

## Implementation of HIV Viral Load Testing

by: Neil Anderson, M.D.

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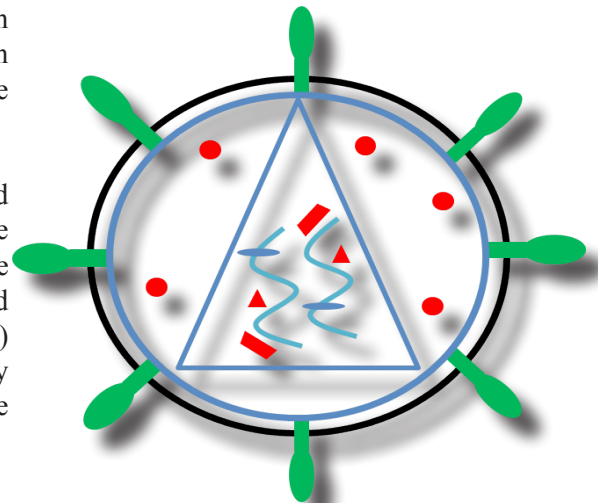
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On October 12th, the BJH Molecular Infectious Diseases Laboratory will begin testing patients for HIV viral load in blood. This will be performed using the COBAS AmpliPrep/COBAS TaqMan platform which is FDA approved for the quantitative testing of HIV-1 from plasma. This is the same molecular assay currently used by our primary send out laboratory, Mayo Medical Laboratories. As such, values obtained from the previous methodology and our new methodology can be directly compared. The benefits of performing this testing at BJH include: improved turnaround time, the ability to consult with local laboratory directors on unusual cases or anomalous results, and the ability to more efficiently manage follow-up testing such as HIV-1 genotyping.

Quantitative measurement of HIV-1 is important for both the diagnosis and management of HIV-1. The 2014 updated HIV-1 screening guidelines recommend the use of HIV-1 molecular testing for confirmation of patients with positive 4th generation antigen/antibody screens and negative HIV-1/HIV-2 differentiation (Multispot) assays. Our practice has been to automatically add HIV-1 viral load testing to specimens that fulfill these criteria. We will be continuing this practice as we bring testing in-house. In patients with a known diagnosis of HIV-1, the viral load at diagnosis and throughout treatment is an important means of monitoring response and disease progression. For this reason, we currently perform viral loads on all patients newly diagnosed with HIV-1 by serology and plan on continuing this process as we bring the testing in-house.

Testing will be performed Monday thru Friday, once per day. The only acceptable specimen for testing is blood collected in an EDTA (purple top) tube. Specimens collected in any other tube type are unacceptable for testing.



*Continued on page 2*



**NATIONAL LEADERS IN MEDICINE**

Importantly, inaccurate results may be obtained when there is a delay in separating plasma from the blood specimen. Thus, specimens must be received by the laboratory within 6 hours of collection. It will not be possible to add on HIV-1 viral load testing to an existing specimen in the laboratory.

If you have any questions, please contact Neil Anderson, M.D., Assistant Medical Director of Microbiology (362-1307) or the laboratory medicine resident on call (747-1320).

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## Update on Transition of BJH Clinical Laboratories to the Institute of Health (IOH)

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by: Charles Eby, M.D.

In September, the BJH clinical laboratories passed their CAP biannual inspection. The inspection was performed by laboratory medicine faculty and staff from Columbia University, and we received positive feedback regarding our current laboratory operations and congratulations on the space and instrumentation improvements that are in progress.

### *Major milestones in last 2 months:*

1. Installation of fully automated chemistry/ hematology instruments and bacteriology culture systems on the 4th floor core laboratory and 5th floor microbiology laboratories respectively.
2. LGM clinical faculty, clinical pathology residents and fellows, and BJH laboratory administration staff moved into new office space on the 4th floor.

Currently HLA and molecular diagnostic laboratories are operating on the 5th floor of the IOH. The remaining clinical laboratories are scheduled to move to the IOH first quarter 2016.

