SERODIAGNOSIS OF SYPHILIS

MODERATOR: DR. Asna Hassan
Nmch patna
**Serological reactions**

- Antigen-Antibody reactions in vitro are known as serological reactions.
- Except during the very early stage of infection, serology remains the mainstay of laboratory testing in syphilis

**Specific antibodies**-
- Produced against *T. pallidum* are IgM (2 weeks) and IgG (4 weeks) antibodies.
- Detected by Treponemal serological procedures

**Nonspecific antibodies**-
- Produced against the protein antigen group common to pathogenic spirochetes and are known as nontreponemal antibodies.
- Detected by nontreponemal serological procedures
Diagnostic Evaluation

• The diagnosis of syphilis is based on the clinical picture in conjunction with demonstration of microorganisms in a lesion and serologic testing.

**Nontreponemal Methods**

• Wasserman test
• Kahn test.
• Venereal Disease Research Laboratory (VDRL)
• Rapid Plasma Reagin (RPR)
• Toluidine Red Unheated serum Test (TRUST)
• Unheated Serum Reagin (USR)
**Treponemal Methods**

- Fluorescent Treponema pallidum-absorbed (FTA-ABS)
- FTA-ABS DS
- Microhemagglutination Treponema pallidum [MHA-TP] or TPHA
- TPPA
- TPI

**Recent advances**  – EIA and Western blot
Nontreponemal tests

• The nontreponemal tests measures anti-lipid antibodies, which are formed by the host in response to lipids released from damaged host cells early in infection with T. pallidum, and lipid-like material form the treponemal cell surface.

• During syphilis infection, an antibody-like substance called reagin can be detected in the patient’s serum or CSF.

• Antigen for test is a nontreponemal antigen composed of cardiolipin, cholesterol and lecithin
## Basis of non treponemal tests

<table>
<thead>
<tr>
<th>Capture system</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes in suspension producing visible flocculation with lipoidal antibodies</td>
<td>VDRL</td>
</tr>
<tr>
<td>Liposomes in suspension + unattached charcoal particles producing dark coloured flocculation due to trapping of charcoal particles in lattice formed by antigen-antibody complex</td>
<td>RPR</td>
</tr>
<tr>
<td>VDRL antigen coated onto wells of microtitre plates and attached antibody detected by enzyme immunoasssay</td>
<td>EIA (Reagin)</td>
</tr>
<tr>
<td>VDRL antigen coated onto well of microtitre plates; attached antibody detected by anti-IgG plus anti-IgM-coated indicator red blood cells</td>
<td>SPEA (solid phase erythrocyte adherence)</td>
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</table>
Nontreponemal Methods-

• Reactive non treponemal tests confirm the diagnosis in the presence of early or late lesion of syphilis, offer a clue in latent subclinical syphilis and used for assessing response to therapy.

• Non treponemal antigen tests *are not entirely specific for syphilis* and do not have satisfactory sensitivity in all stages of syphilis.

• Whenever the results of a non treponemal antigen test disagree with the clinical impression, a treponemal antigen test such as the FTA-ABS should be performed.
Wassermann reaction

• Wasserman (1906) first employed the complement fixation technique for the diagnosis of syphilis.
• He used extracts of liver containing treponemes from infants who had died from early congenital syphilis.
• It was later realized that the test also gave positive results when aqueous or alcoholic extracts of normal liver tissue were used as antigens.
PLATE III

Complement Fixation Test

- Serum with antibodies
- Antigen binds with antibodies
- Unbound Antigen

- Serum without antibodies
- Unbound complement

- Complement binds with Ag/Ab complex
- Hemolysin
- Sensitized red blood cells serve as an indicator
- Hamolysis
- Sensitized RBCs serve as an indicator

- Reactive
  - RBCs settle into a pellet
  - no lysis

- Nonreactive
  - RBCs lysed by unbound complement
  - lysis
THE KAHN PRECIPITATION TEST FOR SYPHILIS

TWO YEARS' EXPERIENCE IN A PUBLIC HEALTH LABORATORY

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Director, Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan

Read before the Laboratory Section of the American Public Health Association at the Fifty-second Annual Meeting, Boston, Massachusetts, October 10, 1923.

