

### General Overview of Spirochaetales

1-Gram-negative **spirochetes**

2- Spirochete from Greek for “coiled hair”

3-**Extremely thin** and can be **very long**

4-Tightly coiled **helical cells with tapered ends**

5- Motile by **periplasmic flagella** (a.k.a., **axial fibrils** or **endoflagella**)

6- **Outer sheath** encloses axial fibrils wrapped around protoplasmic cylinder

7-Axial fibrils originate from insertion pores at both poles of cell

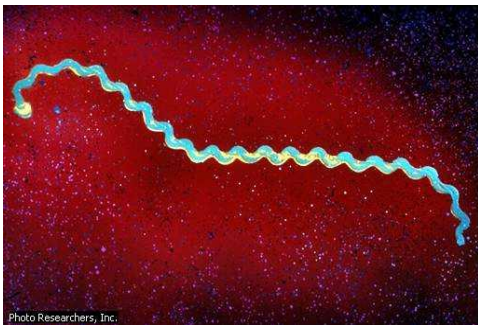
May overlap at center of cell in *Treponema* and *Borrelia*, but not in *Leptospira*

-Deferring numbers of endoflagella according to genus & species

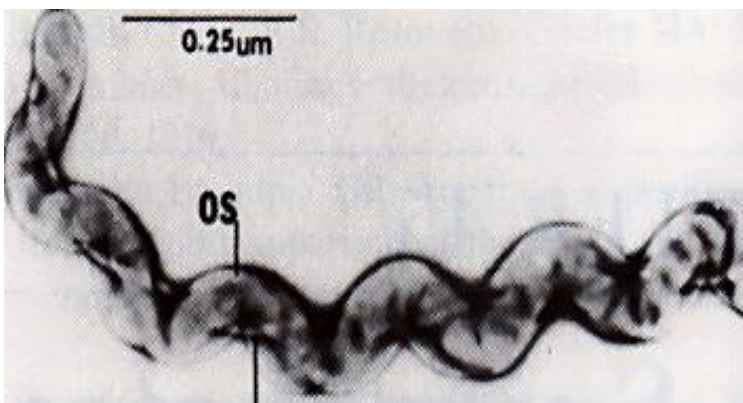
### Morphology

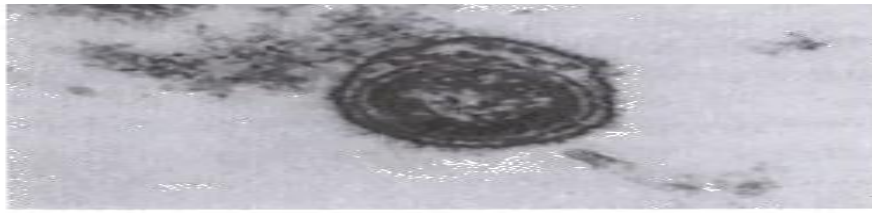
-Have **axial filaments**, which are otherwise similar to bacterial flagella •

-Filaments enable movement of bacterium by rotating in place



### ***Tightly Coiled Spirochete***





## Spirochaetales Associated Human Diseases

| <u>Genus</u>      | <u>Species</u>   | <u>Disease</u>  |
|-------------------|--|---|
| <b>Treponema</b>  | <i>pallidum</i> ssp. <i>pallidum</i><br><i>pallidum</i> ssp. <i>endemicum</i><br><i>pallidum</i> ssp. <i>pertenue</i><br><i>carateum</i> | Syphilis<br>Bejel<br>Yaws<br>Pinta  |
| <b>Borrelia</b>   | <i>burgdorferi</i><br><i>recurrentis</i><br>Many species   | Lyme disease (borreliosis)<br>Epidemic relapsing fever<br>Endemic relapsing fever |
| <b>Leptospira</b> | <i>interrogans</i>   | Leptospirosis<br>(Weil's Disease)   |

### Treponema spp.

#### Nonvenereal Treponemal Diseases

- ✓ Bejel, Yaws & Pinta
- ✓ Primitive tropical and subtropical regions
- ✓ Primarily in impoverished children

#### Treponema pallidum ssp. endemicum

- Bejel (a.k.a. endemic syphilis)
  - Initial lesions: nondescript oral lesions
  - Secondary lesions: oral papules and mucosal patches

- **Late:** gummas (granulomas) of skin, bones & nasopharynx
- **Transmitted person-to-person by contaminated eating utensils**

