ultra (diamonds) is plotted. A 10% CV is achieved at 0.10–0.12 ng/mL (0.10–0.12 µg/L) for cTnI and 0.03–0.04 ng/mL (0.03–0.04 µg/L) for TnI-Ultra.

Consequently, we use a diagnostic cutoff of 0.10 ng/mL (0.10 µg/L), as recommended by the manufacturer.¹⁸

The ADVIA Centaur TnI-Ultra assay is a 3-site sandwich immunoassay using direct chemiluminescent technology with 1 polyclonal antibody and 2 monoclonal antibodies directed against the stable, central region of the TnI molecule. These antibodies are configured to give maximum binding to each troponin molecule. An ancillary reagent is included to reduce nonspecific binding. The manufacturer states an assay range of 0.006–50 ng/mL (0.006–50 µg/L); however, the functional sensitivity at which the CV is 20% was determined to be 0.015 ng/mL (0.015 µg/L). A reference range study, conducted by the manufacturer, using 1,845 fresh samples (serum, EDTA, and lithium heparin plasma) from 648 apparently healthy people ranging from 17 to 91 years of age, demonstrated a 99th percentile of 0.04 ng/mL (0.04 µg/L). A total CV of 10% was obtained at a level of 0.03 ng/mL (0.03 µg/L). Consistent with the ESC/ACC recommendations,¹ we used the manufacturer’s recommended diagnostic cut point of 0.04 ng/mL (0.04 µg/L; 99th percentile) for the TnI-Ultra assay. In addition, we verified the total imprecision using different reagent lots during multiple days by determining the CV at decreasing concentrations of TnI.

Stored specimens from all time points were assayed for both cTnI and TnI-Ultra assays according to the manufacturer’s instructions by trained technologists who were blinded to previous results and patient history. Each patient’s electronic medical record was reviewed, and clinical data were abstracted by an investigator (S.E.F.M.) blinded to the TnI-Ultra results to determine patient demographics and the principal clinical diagnosis.

Results

During the study period, 103 patients had a negative cTnI result followed by a positive cTnI result within 24 hours. A total of 356 serial specimens were analyzed for cTnI and TnI-Ultra. ■ **Table 1** characterizes the study population, including average age, sex, and principal diagnoses. The diagnosis of ACS was based on the guidelines published by the ACC, which includes clinical symptoms, electrocardiographic changes, and/or a typical rise and fall in biochemical markers of necrosis.^{1–3} The performance characteristics of TnI-Ultra were determined. By using the TnI-Ultra, a 10% CV was achieved at a concentration of 0.03 to 0.04 ng/mL (0.03–0.04 µg/L; Figure 1), whereas the cTnI assay has a 10% CV at 0.10 to 0.12 ng/mL (0.10–0.12 µg/L).

By design of this study, all baseline cTnI results using our current assay were less than the diagnostic decision limit. In contrast, TnI-Ultra was positive in the baseline specimens of 63 patients (61.2%) ■ **Figure 2**. The majority of patients did

not have positive cTnI results until 9 to 10 hours after the baseline specimen was drawn. This usually represented the second specimen obtained. However, in 7 patients with suspected myocardial injury, more than 1 specimen was obtained between a positive TnI-Ultra result and a positive cTnI result. There were no specimens in which the TnI-Ultra results were negative and the standard cTnI result was positive. Overall, the TnI-Ultra result was positive (>0.04 ng/mL [0.04 $\mu\text{g/L}$]) before the cTnI result in 66 (64.1%) of 103 patients, allowing the diagnosis of myocardial injury to be made earlier. The difference in the 63 patients noted previously and the 66 patients overall can be explained by the fact that in 3 of the patients, the TnI-Ultra result was positive before the cTnI result but not on the baseline specimen. Most physicians in the study followed the ESC/ACC guidelines and obtained cTnI measurements at initial examination and 6 to 9 and 12 to 24 hours later. For this reason, an average of 564 minutes (9.4 hours) elapsed between a positive TnI-Ultra result and a positive cTnI result in this particular set of serial specimens.