A YEAR ago I discussed before this section the development of the Kahn precipitation test in the laboratories of the Michigan Department of Health and the value of this test as a check on the Wassermann. Our results at that time were based on about 23,000 examinations and we concluded that this test, run parallel with the Wassermann test, gave a more correct laboratory diagnosis of syphilis than the Wassermann alone was capable of giving. We emphasized at that time the responsibility of public health laboratories in giving correct serum diagnosis of syphilis for the reason that such laboratories are usually removed from both the physician and patient and are therefore not able to check up the clinical status in each case, as is true in hospital laboratories. Finally, we the Kahn precipitation test is employed as a routine procedure parallel with the Wassermann also at the Missouri State Board of Health (Dulaney); the Duemling Clinic, Fort Wayne, Indiana (Grant); the Wassermann laboratory of the Washington University, St. Louis (Holmes); and the Toronto General Hospital (Detweiler). Good results have also been obtained with this test by Anderson and Fisher, Fox and Sanderson, Elliott and Todd, and by Babonneix, Boucher and Choay.

The limitations of the procedure discussed last year have recently been summed up by Kahn as follows: the necessity for overnight incubation of a large number of weaker positive reactions and the occasional loss of a positive reaction in cases of unquestionable syphilis.
**VDRL**

- VDRL is a slide flocculation test
- Since the antigen used is cardiolipin which is a lipoid extract from beef heart so it is not a specific test

**VDRL SLIDE** is a glass slide measuring 2 X 3 inch with 12 concave depressions, each measuring 16 mm in diameter and 1.75 mm deep

- Patient’s serum which is first heated at 56\(^\circ\) for 30 min and then cooled to remove non specific inhibitors such as complement.

- CSF sample does not require heating
Procedure

• Wells should be labeled R, WR, NR for reactive, weakly reactive, and nonreactive, respectively.
• Pipette .05ml of specimen into one concavity of an agglutination slide.

• Add one drop (.01 ml) of sensitized antigen suspension to each specimen with a 21 or 22 gauge needle.
• Rotate slides for 8 minutes on a mechanical rotator at 180 rpm.

• Read test immediately after rotation with a 10x objective.
Results for Serum Specimen

Qualitative Testing
Reactive
Weakly Reactive
Nonreactive.

• Verify control sera results for expectation. If reactions are not as expected, the test is invalid and results can not be reported.

Quantitative Testing - Report the titer as the highest dilution that produces a Reactive (not weakly reactive) results.

Titer ≥ 1:8 is taken as positive in a patient with no previous history of syphilis

If history of syphilis is present, then the criteria should be a four fold rise in titer.
Results for CSF Specimen

Qualitative Testing –
• No clumping or very slight roughness (Nonreactive);
• Definite clumps (Reactive)

Quantitative Testing –
Report the titer in terms of the highest dilution that produces a reactive result.
Interpretation

• **Nonreactive VDRL** - with clinical evidence may indicate early primary syphilis, a prozone reaction in secondary syphilis or late syphilis.

• **Nonreactive VDRL** - with no clinical evidence may indicate no current infection or an effectively treated infection.

