CCTC Clinical Chemistry Trainee Council | AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: Diagnosis of Syphilis Using the Reverse Algorithm

PRESENTER: Gregory J. Berry, PhD

Slide 1:
Hello, my name is Gregory Berry. I am a Clinical Microbiology fellow in in the Department of Pathology at University of Texas Medical Branch. Welcome to this Pearl of Laboratory Medicine on “Diagnosis of Syphilis Using the Reverse Algorithm.”

Slide 2:
Syphilis is caused by the spirochete bacterium, *Treponema pallidum*. This bacteria cannot be diagnosed using traditional microbiological methods as it cannot be grown on growth medium and does not take up gram stain. While methods such as dark field microscopy can be used to directly visualize *T. pallidum*, they are not sensitive enough to rule out syphilis and are only useful in cases of chancres and are not useful in the diagnosis of later stages of disease such as secondary, late, or latent syphilis. In addition, few labs have the expertise to perform dark field microscopy. Instead, the clinical laboratory depends on serological methods to determine syphilis infection. Both treponemal and non-treponemal tests are performed to identify a syphilis infection and differentiate between current and past infection.

Slide 3:
When using serologic tests to identify a syphilis infection, two antibody types are detected. These are treponemal antibodies and non-treponemal antibodies. Treponemal antibodies are those that are directed against the causative agent of syphilis, *Treponema pallidum*. Non-treponemal antibodies are those that are instead directed against host antigens being released as a result of active infection. These host antigens include cardiolipin, cholesterol, and lecithin.

Slide 4:
Treponemal serologic tests include assays such as *Treponema pallidum* particle agglutination (TP-PA) test, enzyme immunoassay (EIA), chemiluminescent immunoassay (CLIA), and fluorescent treponemal antibody absorption (FTA-ABS).

Non-treponemal serologic tests all use the same basic antigen formula as the Venereal Disease Research Laboratory (VDRL) test and contain cardiolipin, cholesterol, and lecithin. These tests include VDRL, rapid plasma regain (RPR), unheated serum regain (USR), and toluidine red unheated serum (TRUST) tests. RPR is the most common non-treponemal serologic test used on serum. These non-trepomenal tests are all quantitative and can be used to evaluate treatment progress.

Slide 5:
In order to identify a syphilis infection and see if it is active, both treponemal and non-treponemal tests must be utilized. Traditionally, screening for syphilis was done using a non-treponemal assay, such as RPR. Non-treponemal assays, such as RPR, are sensitive but lack specificity, so a specific treponemal assay, such as TP-PA, is required to confirm the positive screen result. If the RPR was positive in the traditional testing algorithm, an endpoint titer would be determined to get a baseline and monitor treatment progress and a treponemal test would be performed to confirm the presence of antibodies to the causative organism, *Treponema pallidum*.

While the traditional algorithm is effective, many labs are now migrating to the reverse algorithm, where the manual nature of RPR/non-treponemal testing as an initial screen is replaced by an automated treponemal assay. In the reverse algorithm, a treponemal test such as an EIA is used as a screen to identify positive samples. Positive samples are then reflexed to a non-treponemal titer to determine if there is an active infection and to get a baseline to monitor treatment progress. If the first treponemal screening test and the non-treponemal test are discordant (i.e., treponemal positive, non-treponemal negative), this could be due to either past infection, or it could be a false positive treponemal screen result. To determine which of these is the case, an additional treponemal test, such as TP-PA, is performed when the non-treponemal test is negative to confirm the first positive screen result. A positive TP-PA result is indicative of a past syphilis infection, whereas a negative TP-PA result indicates a false positive treponemal screening assay result.

Slide 6
There are several advantages of using the reverse algorithm to do syphilis testing. First of all, treponemal IgG screening tests are highly sensitive and specific and have fewer false negatives than non-treponemal assays, presumably due to detection of early primary syphilis. Another huge benefit is that they can be performed on automated platforms, while non-treponemal assays are still manual assays performed on the benchtop. This shift to the reverse algorithm on an automated platform can increase testing volume while reducing labor costs. Treponemal assays are also more effective for diagnosis of secondary, latent, and late syphilis, presumably because treponemal assays are not subject to prozone reactions which have been reported at a low incidence in RPR assays.

Slide 7:
Studies have been done to look at the sensitivity and specificity of an EIA as an initial screening test over an RPR. Castro et al. showed that when the sensitivity and specificity of a treponemal immunoassay (FTA-Abs) was compared to RPR and an EIA, the EIA outperformed RPR in

primary syphilis (100% for EIA vs. 92% sensitivity for RPR), late syphilis (100% for EIA vs. 97.2% sensitivity for RPR), and past treated syphilis (97.9% vs. 57.9% sensitivity for RPR) diagnosis, and had an overall specificity of 100% (compared to 88.8% in RPR) when compared to FTA-Abs. These data show the validity of using an EIA screening method, as is done in the reverse algorithm.

Slide 8:
While the reverse algorithm has its advantages over the traditional algorithm, there are still important points to consider when instituting its use. The first point is that treponemal tests do not distinguish between present and past infection, so a non-treponemal test (e.g. RPR) result is still required to determine whether infection is active. Another point to consider is that in low prevalence disease populations, tests such as EIA may have higher false positive rates. This necessitates reflex to an additional treponemal test (e.g. TP-PA) and a non-treponemal test, which is included in the reverse algorithm.

Slide 9:
In summary, the reverse screening algorithm enables the laboratory to automate high volume syphilis testing and produce highly sensitive and specific results. Diagnosis of secondary, latent, and late syphilis is also more sensitive with the reverse screening algorithm because it screens using a treponemal assay. And finally, while the reverse algorithm has several advantages, the laboratory must explain the reverse algorithm and interpretation of results to healthcare providers before making any changes in order to avoid confusion.

Slide 10: References

Slide 11: Disclosures

Slide 12: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on “Diagnosis of Syphilis Using the Reverse Algorithm.”