

Syphilis Testing — New Methodology and Algorithm

Setting and History

Syphilis is caused by infection with the spirochete *Treponema pallidum*. It is a systemic infection with periods of latency. These periods of latency, when compounded with the fact that *Treponema pallidum* cannot be isolated in culture, indicate why serologic testing is so critical for the initial diagnosis and in subsequent monitoring of treatment.

In the past, the serologic testing algorithm for syphilis began with a nontreponemal screening test, such as the rapid plasma reagin (RPR) or the venereal disease research laboratory (VDRL) tests. These tests measure the patient's antibody response to nontreponemal antigens. Consequently they are non-specific and can be positive with a host of conditions including autoimmune diseases, aging, injection-drug use, some viral infections and pregnancy.

The nontreponemal tests are not detecting antibody responses to *Treponema pallidum*, but instead are detecting IgG and IgM antibodies in a variety of conditions causing tissue damage. Nontreponemal test titers often correlate with disease activity and usually decline following treatment (often becoming nonreactive over time). A positive result with the RPR (used here in our Bronson Laboratory) required confirmation with a treponemal-specific test including the fluorescent treponemal antibody-absorbed (FTA-ABS) or microhemagglutination assay (MHA-TP). Both of these tests are labor intensive and require subjective interpretation by testing personnel.

EIA Specific for *Treponema pallidum*

More recently, though, enzyme immunoassays (EIA) have become available that are specific for *Treponema pallidum*. The CAPTIA™ Syphilis (T. Pallidum)-G is an EIA for the qualitative detection of IgG antibodies to *Treponema pallidum* in serum specimens. This assay allows an objective interpretation of results and is both highly sensitive and specific (98.4% and 99.3%, respectively).

During the earliest stage of primary syphilis, the first antibodies to appear are IgM while the IgG antibodies reach clinically

significant titers slightly later in the course of primary syphilis. Importantly, IgG titers persist indefinitely irrespective of disease activity or prior therapy. This also means that the EIA test will be positive in latent syphilis disease whereas the nontreponemal RPR test would be negative. Hence, previously untreated syphilis cases will be detected. For these reasons, a positive EIA test will be followed with a nontreponemal RPR in order to distinguish

between active and past infection and to help guide treatment decisions.

Specimen Collection, Interpretation of Results and Cautions

The required specimen is serum, EDTA or citrated plasma samples. Initially reactive or equivocal results should be repeat tested. Any patient with a reactive or equivocal result on initial testing will then have (*continued on back*)

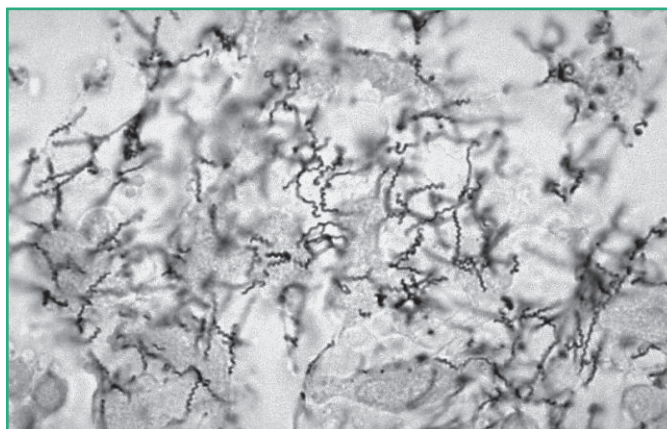


Figure 1. *Treponema pallidum* spirochetes. Modified Steiner silver stain. <http://hardinmd.lib.uiowa.edu/cdc/syphilis.html>

New Instrument to Automate Urinalysis and Urine Microscopy: Improved Turnaround Times Expected

Bronson Laboratory will soon be upgrading our urinalysis instrumentation to the state of the art IQ200 Elite Workcell from Iris Diagnostics. Not only will this save time by integrating the chemistry and microscopy results, but will also greatly reduce the need for manual reviews. Furthermore, with a throughput rate of 70 samples per hour, turnaround times will be significantly decreased. The analyzer performs the urine chemistries, specific gravity, color and clarity.

The IQ200 uses flow cell technology and a built in microscope to take 500 pictures of every specimen at a rate of 24 pictures per second. At that point, all particles in each picture are separated and classified into 12 categories by size, shape, contrast and texture. The IQ200 can classify cells, bacteria, yeast, crystals and casts.

With the IQ200, the technologist will have the ability to review the pictures of each specimen instantly on the computer screen, whereas currently when a manual review is needed, the specimen must be centrifuged and placed on a microscope. In the near future, body fluid testing will be added to the IQ200 also minimizing the number of manual counts being performed by technologists.