• **Quantitative VDRL** - detects changes in reagin titer. Serum samples displaying a fourfold increase in titer on a repeated sample may indicate an infection, reinfection or treatment failure. A fourfold decrease during treatment indicates adequate therapy.
False Negative Reactions

• Technical error - unsatisfactory antigen or technique.
• Low antibody titers
• Presence of inhibitors in the patient’s serum
• Reduced ambient temperature (below $23^0$ to $29^0$)
• Prozone reaction
<table>
<thead>
<tr>
<th>Acute (&lt;6 months)</th>
<th>Chronic (&gt;6 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physiological state:</strong></td>
<td><strong>Physiological state:</strong></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Old age</td>
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<tr>
<td><strong>Infection:</strong></td>
<td><strong>Chronic infection:</strong></td>
</tr>
<tr>
<td><em>Bacterial infection</em>&lt;br&gt;Pneumonococal pneumonia&lt;br&gt;Scarlet fever&lt;br&gt;Infective endocarditis</td>
<td><em>Mycobacterial infection</em>&lt;br&gt;Tuberculosis&lt;br&gt;Leprosy</td>
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<tr>
<td><em>Mycobacterial infection</em>&lt;br&gt;Tuberculosis&lt;br&gt;Leprosy</td>
<td><em>Others</em>&lt;br&gt;Infective endocarditis&lt;br&gt;Malaria</td>
</tr>
<tr>
<td><em>Other STI</em>&lt;br&gt;Chancroid&lt;br&gt;Lymphogranuloma venerum</td>
<td>Connective tissue disease:&lt;br&gt;SLE</td>
</tr>
<tr>
<td><em>Other spirochaetal infection</em>&lt;br&gt;Relapsing fever&lt;br&gt;Leptospirosis</td>
<td>Malignancy:&lt;br&gt;Myeloma</td>
</tr>
<tr>
<td><strong>Viral infection:</strong></td>
<td>Injection drug user&lt;br&gt;Multiple transfusion</td>
</tr>
<tr>
<td>HIV infection&lt;br&gt;Infectious mononucleosis&lt;br&gt;Measles&lt;br&gt;Mumps&lt;br&gt;Chickenpox&lt;br&gt;Viral hepatitis</td>
<td></td>
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**RPR Test**

- The RPR Card antigen suspension is a carbon particle cardiolipin antigen that detects reagin which is an antibody like substance present in serum or plasma from individuals with syphilis.

- The reagin binds to the test antigen which consists of cardiolipin-lecithin coated particles that cause **macroscopic flocculation**.

- When a specimen such as serum or plasma contains antibody, flocculation occurs with the resulting aggregation of the carbon particles.
• The flocculation appears as black clumps against the white background of the plastic coated card.

• The RPR test uses a white plastic coated card that consist of several circles that are 18 mm in diameter.

• The controls which are strongly reactive, moderately reactive, and non-reactive are contained on the control card in a dried form.
**Procedure**

- Label rings on test card with numbers of samples to be tested

- Put 1 drop of serum sample to fall on to each circle

- Reconstitute the antigen bottle, by shaking and place one drop on each test area.

- Rotate card for 8 minutes on a mechanical rotator at 100 rpm and then rotated manually

- Immediately read macroscopically in the “wet” state under a high intensity lamp.
The following reactions should be observed to compare against the test results:

- **Reactive control** - characteristic strong clumping.
- **Reactive moderate control** - moderate clumping.
- **Non-reactive control** - smooth, grayish appearance of unclumped particles.
Results

• A **negative** RPR test may indicate one of the following:

1. The patient does not have syphilis.
2. The infection is too recent for antibodies to be produced.
3. The syphilis is latent or inactive
4. Faulty lab techniques

• A **positive** reaction is not conclusive for syphilis. Several conditions produce biologic false positive results for syphilis
<table>
<thead>
<tr>
<th><strong>VDRL</strong></th>
<th><strong>RPR</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Read under 10x microscopically</td>
<td>Results visible to naked eye</td>
</tr>
<tr>
<td>Done with inactivated serum</td>
<td>Can be done with unheated serum or plasma</td>
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<tr>
<td>Suitable for CSF</td>
<td>Not suitable for CSF</td>
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</tbody>
</table>
# Treponemal Tests

