

Cardiac Troponin: What's the difference between T and I?

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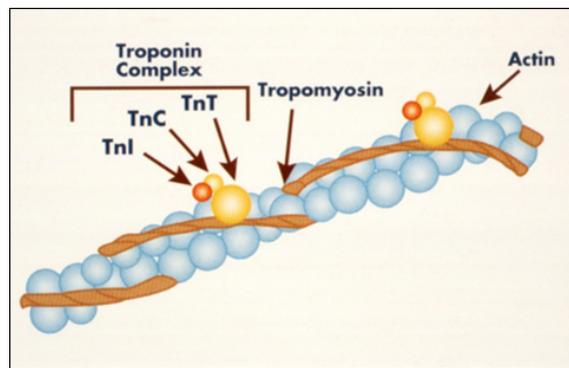
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The diagnosis of myocardial infarction (MI) was revolutionized in the early 1990s by the development of immunoassays for the cardiac troponins. Hugo Katus discovered cardiac troponin T (cTnT) in Germany and Jack Ladenson of the Division of Laboratory Medicine at Washington University developed the first immunoassay for cardiac troponin I (cTnI). cTnI and cTnT are regulatory proteins that sit on the actin/tropomyosin/troponin C complex present in cardiac muscle (See Figure). Both cTnI and cTnT have amino acid sequences that are unique compared to skeletal muscle troponins allowing the development of immunoassays specific to the cardiac forms. With rare exception the cardiac troponins (I and T) are expressed exclusively in cardiac muscle and if they are detected in a patient's blood, it is from cardiac muscle.

Due to the nature of a licensing agreement cTnT assays are only available from Roche Diagnostics. All other manufacturers market cTnI assays using their own set of proprietary antibodies. Since their introduction, both cTnI and cTnT assays have evolved considerably. The analytical sensitivity (the lowest concentration detectable) has improved 50 – 100 fold and precision (repeatability) has similarly improved resulting in highly sensitivity troponin methods that are in use in the rest of the world and are pending FDA clearance in the US. An accepted definition of a highly sensitive cardiac troponin assay is that troponin is detectable in over 50% of healthy subjects.

Currently, in the US, the current cTnT assay from Roche is referred to as the “4th generation” cTnT method while current cTnI methods are referred to as “contemporary sensitive” methods. BJH offers a “contemporary” cTnI method from Abbott.



Clinical guidelines and consensus documents on troponin are absolutely neutral on cTnI vs. cTnT.

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They collectively refer to them as cardiac troponins and make no recommendation about one over the other. cTnT and cTnI have similar sensitivities and specificities for MI and similar prognostic value. Nevertheless, there are differences between troponin assays that users should be aware of.

cTnI assays are not standardized and it is unlikely they ever will be. Thus, different cTnI assays have different reference limits and values will be different by as much as two to three fold between different methods. The reason for the lack of standardization is that manufacturers use different antibodies that recognize different epitopes. This would not be an issue if circulating cTnI only existed as the intact protein, which is not the case. cTnI is subject to proteolytic cleavage in circulation with multiple fragments that are not equivalently detected by all assays. Furthermore, cTnI and its fragments also circulate as phosphorylated and non-phosphorylated forms and as complexes with troponin c each of which can influence binding to different antibodies. Therefore, cTnI results from one cTnI method cannot be compared to another and that it is essential “to know” your assay with respect to its reference interval.

As there is only one manufacturer and one set of antibodies used in cTnT assays the above standardization issues do not exist for cTnT. cTnT is a different protein than cTnI and actual mass concentrations in blood are generally much lower (5-10 fold than those of cTnI. Therefore, the numerical result from a cTnT assay cannot be directly compared to a cTnI result.