### *Upcoming improvements:*

1. Construction will begin soon on a blood distribution center in the current blood bank area on the 2nd floor of the south service building. This center will provide blood to south campus operating rooms, emergency room, and ICUs and will support south campus massive transfusion hemorrhages.
2. Approximately 25 pneumatic tube stations will be upgraded to permit delivery of single units of red cells, FFP, and platelets via secure pneumatic tube system.

2016 will be a year of many advances and changes in delivery of laboratory services to our patients. We look forward to feedback from our healthcare providers as we move forward.

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## Did You Know??

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# 135,000

The square feet of the new Center for Advanced Laboratory Medicine at the Institute of Health.

# 93,000

The square feet of the previous laboratory space.

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# Validating over 100 Laboratory Tests

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by: Khushbu Patel, Ph.D.

What does it take to validate a new lab test? Test validation is a multi-step process, which requires determining the analytical measurement range (AMR), intra-run and inter-run precision, linearity and low-end sensitivity as well as patient comparisons on the old and new methods and analytical interference studies.

As part of the preparation for the move to the new laboratory in the IOH building, over 100 basic chemistry tests and immunoassays are being validated on the new Roche Cobas chemistry analyzers (pictured). After the move, a total of 10 instruments from the current lab will be eliminated.

The new instruments were delivered to BJH in February 2015. Four weeks of initial validation studies were conducted by the manufacturer. Over the past five months, we have conducted additional studies, going above and beyond requirements outlined by the College of American Pathologists (CAP). In particular, we have performed interference studies to test the manufacturer claims for interferences in hemolyzed, icteric and lipemic samples. For each test, we have also conducted at least 40 patient comparisons spanning the linear range to verify our reference ranges.

We estimate that a total of 360 man-hours have been devoted to this task. We anticipate an additional 150 hours for the remaining validation and documentation process. The entire validation process will be completed one month in advance of the move in 1st quarter of next year. We are grateful to the supervisors and technicians who have been involved. They have battled several major obstacles along the way, including difficulty obtaining patient samples necessary for validation of certain tests (for example high TSH samples and negative rubella samples). Their work is critical for ensuring that the chemistry laboratory continues to operate smoothly and turn out important patient results during this time of transition.



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## New: Thyroglobulin, Tumor Marker Reflex Test

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by: Ann Gronowski, Ph.D.

Thyroglobulin (Tg) is a thyroid-specific glycoprotein that serves as the source for thyroxine (T4) and triiodothyronine (T3) production within thyroid follicles. Serum concentrations of thyroglobulin roughly parallel thyroid size (0.5-1.0 ng/mL Tg per gram thyroid tissue, depending on thyroid-stimulating hormone TSH concentration). In situations of disordered thyroid growth (eg, goiter), increased thyroid activity (eg, Graves' disease), or glandular destruction (eg, thyroiditis) larger amounts of Tg may be released into the circulation.

The main use of serum Tg measurements is in the follow-up of differentiated follicular cell-derived thyroid carcinoma. Because Tg is thyroid-specific, serum Tg concentrations should be undetectable, or very low, after the thyroid gland is removed during treatment for thyroid cancer.

The presence of anti-thyroglobulin autoantibodies (TgAb), which occur in 15% to 30% of thyroid cancer patients, can cause misleading Tg results. Traditionally, there have been no reliable means to obtain accurate Tg measurements in patients with TgAb. Recently, Mayo Medical Laboratories introduced a new method that can overcome the interference from TgAb. Serum proteins are digested by trypsin and Tg-specific tryptic peptides are then measured by mass spectrometry.