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Capture system</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact treponemes</td>
<td>Treponemes fixed onto microscope slides</td>
<td>FTA-ABS</td>
</tr>
<tr>
<td>Purified and sonicated</td>
<td>Attached to red blood cells</td>
<td>TPHA</td>
</tr>
<tr>
<td>treponemes</td>
<td>Attached to gelatin particles</td>
<td>TPPA</td>
</tr>
<tr>
<td></td>
<td>Attached to microtitre Plates</td>
<td>EIA</td>
</tr>
<tr>
<td></td>
<td>Proteins separated by PAGE and transferred to filters</td>
<td>Immunoblots</td>
</tr>
<tr>
<td></td>
<td>by Western blotting</td>
<td></td>
</tr>
<tr>
<td>Recombinant antigens</td>
<td>Attached to microtitre plates</td>
<td>Recombinant EIA</td>
</tr>
<tr>
<td></td>
<td>Attached to latex particles</td>
<td>Latex agglutination</td>
</tr>
</tbody>
</table>
TREPONEMAL TESTS-

**TPI- Treponema Pallidum Immobilization test**

- This test determines the ability of patients serum to immobilise motile virulent T. Pallidum.
- The test serum is incubated anaerobically with a suspension of treponemes and complement.
- If antibodies are present the treponemes will be found to be immobilised, when examined under dark field microscope.
- The test is found to be reactive if more than 50% of treponemes are immobilised and non reactive if less than 20%.
- Expensive, time consuming and technically difficult.
Fluorescent treponemal antibody absorption test (FTA-ABS)

• The FTA test is an indirect fluorescent antibody technique.

• In this procedure, the antigen used is *T. pallidum subsp. pallidum* (*Nichols strain*).

• The patient’s serum is first diluted 1:5 in sorbent to remove group treponemal antibodies that are produced in some persons in response to nonpathogenic treponemes.

• Next, the serum is layered on a microscope slide to which *T. Pallidum* has been fixed. If the patient’s serum contains antibody, it coats the treponeme.
• FITC-labeled anti-human immunoglobulin is added and combines with the patient’s antibodies adhering to *T. pallidum*, resulting in FITC-stained spirochetes that are visible when examined by a fluorescence microscope.

• The non specific reactions of the original FTA test are found to arise because of shared antigens common with non pathogenic spirochaete. These are removed by absorption and the improved test is called as FTA-ABS.

• A modification of the standard FTA-ABS test is the FTA-ABS double-staining test used with incident light microscopes, which is the standard treponemal test.
<table>
<thead>
<tr>
<th>Table 2. Documented false positive FTA-ABS serologic tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic infection:</strong></td>
</tr>
<tr>
<td>- Leprosy</td>
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<tr>
<td><strong>Connective tissue disease:</strong></td>
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<tr>
<td>- SLE</td>
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</table>
FTA-ABS double-staining technique

• This technique uses a tetra methylrhodamine isothiocyanate-labeled, anti-human IgG & a counterstain with FITC-labeled anti-T. pallidum conjugate.

• The counterstain was developed to eliminate the need to locate the treponemes by dark-field examination when the patient’s serum did not contain antibodies to T. pallidum.

• Therefore, counterstaining the organism ensures that the nonreactive result is due to the absence of antibodies and not to the absence of treponemes on the slide when it is read with incident illumination.

• Results of both FTA-ABS tests are reported as reactive, reactive minimal, nonreactive, or atypical fluorescence observed.
Treponema pallidum haemagglutination (TPHA)

- Indirect agglutination tests such as the Treponema pallidum haemagglutination (TPHA) test are widely used as they are economical, quick and easy to perform.

- In the TPHA test, erythrocytes sensitized with sonicated *T. pallidum* react with *treponemal antibodies in the serum to form a smooth mat of agglutinated cells in the well of a microtitre plate.

- If antibodies are not present, the cells do not agglutinate and settle to the bottom of the well in a characteristic button shape.
• Many kits are commercially available; the major difference between them being the type of erythrocyte used as antigen carrier.