When restricted to a subset of patients with a principal diagnosis of ACS, the data were highly consistent with the overall results. Of 103 patients, 31 had initial symptoms suggestive of myocardial injury and were eventually diagnosed with ACS (Table 1). The biochemical diagnosis of myocardial injury would have been confirmed earlier by TnI-Ultra in 52% of these patients. Of the patients with ACS with the earlier rise in TnI-Ultra, only 5 (31%) had accompanying physical examination and/or electrocardiographic findings suggestive of ACS. ■Figure 3■ depicts 4 of the 31 patients with ACS and the evolution of their troponin concentrations over time. All patients had significant troponin elevations that were indicated earlier by TnI-Ultra. Because physicians usually waited at least 6 hours to obtain a subsequent troponin level, TnI-Ultra allowed the diagnosis of ACS to be made an average of 570 minutes (9.5 hours) earlier, consistent with the average delay of 9.4 hours in all patients studied.

Discussion

The first report of the use of cTnI for diagnosis of MI was published in 1987.^{5,19} Despite the advances in troponin assays since that time,⁵⁻⁷ improvements are still needed in analytic performance, assay standardization, antibody specificity, and elimination of interference. It is highly probable that troponin will remain the “gold standard” for diagnosis of myocardial injury in the near future. Clinicians and scientists have advocated for better assays with improved clinical sensitivity and precision, and many manufacturers have or are developing new assays to meet these criteria; however, the clinical impact of such changes for diagnostic and prognostic applications needs careful assessment.

Table 1
Baseline Characteristics of 103 Study Patients*

Characteristic	Result
Average (\pm SD) age (y)	68 \pm 15
Men	63 (61)
Principal diagnosis	
ACS [†]	31 (30.1)
Cancer	10 (9.7)
Trauma	9 (8.7)
Stroke	7 (6.8)
Congestive heart failure	6 (5.8)
Cranial bleeding	6 (5.8)
Sepsis	5 (4.9)
Arrhythmia	4 (3.9)
Hypertension	3 (2.9)
Osteoarthritis	2 (1.9)
Diverticulitis	2 (1.9)
Pneumonia	2 (1.9)
Other	16 (15.5)

ACS, acute coronary syndrome.

* Data are given as number (percentage) unless otherwise indicated.

[†] Includes only patients with symptoms suggestive of ACS in which ACS was the principal diagnosis. In the remainder of the patients, although the principal diagnosis was not ACS, myocardial injury was present.

In this study, we evaluated the potential diagnostic advantage of one such assay, TnI-Ultra, and found that it is possible to significantly increase the number of patients with ACS and definitive myocardial injury who are detected at the time of initial examination compared with the use of our established assay.^{18,20} Because, in general, clinicians follow the recommendation to serially measure TnI at initial examination and 6 to 9 and 12 to 24 hours later, in our study population, we found that reporting TnI-Ultra results would allow the diagnosis of cardiac injury to be made an average of 9 hours sooner. Furthermore, in our laboratory, the TnI-Ultra assay provides a CV of 10% at concentrations between 0.03 and 0.04 ng/mL (0.03-0.04 $\mu\text{g/L}$). This result is consistent with recommendations

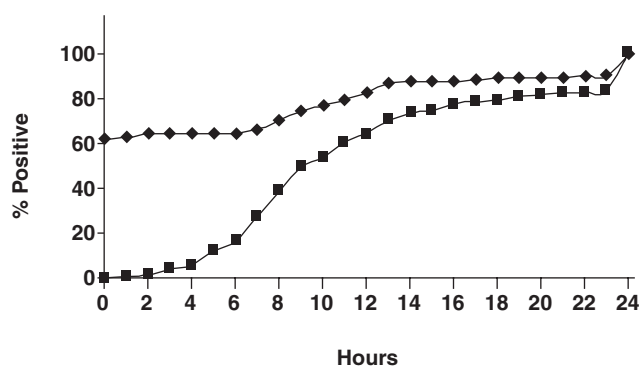


Figure 2 Timing of conversion to positive troponin I (TnI) in patients with myocardial injury. The percentage of patients with positive TnI-Ultra (diamonds) and positive cardiac TnI (cTnI; squares) is shown starting when the baseline specimen was obtained (ie, 0 hours) in a 24-hour period. By study design, all initial specimens for cTnI are negative.