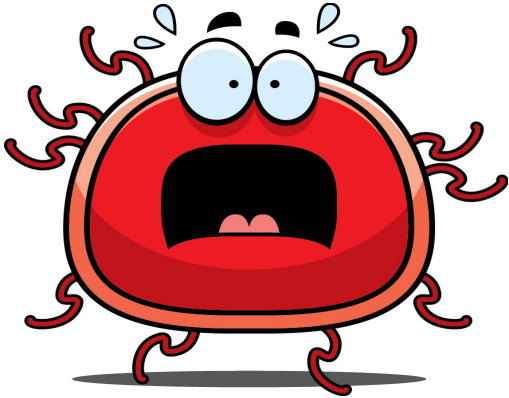
The Thyroglobulin, Tumor Marker Reflex Test begins with the analysis of thyroglobulin antibody by immunoassay. If the thyroglobulin antibody result is negative (<4.0 IU/mL), thyroglobulin testing is performed by immunoassay. If the thyroglobulin antibody result is positive (> or =4.0 IU/mL), then thyroglobulin testing will be performed by mass spectrometry. This test now replaces the old Thyroglobulin, tumor marker test.

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# Avoiding Delay in Compatible Blood Cross Matches: Close Communication between Clinician & Laboratory is Key

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by: Brenda Grossman, M.D., MPH



Many drugs can alter testing for blood compatibility and may delay the release of blood products. We recently encountered an investigational treatment for refractory myeloma which interferes with antibody screening by binding to the RBC membrane. Daratumumab (DARA) is an investigational humanized monoclonal antibody which binds with high affinity to CD38 on the surface of myeloma cells. It leads to myeloma cell death by various mechanisms, including complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity and several others pathways. Clinical trials are ongoing in the US, with at least one trial available at this institution for the treatment of patients with myeloma who have failed previous therapies.

DARA also binds to CD38 on red blood cells and platelets without causing any acute effects on these cells. However, it does interfere with routine blood screening in patients receiving the antibody. This interference was noted in the early clinical trials and several investigators have developed method to minimize the interference. Fortunately CD 38 is denatured by the reducing agent dithiothreitol (DTT) which is commonly used in blood banking to differentiate IgM antibodies from IgG antibodies and to destroy Kell system antigens in difficult antibody identification work ups. DTT works by breaking disulfide bonds and does not interfere with the detection of other clinically significant RBC antibody outside the Kell system. Investigators at Brigham and Women's hospital found that pretreating reagent RBC used in screening with DTT removes the interference in RBC antibody screening. Since Kell antigens are destroyed by DTT and an anti-K may be undetectable, blood which lacks the K antigen must be transfused in these patients. Others have used cord RBCs which do not appear to bind the anti-CD38.

Knowledge that the patient is receiving DARA allows for use of appropriate testing conditions and prevents unnecessary delay in finding compatible blood. If you have a patient that is taking DARA, please contact the blood bank at 362-3887. Many drugs, especially investigational ones, have the potential to adversely affect laboratory tests. If you have questions about potential drug interferences, contact the lab medicine resident on-call at 747-1320.

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## Detecting Erroneous Results

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by: Mitchell Scott, Ph.D.

Most people don't know that the laboratory instruments and information systems have numerous checks and rules to help identify potentially erroneous results. The readout for many tests performed in clinical laboratories is some form of light transmission. Enzymatic and chemistry reactions are determined by light absorption at various wavelengths, immunoassays utilize either light scattering, chemiluminescence, fluorescence or absorption and coagulation testing is performed by measuring changes in absorbance as the clot forms. Because of this, red (hemolyzed), yellow (icteric) and cloudy (lipemic) samples can interfere with many tests and result in erroneously high or low values from the instruments. However, the large chemistry instruments as well as our new automated processing line that will be in the new IOH laboratory, can detect red, yellow and cloudy samples and indicate whether or not to report the result.

Other rules to prevent erroneous results include an extensive "delta" system in which a patient's result is compared to the previous value for that patient. If a result exceeds the delta limit it is not reported until the technologist or quality assurance staff repeat and review the accuracy of the result. For instance, if a sodium result changes by more than 10% in a 24 hour period it is investigated. This system allows us to detect mislabels, samples contaminated with intravenous fluid, gross instrument errors or interfering substances. Low values, below the reportable range of an assay, or those that are biologically implausible are repeated. As an example all albumin values of <1 g/dL are repeated since the most common cause of such a result is "short-sampling" by the instrument. These are just a few of the many automated rules that the laboratory utilizes to assure accurate and meaningful results.

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