SYPHILIS TESTING

RPR AND CONFIRMATORY
What is syphilis and why is it important in this protocol?
Syphilis

- Syphilis is a contagious venereal disease caused by the spirochete Treponema pallidum. The organism enters the body through a break in mucosa or epithelial layer. After a 10-60 day incubation, a painless inflammatory reaction producing a characteristic ulcerated lesion called a chancre usually appears at the site of entry.
These lesions of primary syphilis usually heal spontaneously although the infection persists. Syphilis is usually cured by penicillin, if treated early. If untreated, a generalized skin rash and other abnormalities will begin appearing six weeks to six months following the disappearance of the chancer (secondary stage syphilis).
Again, the clinical symptoms may disappear (latent stage syphilis). The latent syphilis may continue throughout life, it may terminate with spontaneous cure, or it may advance to tertiary syphilis.
Laboratory Diagnosis:

Direct detection of spirochetes:

- **Darkfield microscopy** - Specimen obtained from lesion is evaluated using darkfield microscopy for characteristic corkscrew morphology and flexing motility. Well experienced technician required and non-pathologic morphologically similar organisms must be excluded.

- **Fluorescent Antibody Testing** of specimen - Fluorescent - labeled antibodies bind (direct or indirect methods) with *T. pallidum* organism. Using a fluorescent microscope, specimen evaluated for fluorescence which demonstrates presence of organism. Use of monoclonal antibodies has increased specificity, but subspecies of *T. pallidum* may react. Care must be taken to prevent organisms from washing off slide during preparation.
Laboratory Diagnosis:

Non-specific Reagin tests (includes VDRL and RPR tests). Flocculation and precipitation tests to detect the presence of reagin, an antibody to cardiolipin. Serum specimens may need to be heated to inactivate complement. Appropriately reacting controls and good technique required.
Laboratory Diagnosis:

Specific tests for treponemal antibodies. Requires specific treponemal antigens and direct antibody directed against the *T. pallidum* organism.

- Treponema Pallidum Immobilization Test (TPI) - measures ability of (patient produced) antibody and complement to immobilize live (reagent) treponemes.
Laboratory Diagnosis:

- Fluorescent Treponemal Antibody Absorption Test (FTA-ABS) - an indirect fluorescent antibody test requiring diluted heat-inactivated patient serum. The serum is mixed with non-pathologic Reiter Strain treponemes to remove nonspecific cross-reactive antibodies. The ‘absorbed’ serum is then tested with the Nichols Strain of *T. pallidum*, washed, stained with an antibody conjugate (anti-immunoglobulin with a fluorescein isothiocyanate label) and examined under a fluorescent microscope by an experienced tech. The intensity of the fluorescence is graded 0-4+, with 2+ or greater indicating reactive.
Laboratory Diagnosis:

- Hemagglutination Tests (includes HATTS and MHA-TP) utilize red cells coated with antigens from the Nichols strain of *T. pallidum*. Serum is pre-treated to limit non-specific reactions. Agglutination as indicated by a rough jagged pattern is positive.
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The RPR test utilizes the VDRL cardiolipin antigen modified by the incorporation of choline chloride to inactivate inhibitors (eliminating need for heat treating specimen) and includes charcoal indicator particles to improve reaction visibility (eliminating need to read test microscopically). Antigen supplied comes pre-prepared (an advantage over the VDRL procedure).
Principle: Rapid Plasma Reagin (RPR) test

- Patient sera mixed with a fine particle cardiolipin antigen which has been enhanced with cholesterol, lecithin, and charcoal will result in a macroscopically visible flocculation-type precipitation if the patient’s sera contains reagin - an antibody formed against cardiolipin.
Reagin tests (VDRL & RPR) are considered screening tests. If positive results are obtained, the more specific treponemal testing (FTA-ABS, MHA-TP, etc.) should be performed. WHAT DOES THE PROTOCOL REQUIRE?

Specimens giving any degree of clumping should be subjected to further serological study.
Limitations

- What are some limitations?
Limitations

1. Proper specimen collection, processing and testing procedure must be followed for reliable results.

2. Diseases related to syphilis (yaws, pinta, & nonvenerial endemic syphilis) who’s causative organisms are nearly indistinguishable from *T. pallidum*, can cause positive reactions.
3. **Biological false positives** (BFP), to reagin tests may occur in diseases such as leprosy, malaria, toxoplasmosis, infectious mononucleosis, tuberculosis, lupus erythematosus, and viral pneumonia. The presence of autoimmune or collagen-vascular disease, viral infection or hyperglobulinemia may also produce false positives. IV drug users, pregnant women and the elderly may have false positive reactions.
4. **Negative** serological reactions *may indicate* any of the following:

a. The patient does not have syphilis.
b. The infection is too recent, patient has not produced antibodies.
c. Treatment is underway.
d. Consumption of alcohol prior to testing.
e. The disease is latent, inactive, or patient’s body tolerates the organism.
f. Patient is immunocompromised and unable to respond.
g. Inferior technique.