Syphilis
Introduction

Caused by *Treponema pallidum*

Transmission: Sexual; maternal-fetal, and rarely by other means

Syphilis increases the risk of both transmitting and getting infected with HIV

Preform HIV testing in all patients with syphilis
Laboratory Diagnosis

Identification of *Treponema pallidum* in lesions

- Darkfield microscopy
- Direct fluorescent antibodies - *T. pallidum* (DFA-TP)

Serology tests

- Nontreponemal tests
- Treponemal tests
The Nontreponemal tests, Venereal Disease Research Laboratory test (VDRL) and Rapid Plasma Reagin (RPR) should have the result confirmed by specific treponemal testing

1. Fluorescent treponemal antibody absorption test (FTA-ABS)

2. EIA.
Test to Confirm

Syphilis may be confirmed either via blood tests or direct visualization using microscopy. Typical diagnosis is with blood tests using nontreponemal and/or treponemal test.

Nontreponemal test are used initially and include VDRL and RPR however as these test occasionally are falsely positive confirmation is required with a treponemal test such as treponemal pallidum particle agglutination (TPHA) or fluorescent treponemal absorption test (FTA-ABS).
The VDRL is a confirmatory serological microflocculation slide test used for the detection of syphilis antibodies. In a VDRL procedure, the patient’s serum is heat-inactivated and mixed with a buffered saline suspension or VDRL antigen containing cardiolipin, lecithin and cholesterol that binds with Reagin, an antibody-like protein. A combination of Reagin and VDRL Antigen form microscopic clumping called flocculation. The basis of the test is that an antibody produced by a patient with syphilis reacts with an extract of ox heart. It therefore visualizes through foaming of the test tube fluid, or Flocculation.
RPR test uses the same antigen as the VDRL, but in that test it has been bound to several other molecules including a carbon particle to allow visualization of the flocculation reaction without the need of a microscope.
FTA-Abs (Fluorescent Treponemal Antibody absorption test)

FTA-Abs is a treponemal test for syphilis. Using antibodies specific for the treponemal pallidum species, such tests are more specific than non-treponemal testing such as VDRL.

FTA-Abs turns positive earlier and remains positive longer than VDRL.

FTA-Abs should always be followed to confirm a positive RPR and/or VDRL test for syphilis.
The FTA-ABS blood test (fluorescent treponemal antibody absorption test) is used to detect the presence of antibodies that react to the bacteria *Treponema pallidum*.

A normal test result will give a **negative** reading for the presence of antibodies. This means that the person is not currently infected with syphilis, and he has not been previously infected with this disease.

If the test result is **positive**, the patient has contracted a syphilis infection. The test result will always be positive even if he has previously been diagnosed with syphilis and it has been successfully treated.

For this reason, the FTA-ABS test cannot be used to monitor the effectiveness of syphilis treatments.
Treponemal pallidum particle Hemagglutination assay (TPHA test)

is an indirect agglutination assay used for detection and titration of antibodies against the causative agent of syphilis, *Treponema pallidum*

In the test, gelatin particles are sensitized with *T. pallidum* antigen. Patient serum is mixed with the reagent containing the sensitized gelatin particles. The particles aggregate to form clumps when the patient serum is positive for syphilis. In other words, the patient's serum contains antibodies to *T. pallidum*.

A negative test shows no clumping of gelatin particles. This is a type of specific treponemal test for syphilis.
One drop (75 µl) of test cells are added to wells 2-8 in each row.
Chlamydia

- Gram-negative cocci
- Obligate intracellular coccoid parasites
- Small, non motile, Gram negative.
- Contain DNA and RNA, and ribosomes
- Lack ATP, biosynthetic pathways
Order – Chlamydiales
Family – Chlamydiaceae (only one family)

4 species in the Genus Chlamydia

C. trachomatis
C. pneumoniae
C. psittaci

C. pecorum

Affects human
Affects ruminants
C trachomatis

- Trachoma
- conjunctivitis
- proctitis
- urethritis
- salpingitis
- Lymphogranuloma venereum

C psittaci & C pneumoniae

- Upper respiratory infection
- Bronchitis
- Pneumonia
Laboratory diagnosis

4 approaches available:

1. Microscopic demonstration of inclusion or elementary bodies.
2. Isolation of Chlamydia
3. Demonstration of Chlamydial Antigens
4. Demonstration of antibodies or hypersensitivity
- Gram stain - gram negative
- Stain better with Giemsa Stain.
- Inclusion bodies are basophilic and present in cytoplasm.
- Inclusion bodies can be stained with Logol’s iodine.
- Immunofluorescence staining.
2. Culture

Animal inoculation
Yolk sac of 6 – 8 days old chick embryo.
Tissue culture
Mycoplasma

* Smallest free living microorganisms.
* Can pass through bacterial filters.
* Pleomorphic.
* May present as small spherical or branching filaments.
* Lack cell wall but have triple layered cell membrane rich in cholestrol and other lipids.
* Gram negative.
  - Better stained by Giemsa stain.
  - Non sporing, Non-flagellated

* Normal flora of upper respiratory tract
Laboratory Diagnosis

Specimens
- Throat swab
- Respiratory secretions
- Genital secretions

Microscopy
- Highly pleomorphic, Varying from small spherical shapes to longer branching filaments
- Gram negative, but better stained with Giemsa
Isolation of Mycoplasma (Culture)

1. Semi solid enriched medium containing 20% horse or human serum, yeast extract and DNA.

2. Incubate aerobically for 7 – 12 days with 5 – 10% CO₂ at 35 – 37 °C.

3. Typical fried egg appearance of colonies, central opaque granular area of growth extending into depth of the medium, surrounded by a flat, translucent peripheral zone. Colonies best seen with a hand lens after staining with Dienes method.
4. Produce beta hemolytic colonies, can agglutinate guinea pig erythrocytes.

Other investigation test

PCR and immunohistochemistry
1. Obligate intracellular pathogens.
2. Cause spotted fevers and typhus in human.
3. All species are transmitted to human via arthropod vectors.
4. Small gram negative bacteria.
5. Slim layer around intracellular growth.
7. Can produce their own ATP but in limited amount and will utilize host’s ATP and NAD.
Diagnosis

* Use immunofluorescent antibodies to examine a biopsy

* The organism can be inoculated into tissue culture and grown over 4-7 days but this is very hazardous to personnel.