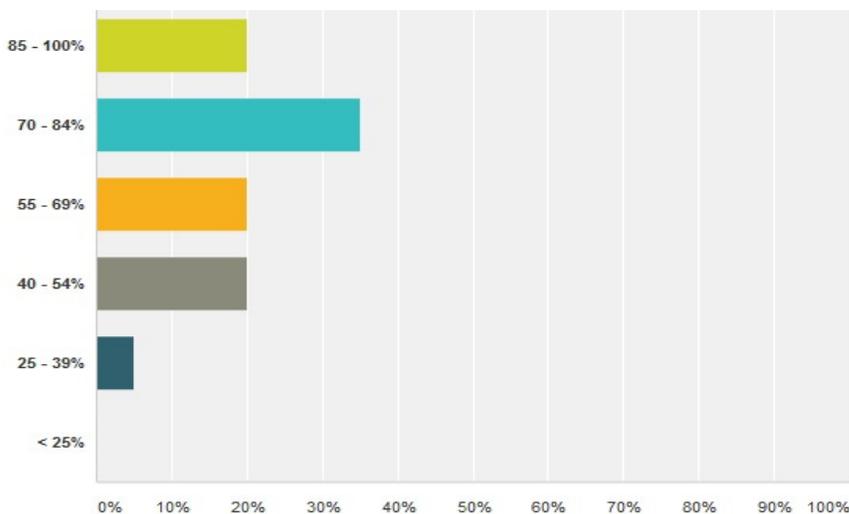
As mentioned previously, both cTnI and cTnT are cardiac specific with one rare exception. In the fetus, cTnT is expressed in both skeletal and cardiac muscle but in adults cTnT is only expressed in cardiac muscle. However, several studies have shown that in patients with skeletal muscle pathologies such as muscular dystrophy, myositis and other rare myopathies cTnT is reexpressed in diseased and regenerating skeletal muscle. This does not occur for cTnI. While rare, this potential cause of false positive cTnT should be kept in mind.

One major difference in cTnT vs cTnI is the susceptibility of the cTnT assay to a substantial negative interference in mildly hemolyzed samples. Interestingly, this negative interference is due to proteolytic cleavage of cTnT by proteases present in RBC. Hemolysis above 100 mg/dL of soluble hemoglobin (mild but visible hemolysis) results in a negative interference. The negative interference in the cTnT increases with the extent of hemolysis. This is significant because 20% of samples from the BJH ED are hemolyzed above 60 mg/dL, 14% above 100 mg/dL and 6% above 200 mg/dL.

While clinical guidelines are neutral on cTnT vs. cTnI, there may be advantages to cTnI, particularly in the susceptibility to interference from hemolyzed samples.

Did You Know??

In 1996 Forsman et al wrote that 60-70% of critical medical decisions involve clinical laboratory data, although they had no data to back up this statement (Clin Chem 1996;42:813-6). Since then, this has become a frequently used “factoid”. We asked the BJH medical staff what percentage of the medical decisions that they make are based partly or fully on laboratory results. The data is shown below. Our n is small, but these data support Forsman’s statement and underscore the importance of laboratory results for patient care.



Percentage of the medical decisions that are based partly or fully on laboratory results

Note if you did not respond to this survey and still would like to, please go to:

<https://www.surveymonkey.com/s/NHPVHLD>

Hematologic Malignancy Testing Algorithms (HMTA) to Optimize Molecular

Blood or bone marrow from patients with known or suspected hematologic malignancies is commonly submitted to the BJH Molecular Diagnostics Laboratory (MDL) for molecular analysis. In patients with acute leukemia and myeloproliferative neoplasms, specific somatic mutations may have diagnostic, predictive, or prognostic value. Examples of useful molecular testing in hematologic malignancies include:

- FLT3 internal tandem duplication and NPM1 mutation status at first diagnosis of normal-karyotype acute myeloid leukemia, for prognostic purposes
- FLT3 mutation status (but not NPM1 status) in relapsed AML patients, regardless of karyotype and FLT3 status at diagnosis, for predictive purposes (i.e. use of FLT3 inhibitors)
- KIT exon 8 and 14 mutation testing in patients with new diagnosis of core binding factor AML, for prognostic purposes
- Quantitative BCR-ABL testing by RT-PCR in patients with CML, for disease monitoring

However, testing is only appropriate at certain time points, such as at first diagnosis or at relapse. The same tests performed in different contexts—for example, FLT3 mutation testing on a chemoablated marrow—may be unnecessary, wasteful, and even confusing or harmful. In many contexts—for example, myelodysplastic syndromes—no molecular testing is recommended, though that may change in the future.