• The TPHA test is highly sensitive in all stages of the disease except possibly in early primary syphilis.

• It also has very high specificity with as few as 0.07% false positives.

• These false positives have been reported in some patients with infectious mononucleosis, connective tissue diseases, leprosy and with intravenous drug use.
Treponema pallidum agglutination test (TPPA)

- The most recent modification is the use of gelatin or polymer particles, rather than erythrocytes, as carrier for the *T. pallidum antigen*.

- The use of gelatin particles has almost eliminated non-specific agglutination reactions and it is claimed that the sensitivity of the test in primary syphilis has increased due to the improved IgM binding capacity of the sensitized gel particles.

- As a consequence, many laboratories have replaced the TPHA with the TPPA.
Enzyme Immuno Assays

• Recent studies suggest that certain new recombinant antigen-based EIAs are now among the most sensitive and specific treponemal tests.

• Serum is added to a microwell coated with a treponemal antigen.

• After incubation, an enzyme labelled antihuman immunoglobulin conjugate and enzyme substrate are added to detect antigen antibody reaction.

• The test can be modified to detect specific IgM antibody.
**Immunoblotting**

- Immunoblotting allows for the detection of antibodies to individual proteins.

- It is generally agreed that detection of antibodies to immunodeterminants with molecular masses of 15, 17, 44.5 and 47kDa are diagnostic for acquired syphilis.

- Studies suggest that the assay (using IgG conjugate) is more sensitive and specific than the FTA-ABS test.
Treponemal tests for specific IgM

19S (IgM) FTA-ABS test

- Early serological tests for the diagnosis of congenital syphilis lacked both sensitivity and specificity due to interference from high levels of maternal IgG and from rheumatoid factor produced by the fetus in response to maternal IgG.

- The first test to largely overcome these problems was the 19S (IgM) FTA-ABS test.

- This test is performed on fractionated serum using the FTA-ABS technique with conjugated antibody specific for IgM.
**IgM capture EIA**

- IgM EIA assays are mainly used to assist in the diagnosis of congenital syphilis.

- In symptomatic congenital syphilis the IgM capture EIA has a sensitivity of between 88-92% and a specificity of 95%.

- The assay may also be useful for differentiating past infection from current or recent infection and for detection of early syphilis.

- The sensitivity of the test is 93% in primary syphilis, decreasing as the disease progresses.
POLYMERASE CHAIN REACTION

- Most of the PCR are based on the membrane lipoproteins.

- Amplification of a 658-bp segment of the gene encoding 47 kDa surface antigen is done.

- PCR is extremely valuable in congenital syphilis, neurosyphilis, early primary syphilis, syphilis in HIV patients and distinguishing new from old infections.

- However it is very costly and technically demanding
TESTING STRATEGY:

• The screening test should have high sensitivity and the confirmatory test should have high specificity and should be methodologically independent.

• Serology cannot distinguish between the different treponematoses.

• The testing strategy varies by using either a non-treponemal or a treponemal test or both in combination, depending on a number of factors.

• In India, USA and some European countries non treponemal tests are used for screening whereas a combination (VDRL and TPHA) is used in UK.
• If VDRL is used as a screening test then it does not detect most adequately treated cases, thus simplifying patients assessment.

• The disadvantage is that it can give false negative results due to prozone phenomenon, false positive results and they lack sensitivity in late stage infection.

• A combination of VDRL and TPHA provides sensitive and specific screening for all stages of syphilis except very early stage.

• However it is more expensive, labourous and requires subjective interpretation.
• EIA is being increasingly used as a screening test in place of combination because it gives comparable result to the VDRL-TPHA combination and also useful in patients with HIV infection.

• FTA-ABS is considered as a gold standard but due to its limitations TPHA is preferred.