Primitive tropical/subtropical areas (Africa, Asia & Australia)

**Treponema pallidum ssp. pertenue**

- **Yaws: granulomatous disease**
  - **Early:** skin lesions (see below)
  - **Late:** destructive lesions of skin, lymph nodes & bones
- **Transmitted by direct contact with lesions containing abundant spirochetes**

Primitive tropical areas (S. America, Central Africa, SE Asia)

**Treponema carateum**

- **Pinta:** primarily restricted to skin
  - **1-3 week incubation period**
  - **Initial lesions:** small pruritic papules
  - **Secondary:** enlarged plaques persist for months to years
  - **Late:** disseminated, recurrent hypopigmentation or depigmentation of skin lesions; scarring & disfigurement
- **Transmitted by direct contact with skin lesions**
- **Primitive tropical areas**

(Mexico, Central & South America)

**Treponema pallidum ssp. pallidum**

**Venereal Treponemal Disease**

- **Syphilis**
- Primarily **sexually transmitted disease (STD)**
- May be **transmitted congenitally**

**General Characteristics of Treponema pallidum**

- Too thin to be seen with light microscopy in specimens stained with Gram stain or Giemsa stain
  - Motile spirochetes can be seen with **dark field microscopy**

- Staining with anti-Treponemal antibodies labeled with fluorescent dyes
- Intracellular pathogen
- Cannot be grown in cell-free cultures in vitro
- Do not survive well outside of host
- Care must be taken with clinical specimens for laboratory culture or testing

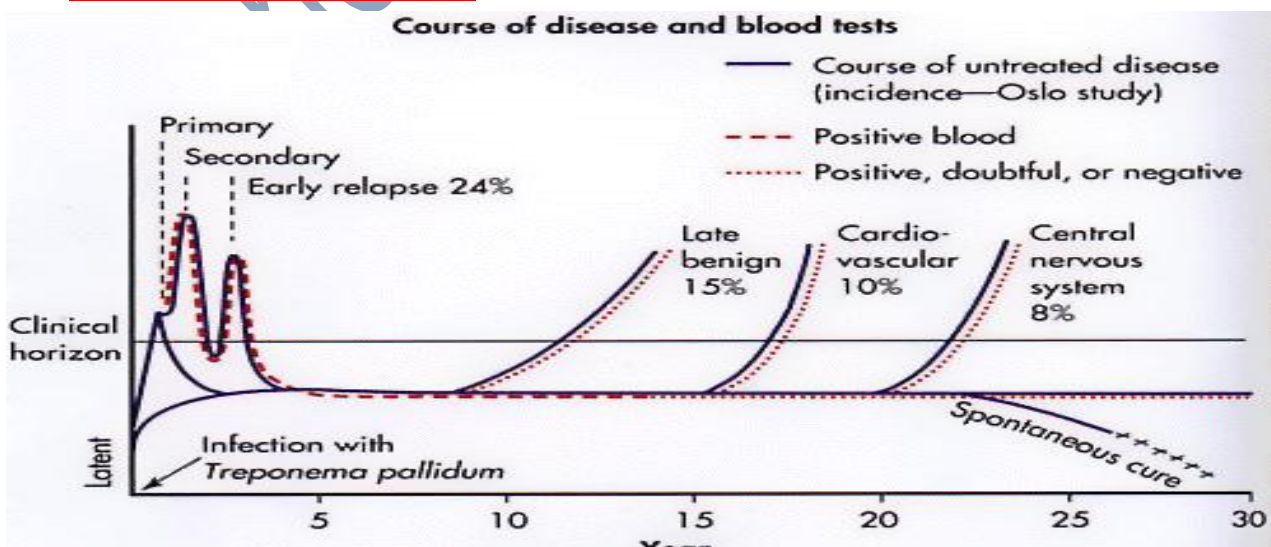
### Pathogenesis of T. pallidum

- Tissue destruction and lesions are primarily a consequence of patient's immune response
- Syphilis is a **disease of blood vessels and of the perivascular areas**
- In spite of a **vigorous host immune response** the organisms are capable of persisting for decades
  - Infection is **neither fully controlled nor eradicated**
  - In **early stages**, there is an inhibition of cell-mediated immunity
  - Inhibition of CMI abates in **late stages** of disease, hence late lesions tend to be localized

### Virulence Factors of T. pallidum

- Outer membrane proteins promote adherence
- Hyaluronidase may facilitate perivascular infiltration
- Antiphagocytic coating of fibronectin
- Tissue destruction and lesions are primarily result of host's immune response (immunopathology)