Analyses performed in early 2012 suggested an opportunity to optimize test utilization. The MDL worked with the Section of Bone Marrow Transplantation and Leukemia to develop an algorithm for molecular testing in hematologic malignancies based on the European LeukemiaNet (ELN) guidelines, with separate pathways for newly-diagnosed AML (Figure 1), recurrent AML and other diagnoses. This algorithm was implemented in August 2012. Clinicians invoke the HMTA via a specific checkbox on the requisition form for blood and bone marrow testing. Specimens are triaged by the laboratory medicine resident on call, in conjunction with an MDL medical director, to select appropriate testing.

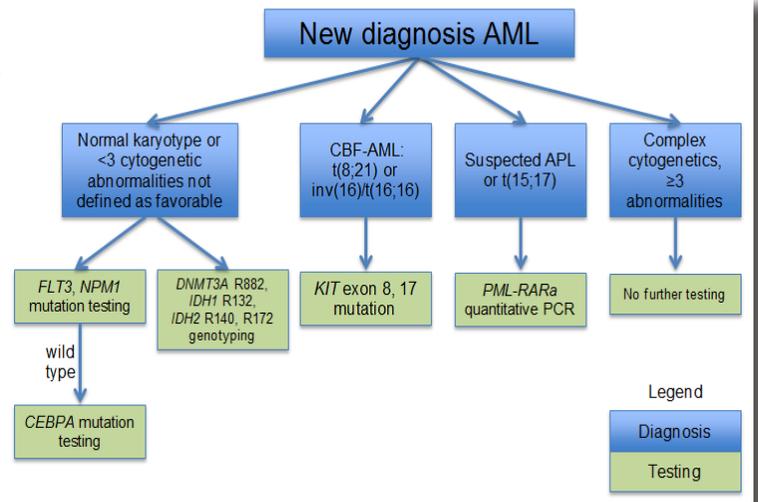


Figure 1. HMTA for new diagnosis of acute myeloid leukemia. CBF, core binding factor; APL, acute promyelocytic leukemia.

Post-implementation review showed that the HMTA reduced unnecessary testing while increasing and streamlining the use of indicated tests. For example, FLT3 testing volume dropped by 81% in the first 125 days after implementation (Figure 2), resulting in savings of \$32,000. Utilization of reflex CEBPA and KIT testing increased commensurately. The algorithm has

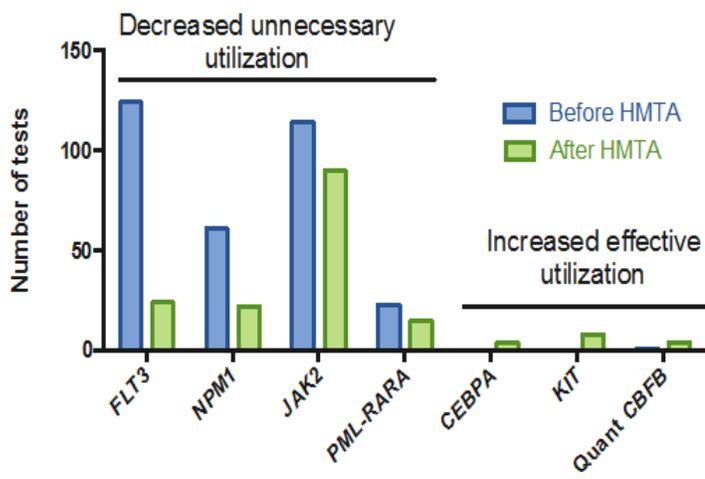


Figure 2. Test volumes in the 125 days after implementation of the HMTA (green) and in a comparable period one year prior (blue). Courtesy of Christina Lockwood, Ph.D., and Mohammad Hussaini, M.D.

also simplified test ordering because clinicians can rely on Laboratory Medicine to select appropriate testing using a mutually agreed-upon algorithm.