• If TPHA is used as a screening test then EIA can be used as a confirmatory test.
Assessment of stage of infection

• Quantitative non treponemal test – VDRL in dilution

• Antitreponemal IgM

IgM becomes undetectable within 3-9 months after adequate treatment of early syphilis. It may persist for 1-1.5 yrs after treatment of later disease.

• Reinfection-A four-fold or greater rise in VDRL or RPR titre is indicative of reinfection and response to treatment is much slower in patients with reinfections.
Monitoring effect of therapy

• Quantitative non treponemal test (VDRL) remain the method of choice.

• Done at 3 month interval for minimum of 1 year.

• There should be at least 4 fold decline in titre by 3rd or 4th month and an eightfold decline in 6-8 months.

• 95% of patients with early latent syphilis become seronegative by two years and all within four years.

• Failure to decline after adequate treatment occurs in Late stage or Latent stage of syphilis, HIV patients and in patients treated for reinfection.
• Following treatment at birth for congenital syphilis, the nontreponemal tests should become nonreactive within 3-6 months.

• Infants treated later may require a year for the nontreponemal tests to become seronegative. The treponemal tests will remain reactive.

• It may take years for the VDRL-CSF to become non-reactive.
Sero Diagnosis Of Different Stages Of Syphilis-

PRIMARY SYPHILIS-

• Dark field microscopic examination of fluid obtained from the surface of chancre is the most specific and sensitive for diagnosing primary syphilis.

• Non treponemal test are positive in about 80% of patients at the time of patient’s visit.

• However it must be done in all patients for providing baseline for follow-up after therapy.
• Treponemal tests also have similar sensitivity, thus a negative result does not rule out the diagnosis.

• Once treponemal tests are positive due to disease they remain so for rest of life. Thus a positive result does not establish the diagnosis.
Secondary syphilis -

• Treponemes can be found by dark field microscopy in wet skin lesions of secondary syphilis.

• Serological tests gives distinctive results as antibody to cardiolipin are present in all patients of secondary syphilis in high titre.

• Prozone phenomenon may occur.

• Trponemal tests are always positive
Latent syphilis

- Reactive non treponemal and treponemal test in the absence of any apparent signs of the disease.

- It may indicate partially treated syphilis, asymptomatic neurosyphilis or adequately treated syphilis in which the serological abnormalities persist.

- Hence history is very important and lumbar puncture should be done.
Late Syphilis

- Sensitivity of VDRL and RPR test are low in late syphilis whereas the sensitivity of specific tests are more than 90%.

NEUROSYPHILIS-
- The serum tests are positive in most of the cases.
- CSF-VDRL is highly specific (99.8%) but it is non reactive in 50% of cases particularly in asymptomatic neurosyphilis, isolated 8th nerve involvement and in tabes dorsalis.
- Positive in all patients of paresis.
- CSF changes
• Treponemal tests in CSF are more sensitive but less specific and if non reactive they rule out neurosyphilis.

• False positive may occur due to diffusion of IgG across blood/CSF barrier or contamination from blood

• CSF IgG index = \frac{\text{CSF to serum IgG ratio}}{\text{CSF to serum albumin ratio}}

• More than 0.7 indicate synthesis in CSF

• T. Pallidum antibody index >100

\[
\text{CSF TPHA Titres} \quad \frac{\text{CSF albumin } \times 1000}{\text{Serum albumin}}
\]
<table>
<thead>
<tr>
<th>TEST</th>
<th>PRIMARY</th>
<th>SECONDARY</th>
<th>LATENT</th>
<th>LATE</th>
<th>% SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDRL</td>
<td>78</td>
<td>100</td>
<td>95</td>
<td>71</td>
<td>98</td>
</tr>
<tr>
<td>RPR</td>
<td>86</td>
<td>100</td>
<td>98</td>
<td>73</td>
<td>98</td>
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<tr>
<td>TEST</td>
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<td>FTA-ABS</td>
<td>84</td>
<td>100</td>
<td>100</td>
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<tr>
<td>MHA-TP</td>
<td>76</td>
<td>100</td>
<td>97</td>
<td>94</td>
<td>99</td>
</tr>
<tr>
<td>FTA-ABS DS</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>99</td>
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</table>
**Pregnancy**

- Optimally pregnant women should be screened for syphilis at their first prenatal visit, during third trimester, and at the time of delivery with a nontreponemal test (VDRL or RPR).

