SPIROCHETES

Treponema pallidum:

- Morphology:
  - It is a gram-negative bacteria.
  - It is about 10µm long and 0.1-0.2µm wide.
  - It is actively motile, showing rotation round the axis, backward and forward movements and flexion of the whole body.

  - Covering of T.pallidum:
    - ✓ Cytoplasm covered by a trilaminar cytoplasmic membrane.
    - ✓ It is enclosed by a cell wall containing peptidoglycan.
    - ✓ Over which is an outer membrane layer rich in lipid.
    - ✓ Now between the outer membrane layer and the inner cell wall containing peptidoglycan , three of four endoflagella is present.

- Pathogenesis:
  - Infection with T.pallidum occurs only in human beings.
  - The bacteria enters the body through minute abrasions on the skin or mucosa.
  - In acquired syphilis; the treponema enters the body by sexual contact or because of contaminated needles.
  - Clinical disease sets in after an incubation period of about a month.
  - There are three clinical stages of the disease in an untreated case- **primary**, **secondary and tertiary.**

    1. **Primary syphilis(localised stage):**
       - ✓ Classical symptom is the presence of a **hard chancre**. It is painless, relatively avascular, indurated and a circumscribed lesion.
       - ✓ The chancre appears on the genital area.
       - ✓ These chancre are rich in spirochetes and they heal on their own. within 10-40 days.
       - ✓ The spirochetes found in the chancre move to nearby lymph nodes resulting in swollen, discrete, non-tender and rubbery lymph nodes.

    2. **Secondary syphilis (Disseminated stage):**
       - ✓ After healing off primary lesion, the patient remains asymptomatic for 2 to 6 months, then secondary syphilis sets in.

- ✓ At this stage, the spirochetes enter into the blood stream.
- ✓ There is widespread multiplication of treponemes inblood, because of which the patient is most infectious during the secondary stage.
- ✓ Also, the mucous membrane and skin which contains numerous treponemes there is presence of macular rashes.
- ✓ The rash may coalesce together in intertriginous area especially in the perianal region, producing wart like condylamata.
- ✓ The oral lesions called 'mucous patches' are mainly seen on the tongue, buccal mucosa or gingiva.
- ✓ They are usually painless, greyish white plaques overlying an ulcerated surface.
- ✓ These lesions are highly infectious as they contain a large number of microorganism.

3. **Tertiary Stage:**
   - ✓ It contains few spirochetes.
   - ✓ These spirochetes are present in the capillaries and the organs produce severe immune response.
   - ✓ This may result in cardiovascular lesion including aneurysms, chronic granulomata and meningovascular lesion.

- Culture:
  - Pathogenic treponemes (T.pallidum) cannot produce growth in artificial culture media but are maintained by subculture in susceptive animals.
  - Nichol's strain is a virulent T,palidum strain.
  - It is maintained in rabbit testes for several decades by serial testicular passage.
  - Cultivable treponemes such as T.phagedenis(Reiter's reponemes) and T.refringans(Noquchi strain) are non pathogenic.

- Laboratory Diagnosis:
  - It does not take ordinary bacterial strains because of the thinness of spirals.
  - Moreover because of its thinness, it cannot be seen under light microscope.
  - The diagnosis of syphilis consists of demonstration of treponemes and detection of antibodies by serological tests.
  - Specimens include collection of exudates from the lesion for direct demonstration of treponemes and serum for serological tests.

i. Demonstration of Treponemes:
   - ✓ Applicable in primary and secondary stage and in cases of congenital syphilis with superficial lesions.
   a. **Dark-ground microscopy**:

- Treponema pallidum, appears as a slender, spiral organism showing rotational as well as flexion and extrusion movement.
- A treponemal concentration of 104 per ml is requires for the test to become positive.

b. Direct fluorescent-antibody staining for T.pallidum:

- Smear of the material to be tested is made on a glass slide.
- It is stained with fluorescent labelled monoclonal antibody against T.pallidum.
- The treponemes appear distinct , sharp outline and exhibit an appear green florescence.
- It is a better and safer method for microscopic diagnosis.

c. Treponemes in tissues:

- They can be demonstrated by silver impregnation method of staining or by immunofluorescence staining.
- The treponemes reduce silver nitrate to metallic silver that is deposited on the surface enlarging the diameter of the organism.

d. Serological tests:

- These tests form the mainstay of laboratory diagnosis.
- Depending upon the antigen used, serological tests are divided into non-treponemal tests.
  (cardiolipin or lipoidal antigen is used) and treponemal test(treponemes are used as antigens).