Building on the success of the AML HMTA, an algorithm has now been implemented for myeloproliferative neoplasms. The MPN algorithm is intended to facilitate utilization of BCR-ABL RT-PCR and JAK2 V617 and exon 12, CALR exon 9, and MPL W515 mutation testing. The HMTAs show that collaboration between clinical services and the Division of Laboratory and Genomic Medicine can improve the quality and cost-effectiveness of care. For further information, please contact Dr. Jacqueline Payton, Director of Molecular Diagnostics Laboratory jpayton@path.wustl.edu (314) 362-5935.

HIV Fourth Generation Algorithm: The BJH Experience

In October 2014 BJH transitioned to a new testing algorithm to screen for Human Immunodeficiency Virus (HIV). This updated methodology improves the detection of acute HIV and is consistent with the Centers of Disease Control (CDC) HIV screening guidelines (<http://stacks.cdc.gov/view/cdc/23447>). Previously, a 3rd generation enzyme immunoassay to detect HIV antibodies was performed, with a Western Blot confirmatory testing on reactive specimens. The new 4th generation screening assay is a combined antigen/antibody detection test. All specimens that are reactive in this screening assay are tested by a secondary antibody assay that detects and differentiates HIV-1 and HIV-2 antibodies (the HIV1/HIV2 Multispot test). A major advantage of this new algorithm is that detection of both p24 antigen and HIV antibodies allows for detection of HIV approximately 5 to 7 days earlier than the 3rd generation assay. In fact, since the antigen/antibody screen can produce a positive result earlier in the course of infection than the Multispot differentiation assay, it is recommended that screen positive, Multispot negative specimens be confirmed using nucleic acid amplification testing.

During the past nine months, we have tested more than 10,000 specimens using this algorithm (Figure 1). Approximately 1% of initial antigen/antibody screens were reactive. However, nearly 25% (16 total) of these specimens were ultimately considered to be HIV negative following confirmatory testing. This is similar to the false positive rate reported in the literature, and an important statistic to keep in mind whenever a patient is being tested for HIV1,2. While the antigen/antibody test is highly sensitive, making it an effective screen, the relatively high rate of false positives precludes its use as the sole diagnostic assay for HIV. Thus, it is important that the results of the screening assay are considered preliminary, that confirmatory testing is closely monitored, and that all findings are considered in the context of the patient's clinical presentation.

At this time, the screening assay and the Multispot differentiation assay are performed in the BJH laboratory 24 hours a day, 7 days per week; samples that are reactive in the screening assay are automatically reflexed for confirmatory testing. Both tests are usually completed within 12 hours of specimen receipt by the laboratory. While HIV viral load testing is currently a send out test that is performed at Mayo Clinic, it will be performed on-site in-house in the near future. If you have any questions about this testing algorithm please contact Adrain McClellan, Supervisor of Special Chemistry at 362-5009 or Neil Anderson, MD, Assistant Medical Director of Clinical Microbiology at 362-1307.

¹ Kim S, et al. False-positive rate of a "fourth-generation" HIV antigen/antibody combination assay in an area of low HIV prevalence. Clin Vaccine Immunol. 2010;17:1642-4.

² Dubravac T, et al. Use of the Abbott Architect HIV antigen/antibody assay in a low incidence population. J Clin Virol. 2013;58: Suppl 1:e76-8.