- A confirmatory FTA-ABS test should be performed if VDRL or RPR is positive near delivery.
• For mothers with:
  1. Positive VDRL or RPR at the time of delivery and/or
  2. A history of syphilis which is untreated, and/or
  3. A treatment history which cannot be documented.

The infant should receive an evaluation for clinical signs and symptoms of syphilis.
• This evaluation must include a nontreponemal serological test performed on infant serum, not cord blood.

• Titers may increase slightly in serofast women who were previously treated for syphilis become pregnant. It is generally less than fourfold increase.

• Serological response to treatment is similar to that of non-pregnant women.
**Congenital syphilis**

- Irrespective of intrauterine infection, the infant’s serological results will be similar to those of the mother.
- A lower RPR or VDRL titre in the infant’s serum than in the mother’s does not exclude congenital infection.
- Demonstration of anti-treponemal IgM in the blood of neonates is considered presumptive for the diagnosis of congenital syphilis.
- Serum from the neonate is the preferred specimen.
- A negative IgM result, however, does not exclude a diagnosis of congenital syphilis.
• Demonstration of treponema pallidum by direct examination from the nasal discharge or from early lesions of congenital syphilis will confirm the diagnosis.

• A positive non treponemal test in a titre higher than the mother or a rising titre in serial monthly tests suggests a perinatal infection & the infant should be treated.

• Blood taken from the umbilical cord or even the infant may give positive results due to the presence of maternal antibodies in fetal circulation.
• An active infection can be ruled out in these cases from an FTA-ABS test using fluorescent labelled IgM conjugate.

• New diagnostic techniques include western blot supplementing FTA-ABS IgM tests on serum & PCR tests on cerebrospinal fluid.

• Pathological examination of the placenta or umbilical cord using specific fluorescent anti treponemal staining is suggested.
• False negatives may be caused by:

1. The mother being infected late in pregnancy
2. Suppression of IgM synthesis in the neonate because of high levels of maternal IgG, or
3. The immunological system not being sufficiently developed at birth
• If the specific IgM test is negative at birth, it is therefore advisable to repeat the test at monthly intervals for the first three months to ensure there is no late production of IgM.

• If the infant is not treated, the diagnosis is also confirmed by failure of the infant’s RPR or VDRL to become non-reactive in the first 3-6 months of life.

• In practice, however, the diagnosis of congenital syphilis is usually made on the basis of maternal serology and whether or not the mother has been adequately treated during pregnancy.
**Syphilis and HIV infection**