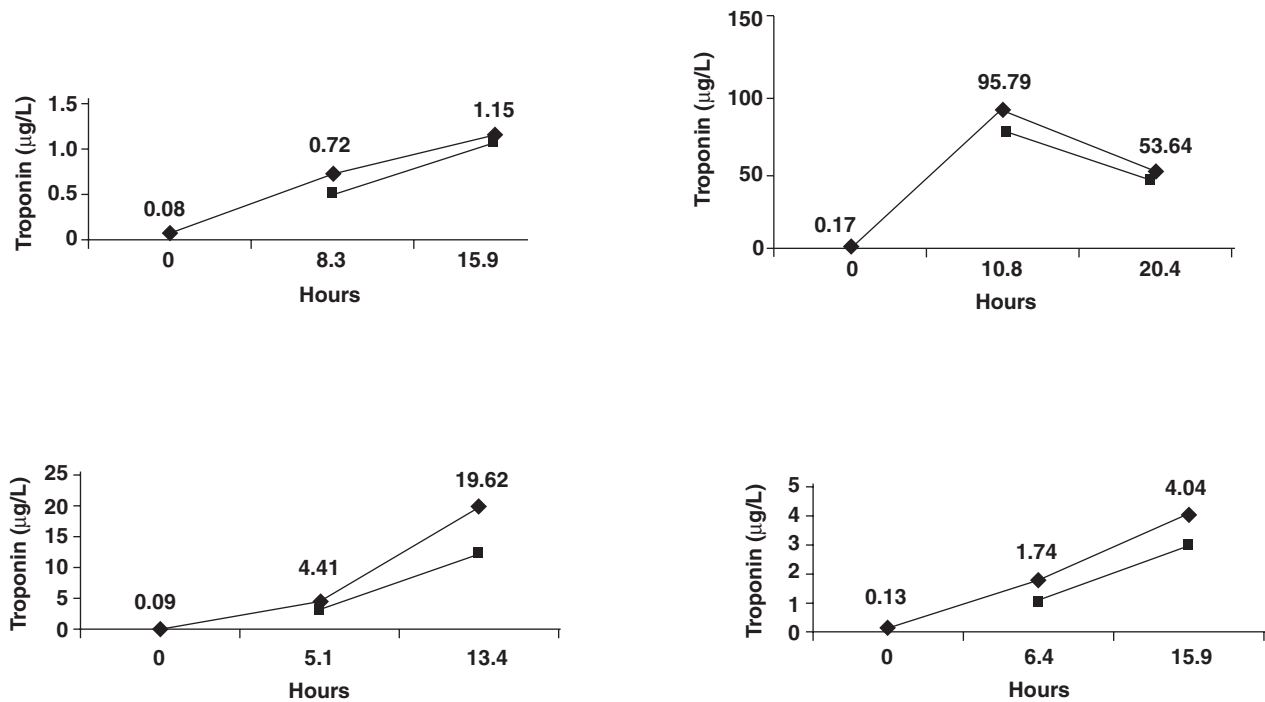


Figure 3 Troponin levels in 4 patients with acute coronary syndrome. The troponin I (TnI)-Ultra (diamonds) and cardiac TnI (cTnI; squares) concentrations are plotted over time; values for TnI-Ultra are depicted. Zero hours indicates the time the first specimen was obtained. By definition, all cTnI values were negative at time zero, so a data point was not plotted. Values for cTnI are given in Système International units; to convert to conventional units (ng/mL), divide by 1.0.

to achieve this optimal CV at the level of the 99th percentile and illustrates improved analytic performance of the TnI-Ultra compared with the current cTnI assay.

It is important to note that the ability to provide earlier detection for more than 50% of patients who were subsequently confirmed to have ACS at initial examination has the potential to substantially enhance early triage in the emergency setting and provide clinicians the opportunity to begin appropriate therapy for high-risk patients as early as possible. A rise and/or fall in cardiac troponin levels together with clinical evidence of myocardial ischemia is required for the diagnosis of ACS. The combination of the enhanced sensitivity and precision of this more sensitive troponin assay should allow significant changes in troponin levels (ie, presence or absence of ACS) to be detected in shorter intervals than the 6- to 9- and 12- to 24-hour windows that are currently recommended. In the present era of demands that threaten to exceed resources in acute care, emergency physicians, in particular, place very high value on rapid turnaround of results that influence triage.²¹ Thus, the ability to obtain a potentially treatment-defining result earlier is of clinical importance.