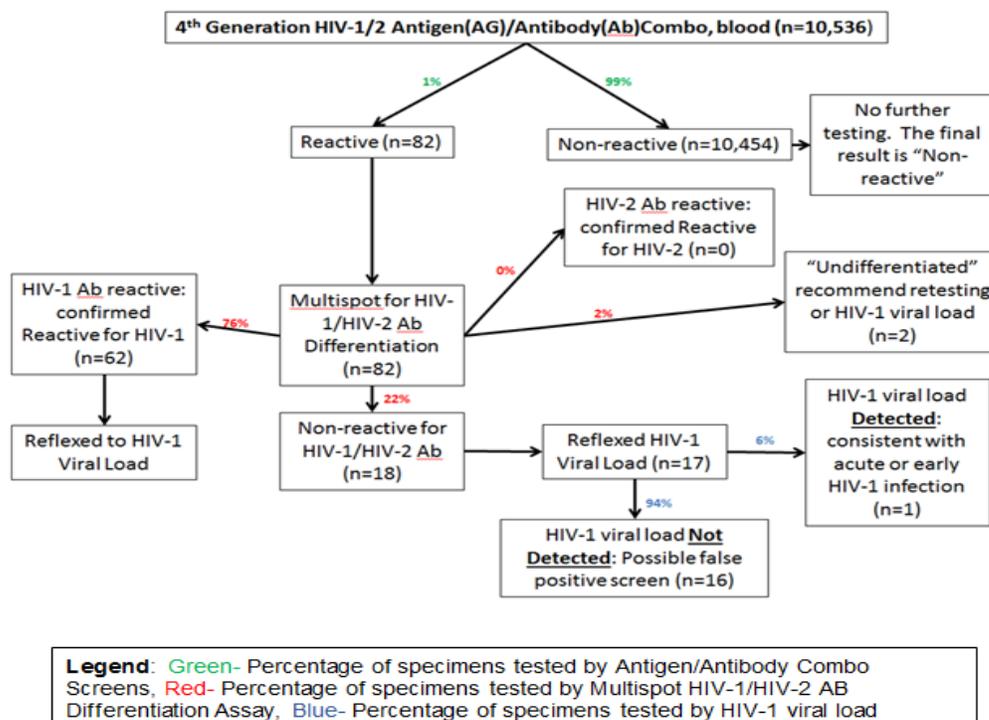


Figure 1. HIV Screening at BJH from 10/13/14 to 7/6/15

Update on Moving Plans for BJC Clinical Laboratories

The new homes for BJH clinical laboratories on the 4th and 5th floors of the Institute of Health (IOH) building are nearing completion. This photo shows the future location of the Core Laboratory on the 4th floor which will be the site for delivery of most patient samples via an upgraded pneumatic tube system and highly automated chemistry, hematology, and coagulation testing. Additional laboratory services on this floor will include blood bank, flow cytometry, serology, urinalysis, and toxicology. Construction of the new microbiology laboratory on the 5th floor is also nearing completion. During the next few months advanced automated analytical instruments will be installed and their performance validated. We anticipate moving these laboratories from their current locations on the 2nd floor of BJH south to the IOH in the 4th quarter of 2015 and 1st quarter of 2016. Look for opportunities to tour the new laboratories during an open house later this year.



A northeast view of the new Core Laboratory, 4th floor, IOH Building.

BJC Computer Goes Down: Laboratory Staff Steps Up

On Tuesday, July 28th at ~ 6 pm, all BJC computer applications were non-functional and remained that way for ~20 hours. This included employee time clocks, pharmacy, HMED, Compass, Clinical Desktop, and the laboratories, just to mention a few.

What does this mean for the laboratories? The positive patient identification (PPID) system was non-functional and all tests had to be ordered via manual requisition forms. This lack of automation led to >30 mislabeled specimens sent to the chemistry lab (equal to ~1 month of mislabeled specimens) demonstrating the value of the PPID system.

Once received in the laboratory, a downtime accession number sticker was placed on each tube and on the requisition. Each ordered test was hand written on the tube. Patient name, demographics and test orders



Thousands of requisition forms that required manual entry

were manually entered into instruments. Results were printed and immediately faxed to the ICU & ED. As the breakdown continued, paper copies of routine results were sent to the floors via couriers ever 2 hours. When requested, results were given verbally by phone. During this period, all laboratory staff, lab medicine residents and fellows were on hand helping to phone and fax results.

After the computers returned to normal function, each test had to be ordered manually in the computer using the downtime accession number, so that the results could be released from the instruments to the EMR. Manual order entry took an additional ~40 hours to complete.

Thanks to all the laboratory staff, lab medicine residents and fellows that helped deliver patient care during this difficult outage!

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