1. Confusing clinical signs and symptoms.
2. Rapid progression of disease and neurological involvement.
3. Lack of serological response in a patient with active syphilis.
4. Failure of non-treponemal test titres to decline after treatment with standard regimens.
5. Unusually high titres in non-treponemal tests.
6. Disappearance of treponemal test reactivity over time.
7. Biological false positive reactions for VDRL and RPR.
<table>
<thead>
<tr>
<th>Category</th>
<th>VDRL</th>
<th>FTA-ABS</th>
<th>TPPA</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Non-Reactive</td>
<td>Non-Reactive</td>
<td>Non-Reactive</td>
<td>Non-Reactive</td>
<td>Note - this test cannot detect primary syphilis in the early stages of disease. Suggest retesting if necessary.</td>
</tr>
<tr>
<td>B. Non-Reactive</td>
<td>Weakly Reactive</td>
<td>Non-Reactive</td>
<td>Non-Reactive</td>
<td>Weakly reactive VDRL with negative FTA-ABS. Possibly a cross reaction or early disease. If clinically indicated retest in 14-21 days. False positive tests are seen in certain acute or chronic infections, following immunization, in autoimmune diseases, pregnancy, tuberculosis, hepatitis, Lyme disease, rheumatoid arthritis, drug addiction, cross reaction with other treponemes and other conditions.</td>
</tr>
<tr>
<td>C. Weakly reactive VDRL and Reactive FTA with Negative TPPA</td>
<td>Weakly Reactive</td>
<td>Reactive</td>
<td>Non-Reactive</td>
<td>Weakly reactive VDRL, reactive FTA, and non-reactive TPPA. Possibly a cross reaction or early disease. Consider results in view of clinical history. If clinically indicated retest in 14-21 days. False positive tests are seen in certain acute or chronic infections, following immunization, in autoimmune diseases, pregnancy, tuberculosis, hepatitis, Lyme disease, rheumatoid arthritis, drug addiction, cross reaction with other treponemes and other conditions.</td>
</tr>
<tr>
<td>D. Reactive (WR VDRL)</td>
<td>Weakly Reactive</td>
<td>Reactive</td>
<td>Reactive</td>
<td></td>
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<tr>
<td>-----------------------</td>
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</tr>
<tr>
<td><strong>in the TPPA and FTA with sera weakly reactive in the VDRL screening test suggests a current or past infection. However, false positive results have rarely been observed.</strong></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E. Reactive</th>
<th>Reactive</th>
<th>Reactive</th>
<th>Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>in the TPPA, FTA, and VDRL tests suggests a current or past infection with syphilis. However, false positive results have rarely been observed. False positive tests are seen in certain acute or chronic infections, following immunization, in autoimmune diseases, pregnancy, tuberculosis, hepatitis, Lyme disease, rheumatoid arthritis, drug addiction, cross reaction with other treponemes and other conditions.</strong></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F. Non – Reactive (reactive VDRL not confirmed by FTA and TPPA)</th>
<th>Reactive</th>
<th>Non-Reactive</th>
<th>Non-Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-reactive in the TPPA and FTA with sera that are reactive in VDRL test should be considered a qualitative false positive and does not indicate syphilis. False positive tests are seen in certain acute or chronic infections, following immunization, in autoimmune diseases, pregnancy, tuberculosis, hepatitis, Lyme disease, rheumatoid arthritis, drug addiction, cross reaction with other treponemes and other conditions.</strong></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>G. Non – Reactive (probable False Positive FTA)</th>
<th>Non-Reactive</th>
<th>Reactive</th>
<th>Non-Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VDRL and TPPA with a reactive FTA suggests possible reactive FTA, consider repeat testing in 14-21 days. False positive tests are seen in certain acute or chronic infections, following immunization, in autoimmune diseases, pregnancy, tuberculosis, hepatitis, Lyme disease, rheumatoid arthritis, drug addiction, cross reaction with other treponemes and other conditions.</strong></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Non-Reactive</td>
<td>Reactive</td>
<td>Reactive</td>
</tr>
<tr>
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</tr>
<tr>
<td><strong>H. Reactive</strong></td>
<td></td>
<td></td>
<td><strong>Positive</strong> VDRL test usually becomes negative, whereas TPPA and FTA usually remain positive for a lifetime.</td>
</tr>
<tr>
<td><strong>I. Reactive</strong></td>
<td>Not Available</td>
<td>Reactive</td>
<td>Reactive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VDRL result not available for interpretation. Reactive in the TPPA and FTA test suggests a past infection. However, false positive results have rarely been observed. False positive tests are seen in certain acute or chronic infections, following immunization, in autoimmune diseases, pregnancy, tuberculosis, hepatitis, Lyme disease, rheumatoid arthritis, drug addiction, cross reaction with other treponemes and other conditions.</td>
</tr>
</tbody>
</table>
Thank you