There are several limitations in our study. It is a retrospective study with a focused objective regarding the timing of detection of myocardial injury in patients with established cardiac injury. The study was not designed to evaluate prognosis, the impact of therapy, or diagnostic specificity (or negative predictive value) among patients with MI vs control subjects. In

addition, patients with underlying diagnoses other than ACS were not excluded. Although troponins offer high tissue specificity and reflect irreversible myocardial necrosis, they do not indicate the mechanism of myocardial injury.²²⁻²⁴ Many conditions other than ACS are associated with an increase in troponin levels, such as myocarditis, pulmonary embolism, acute heart failure, septic shock, and renal failure.^{22,23,25,26} Our results show that, as expected, myocardial injury will also be detected earlier in cases in which the mechanism of injury is not ACS. Additional studies to characterize diagnostic and prognostic performance in the broad population of patients with nontraumatic chest pain are likely to be useful. In addition, more frequently obtaining blood samples in the first several hours after initial examination would be needed to more accurately quantify the average time benefit of TnI-Ultra. From previous studies, we estimate that owing to its enhanced sensitivity, TnI-Ultra will detect injury at least 30 minutes earlier than cTnI.²⁷⁻²⁹

We found that the new more sensitive assay for cTnI (TnI-Ultra) offers superior analytic performance and detects myocardial injury earlier than the present generation assay from the same manufacturer. Implementation of the newer assay is likely to facilitate earlier identification of and intervention in high-risk patients with ACS.

From the ¹Department of Pathology, Division of Clinical Laboratories, and the ²TIMI Study Group, Cardiovascular

Division, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, MA.

Address reprint requests to Dr Jarolim: Brigham and Women's Hospital, 75 Francis St, Amory 2, Boston, MA 02115.

All reagents were provided by Siemens Medical Solutions (formerly Bayer Diagnostics).