### Progression of Untreated Syphilis





**Stages of Disease**

- Primary
- Secondary
- Latent
- Tertiary
- Congenital Syphilis

**Primary Syphilis**

- -Organism enters directly through skin or through mucosal tissue.
- -Carried by blood throughout the body.
- -Organisms remaining at the site begin to multiply.
- Syphilis cannot be spread by toilet seats, door knobs, swimming pools, hot tubs, bath tubs, shared clothing, or eating utensils

**Primary Syphilis - Chancre**

- -Variable incubation period of 10 days to several months, a primary lesion, *chancre*, forms at the entrance site.
- -Chancre begins as a small, usually singular nodule; as it enlarges, the overlying epithelial tissues begins to necrosis, resulting in a relatively painless ulcer.

Unlike other bacterial infections, there is *no* formation of pus unless a secondary bacterial infection sets in

**Secondary Syphilis**

- -Occurs 6-8 weeks after initial chancre, *becomes systemic, patient highly infectious*.
- -Characterized by localized or diffuse mucocutaneous lesions, often with generalized lymphadenopathy.
- -Primary chancre may still be present.
- -Secondary lesions subside in about 2-6 weeks.
- -Serology tests *nearly 100%* positive.

**Latent Syphilis**

- -Stage of infection in which organisms persist in the body of the infected person without causing symptoms or signs (asymptomatic).
- -This stage may last for years.

- -One-third of untreated latent stage individuals develop signs of tertiary syphilis.
- After four years it is rarely communicable sexually but can be passed from mother to fetus
- This stage may be further subdivided.
  - -Early latent, initial infection occurred within previous 12 months.
  - -Late latent, initial infection occurred greater than 12 months.
  - -Latent of unknown duration, date of initial infection cannot be established as having occurred in the previous year.

#### **Tertiary Syphilis**

- -Divided into three manifestations:
  - -Gummatous syphilis
  - -Cardiovascular syphilis
  - -Neurosyphilis

#### **Congenital Syphilis**

- -Bone deformities
- -Blindness
- -Deafness
- -Deformed faces
- -Dental deformities Skin rashes Neonatal death
- 

#### **Diagnosis of Syphilis**

- -Evaluation based on three factors:
  - 1-Clinical findings.
  - 2-Demonstration of spirochetes in clinical specimen.
  - 3-Present of antibodies in blood or cerebrospinal fluid.
- -More than one test should be performed.
- -No serological test can distinguish between other Treponemal infections.
- -Direct examination of clinical specimen by dark-field microscopy or fluorescent antibody testing of sample.

## Spirochetes spp. 3rd class lab.no.13

- -Non-specific or non-treponemal serological test to detect reagin, utilized as screening test only.
- -Specific Treponemal antibody tests are used as a confirmatory test for a positive reagin test.

| Diagnostic Test | Method or Examination  |
|-----------------|--|
| Microscopy      | Darkfield<br>Direct fluorescent antibody staining  |
| Culture         | Not available  |
| Serology        | Nontreponemal tests<br>Venereal Disease Research Laboratory (VDRL)<br>Rapid plasma reagin (RPR)<br>Treponemal tests<br>Fluorescent treponemal antibody absorption (FTA-ABS)<br>Microhemagglutination test for <i>Treponema pallidum</i> (MHA-TP) |

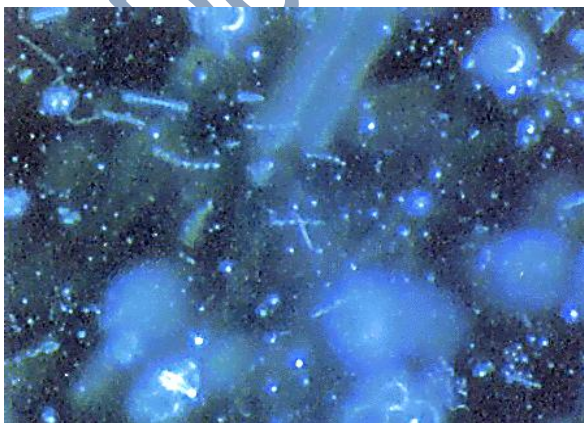
### Microscopy

- Spiral shaped and motile due to periplasmic flagella.