The transition to the new instrumentation should be relatively seamless outside the laboratory as patient reports will remain the same. For more information, please contact Dr. Herzog at (269) 341-8997, core lab manager Paul Guthrie at (269) 341-7194, or hematology senior tech Amy Schmidt at (269) 341-8506.

— Amy Schmidt, MT ASCP

Syphilis Testing — New Methodology and Algorithm *(continued)*

(continued) the RPR test performed in order to distinguish between active and past infection and to assist in ruling out false positives.

1. IgG-positive and RPR-positive: Untreated, current syphilis (unless ruled out by treatment history). Patients treated in the past are considered to have new syphilis infection if RPR titers show a four-fold (or greater) increase between acute- and convalescent-phase specimens.
2. IgG-positive and RPR-negative: Past, successfully treated syphilis in patients with history of appropriate treatment. In patients without history of treatment, a second treponemal assay (i.e. FTA-ABS) should be performed to determine if the EIA results were actually positive, rare possibility of false negative RPR due to prozone and late syphilis/neurosyphilis.
3. IgG-negative (no RPR performed). No evidence of infection. If clinically indicated, a second specimen should be submitted in one to two weeks for follow-up testing.
4. IgG-negative and RPR-positive: Current infection unlikely, most likely false positive secondary to other medical disease.
5. Infants less than six months old with IgG antibodies to *Treponema pallidum*, or a positive RPR, probably have maternal antibody. Therefore, testing for IgM antibody should be performed to assess

for acute-phase infection in infants less than six months old.

6. RPR results are useful to determine the current disease status and response to therapy. Response to treatment may be indicated by a decrease in the RPR titers or by a conversion of the RPR from positive to negative. As a reminder, the EIA IgG antibody test will remain positive for the lifetime of the patient and it should not be used to determine response to therapy.

Several cautions are warranted. Serologic testing for syphilis may be negative in severely immunosuppressed patients. Very early cases of primary syphilis may be negative with both IgM and IgG serologies. Rare cases of old, successfully treated infection (more than 10 years



earlier) may be negative for both IgM and IgG serologies.

Anticipated Start Date

It is anticipated that the Bronson Laboratory will begin using the CAPTIA™ Syphilis (T. Pallidum)-G EIA in June 2011.

References:

1. Larsen S, Steiner B, Rudolph A. Laboratory diagnosis and interpretation of tests for syphilis. *Clinical Microbiology Reviews*. 1995; 8(1):1-21.
2. CDC. 2010 STD Treatment Guidelines. <http://www.cdc.gov/std/treatment/2010>
3. Mayo Medical Laboratories, Online Test Catalog, Unit Code 81814: Syphilis IgG Antibody with Reflex RPR, Serum
4. Leferve JC, Bertrand MA, Baurtaud FL. Evaluation of the CAPTIA™ Enzyme Immunoassays for Detection of Immunoglobulins G and M to *Treponema pallidum* in Syphilis. *1990 Clinical Microbiology*, 1990; 28:1704-1707.

— Jeff Hinson, DO

Figure 2. Syphilitic chancre of the finger. <http://hardinmd.lib.uiowa.edu/cdc/syphilis.html>

LabWire is published by Bronson Laboratory Services. If you have a topic you would like addressed in this publication, call 341-8997 or send your request to Jeff Pearson, MD (pearsonj@bronsonhg.org).

Teamwork Brings New Lab Equipment Online

Bronson Laboratory is the first in the nation to have a Roche cobas 8000 analyzer up and running. This large piece of equipment performs a variety of chemistry and immunoassay tests, including basic and comprehensive profiles and testing for cardiac markers, drug levels, and hormones.



The new Roche cobas 8000 analyzer now operating in the Bronson Laboratory can run critical tests faster than the equipment it replaces.

While other labs in the nation have had this equipment installed, Bronson has been the first to have it functioning. According to laboratory manager Paul Guthrie, MLS(ASCP), this was due to the excellent team and work processes in place. In less than two months, five

employees each received 35 hours of off-site training and 40 employees each received 1.5 hours of CBT training along with three to five hours of hands on training. Nearly 100 technical procedures were written, and 2,000 samples were gathered and tested for correlation studies. 4,000 tests were run for precision

studies, and more than 1,000 samples were run for linearity studies. Inventory control was established for nearly 150 new reagents and supplies, and a variety of logistics (water, electric, IT, cooling, supply room, etc.) were coordinated to keep both the old and new equipment running in the limited space available.

This was the largest project the laboratory has ever undertaken. In addition to technical and software improvements not available in the older equipment being replaced, the new analyzer can perform critical tests like cardiac troponin (to detect myocardial damage) nine minutes faster.

Congratulations to the staff of the Bronson Laboratory for this complex achievement and the improvement in patient care it provides!