References

- Alpert JS, Thygesen K, Antman E, et al. Myocardial infarction redefined: a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol*. 2000;36:959-969.
- Braunwald E, Antman EM, Beasley JW, et al. ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). *J Am Coll Cardiol*. 2002;40:1366-1374.
- Apple FS, Quist HE, Doyle PJ, et al. Plasma 99th percentile reference limits for cardiac troponin and creatine kinase MB mass for use with European Society of Cardiology/American College of Cardiology consensus recommendations. *Clin Chem*. 2003;49:1331-1336.
- Newby LK, Alpert JS, Ohman EM, et al. Changing the diagnosis of acute myocardial infarction: implications for practice and clinical investigations. *Am Heart J*. 2002;144:957-980.
- Eriksson S, Ilva T, Becker C, et al. Comparison of cardiac troponin I immunoassays variably affected by circulating autoantibodies. *Clin Chem*. 2005;51:848-855.
- Jernberg T, Venge P, Lindahl B. Comparison between second and third generation troponin T assay in patients with symptoms suggestive of an acute coronary syndrome but without ST segment elevation. *Cardiology*. 2003;100:29-35.
- Pagani F, Stefani F, Panteghini M. Innotrac aio! Second-generation cardiac troponin I assay: imprecision profile and other key characteristics for clinical use [letter]. *Clin Chem*. 2004;50:1271-1272.
- Adams JE III, Sicard GA, Allen BT, et al. Diagnosis of perioperative myocardial infarction with measurement of cardiac troponin I. *N Engl J Med*. 1994;330:670-674.
- Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. *N Engl J Med*. 1996;335:1342-1349.
- D'Costa M, Fleming E, Patterson MC. Cardiac troponin I for the diagnosis of acute myocardial infarction in the emergency department. *Am J Clin Pathol*. 1997;108:550-555.
- Haastrup B, Gill S, Kristensen SR, et al. Biochemical markers of ischaemia for the early identification of acute myocardial infarction without ST segment elevation. *Cardiology*. 2000;94:254-261.
- Huggon AM, Chambers J, Nayeem N, et al. Biochemical markers in the management of suspected acute myocardial infarction in the emergency department. *Emerg Med J*. 2001;18:15-19.
- Tucker JF, Collins RA, Anderson AJ, et al. Early diagnostic efficiency of cardiac troponin I and troponin T for acute myocardial infarction. *Acad Emerg Med*. 1997;4:13-21.
- Zaninotto M, Altinier S, Lachin M, et al. Strategies for the early diagnosis of acute myocardial infarction using biochemical markers. *Am J Clin Pathol*. 1999;111:399-405.
- Newby LK, Christenson RH, Ohman EM, et al, for the GUSTO-IIa Investigators. Value of serial troponin T measures for early and late risk stratification in patients with acute coronary syndromes. *Circulation*. 1998;98:1853-1859.
- Neumann FJ, Kastrati A, Pogatsa-Murray G, et al. Evaluation of prolonged antithrombotic pretreatment ("cooling-off" strategy) before intervention in patients with unstable coronary syndromes: a randomized controlled trial. *JAMA*. 2003;290:1593-1599.
- Eggers KM, Oldgren J, Nordenskjold A, et al. Diagnostic value of serial measurement of cardiac markers in patients with chest pain: limited value of adding myoglobin to troponin I for exclusion of myocardial infarction. *Am Heart J*. 2004;148:574-581.
- Morrow DA, Rifai N, Tanasijevic MJ, et al. Clinical efficacy of three assays for cardiac troponin I for risk stratification in acute coronary syndromes: a Thrombolysis In Myocardial Infarction (TIMI) 11B substudy. *Clin Chem*. 2000;46:453-460.
- Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. *Am Heart J*. 1987;113:1333-1344.
- Morrow DA, Cannon CP, Rifai N, et al. Ability of minor elevations of troponins I and T to predict benefit from an early invasive strategy in patients with unstable angina and non-ST elevation myocardial infarction: results from a randomized trial. *JAMA*. 2001;286:2405-2412.
- Han JH, Gibler WB. Biomarkers in the emergency department. In: Morrow D, ed. *Cardiovascular Biomarkers Pathophysiology and Disease Management*. Totowa, NJ: Humana Press; 2006.
- Hamm CW, Giannitsis E, Katus HA. Cardiac troponin elevations in patients without acute coronary syndrome [editorial]. *Circulation*. 2002;106:2871-2872.
- Roongsritong C, Warraich I, Bradley C. Common causes of troponin elevations in the absence of acute myocardial infarction: incidence and clinical significance. *Chest*. 2004;125:1877-1884.
- Scirica BM, Morrow DA. Troponins in acute coronary syndromes. *Semin Vasc Med*. 2003;3:363-374.
- Angheloiu GO, Dickerson RP, Ravakhah K. Etiology of troponin I elevation in patients with congestive heart failure and low clinical suspicion of myocardial infarction. *Resuscitation*. 2004;63:195-201.
- Edouard AR, Felten ML, Hebert JL, et al. Incidence and significance of cardiac troponin I release in severe trauma patients. *Anesthesiology*. 2004;101:1262-1268.
- Bertinchant JP, Larue C, Pernel I, et al. Release kinetics of serum cardiac troponin I in ischemic myocardial injury. *Clin Biochem*. 1996;29:587-594.
- de Winter RJ, Fischer J, Bholasingh R, et al. C-reactive protein and cardiac troponin T in risk stratification: differences in optimal timing of tests early after the onset of chest pain. *Clin Chem*. 2000;46:1597-1603.
- Mair J, Thome-Kromer B, Wagner I, et al. Concentration time courses of troponin and myosin subunits after acute myocardial infarction. *Coron Artery Dis*. 1994;5:865-872.

First and Only FDA Cleared Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager



Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System



Click or Scan
to discover more

ADS-04159-001 Rev 001 © 2024 Hologic, Inc. All rights reserved. Hologic, Genius, and associated logos are trademarks and/or registered trademarks of Hologic, Inc. and/or its subsidiaries in the United States and/or other countries. This information is intended for medical professionals in the U.S. and other markets and is not intended as a product solicitation or promotion where such activities are prohibited. Because Hologic materials are distributed through websites, podcasts and tradeshows, it is not always possible to control where such materials appear. For specific information on what products are available for sale in a particular country, please contact your Hologic representative or write to diagnostic.solutions@hologic.com.

genius™
DIGITAL DIAGNOSTICS