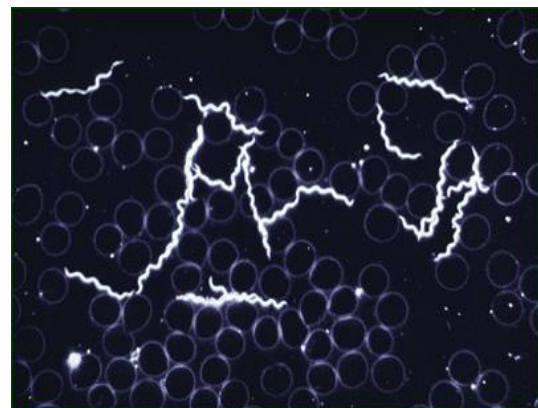
Variable length



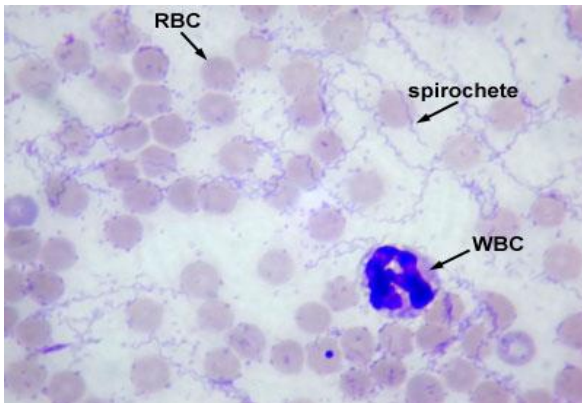
*Fluorescence antibody staining*



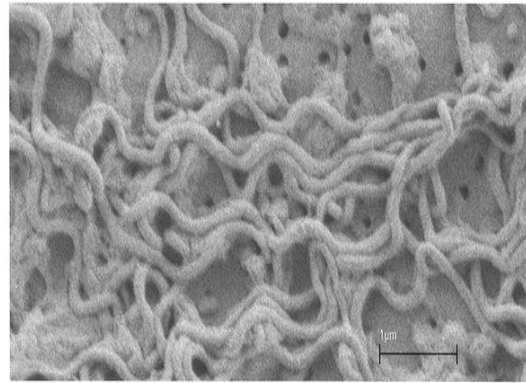
*dark field microscopy*



Spirochetes in Blood



Scanning Electron Micrograph of T. pallidum



### Serological Testing

- -Important in diagnosis
- -Isolation of organism very difficult
- -Clinical symptoms not always apparent.

### A-Nontreponemal Reagin Tests

- 1-VDRL
- 2-RPR
- 3-USR-unheated serum reagin test
- 4-RST-reagin screen test
- 5-ELISA

### Nontreponemal Reagin Tests

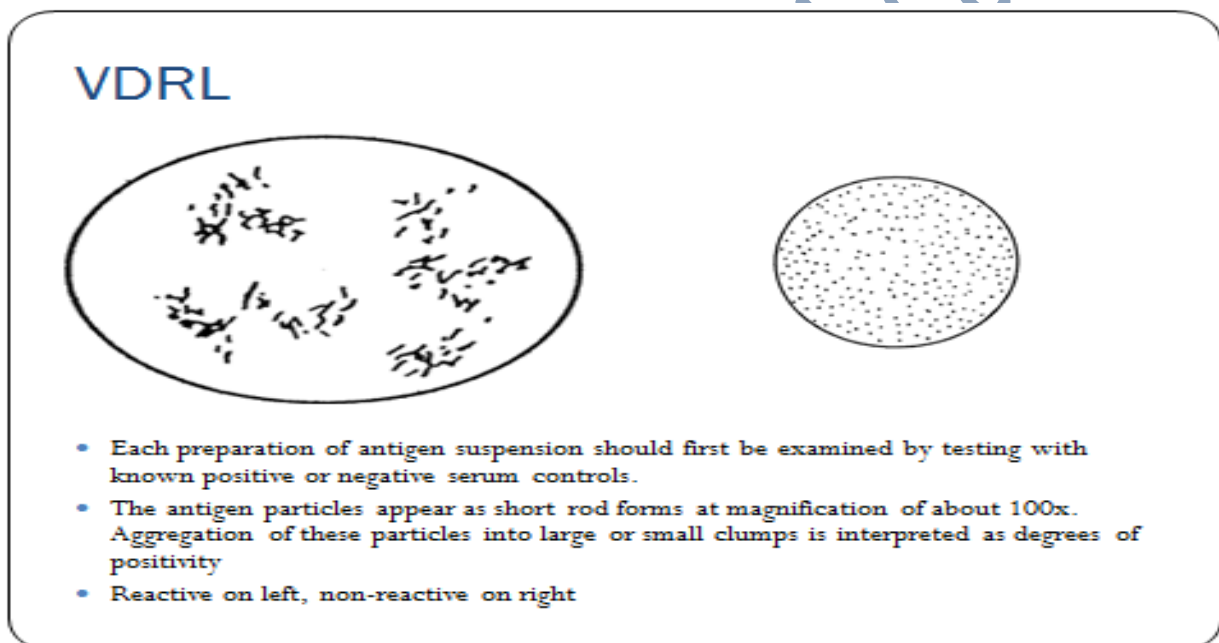
Non-specific or non-Treponemal serological test to detect **reagin**, utilized as screening test only.