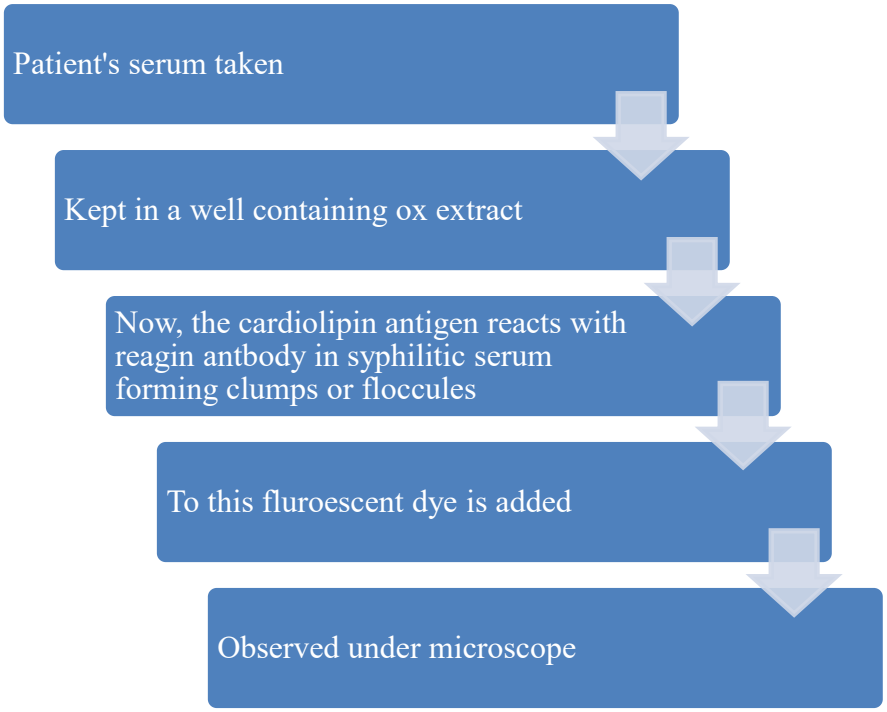
1. Non-treponemal tests:
   - ⇾ They are screening tests.
   - ⇾ Here the regain antibodies are detected by cardiolipin antigen.
   - ⇾ Cardiolipin is an antigen which is present in the extract of a bull and its structure is similar to that of treponemes. Because of which, the regain antibodies mistakenly binds to the cadiolipin.

Non treponemal includes:

- Veneral disease research laboratory test(VDRL)
- Rapid plasma reaction test(RPR)
- Kahn tests
- Wasserman reaction

↠ All these tests are **flocculation tests** except wasserman's reaction which is a Complement Fixation Test(CFT). The Wassermsn's reaction and the Kahn tests are no longer in use .

- VDRL Tests:



↠ RPR tests: same as VDRL with two changes:

↠ Here, carbon particles are added. As a result of which there is visualization of the fluorescent region without the use of microscope.

- Disadvantages of non-treponemal tests:

↠ Non treponemal tests are not specific for syphilis organism eg: cardiolipin is also released by damaged cell in the body.

↠ This accounts for the biological false positive result.

↠ Conditions in which BFP reactions occur include:

- Leprosy
- Malaria
- Relapsing fever
- Infectious mononucleosis
- Tropical eosinophilia

- Hepatitits

→ To eliminate these false positive result, tests have been developed using treponemal antigens.

- **Treponemal tests(confirming tests):**

Tests using Reiter treponems	• Reiter protein complement fixation test.
Tests using T.pallidum(Nichol's strain)	• T.pallidum immobilisation (TPI)
Using killed T.pallidum	• Trepnema pallidum agglutination test(TPA) • Treponema pallidum immune adherence test(TPIA) • Fluorescent treponemal antibody (FTA) tests
Using an extract of T.pallidum	• Treponema pallidum haemo-agglutination assay(TPHA) • Enzyme immunology

- **Treponema pallidum immobilization (TPI) test**:
  - ✓ This test employs live T.pallidum.
  - ✓ The test serum is mixed with actively motile Nichol's Strain of T.pallidum and incubated anaerobically.
  - ✓ If patient's blood contains antibodies then, the treponemes are seen immobilised when it is viewed under dark ground illumination.

- **Treponema pallidum agglutination test(TPA):**
  - ✓ Suspension of live T.pallidum which is inactivated by formalin + patient's serum.
  - ✓ If antibody is present in then treponemes become agglutinated when it is viewed under dark ground illumination.

- **Treponema pallidum immune adherence test(TPIA):**
  - ✓ Suspension of treponemes of inactivated T.pallidum – patient's serum, complement and fresh heparinization of whole blood from a normal individual and incubated.
  - ✓ If antibody is present in the serum, then the treponemes will adhere to the erythrocytes. Which then will get phagocytised and then will disappear.
  - ✓ TPA and TPIA are no longer used.

- **Fluorescent treponemal antibody (FTA) tests:**
  - ✓ Killed T.pallidum smears + patient's serum + labelled antihuman immunoglobin fluorescent conjugate.
  - ✓ If patient's serum has antibody then treponems fluoresce when it is viewed under immunofluorescence microscope.

- **FTA – absorption test (FTA-Abs):**
  - ✓ Same as FTA except that the patient's serum is mixed with an absorbent containing an extract of a non-pathogenic treponeme (Reiter treponeme).
  - ✓ The purpose of the absorbent is to remove anti-treponemal antibodies that are not specific for the syphilis bacteria.
  - ✓ FTA-Abs has high specificity and sensitivity.