- **Reagin** is an antibody formed against cardiolipin.
- -Found in sera of patients with syphilis as well as other diseases.
- -This type of reagin not to be confused with same word originally used to describe IgE.
- -Non Treponemal tests become positive 1 to 4 weeks after appearance of primary chancre.
- -in secondary stage may have false negative due to Prozone, in tertiary 25% are negative, after successful treatment will become nonreactive after 1 to 2 years



— **1-Venereal Disease Research Laboratory - VDRL**

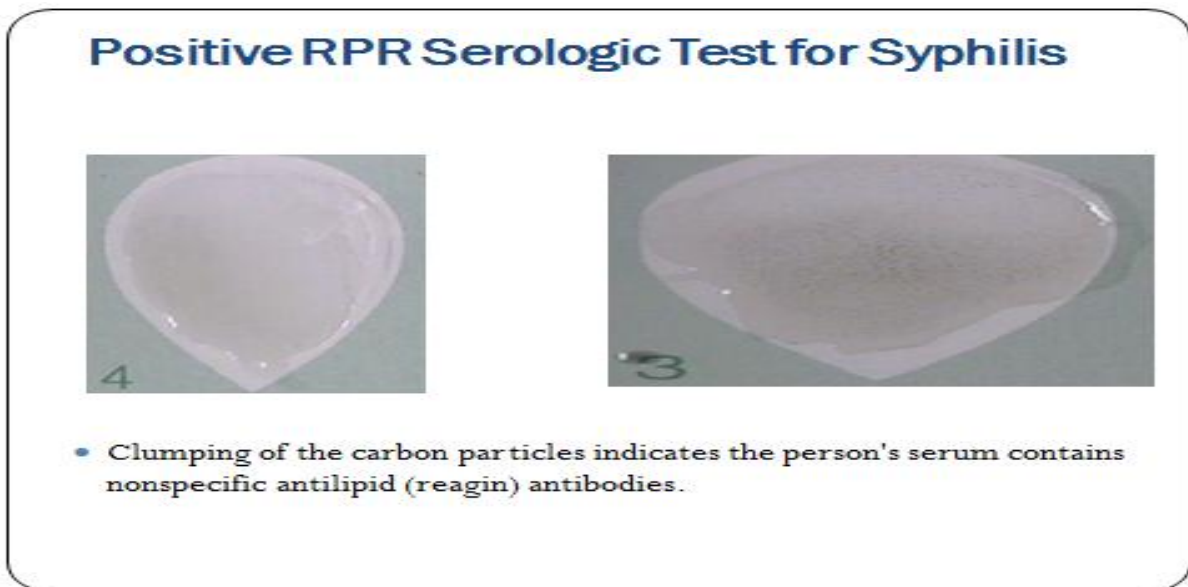
- -Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin.
- -Utilizes an antigen which consists of **cardiolipin, cholesterol and lecithin**.
  - Antigen very technique dependent.
  - Must be made up fresh daily.
- -**Serum must be heated to 56 C for 30 minutes** to remove anti-complementary activity which may cause false positive, if serum is not tested **within 4 hours** must be **reheated for 10 minutes**.
- --VDRL used primarily to screen cerebral spinal fluid.



**2-Rapid Plasma Reagin Test - RPR**

- -General screening test, can be adapted to automation.
- -**CANNOT** be performed on CSF.
- -**CANNOT** be performed on cord blood.
- -Antigen
  - =VDRL cardiolipin antigen is **modified with choline chloride** to make it more stable
  - -attached to charcoal particles to allow macroscopic reading
  - -antigen comes prepared and is very stable.

- -Serum or plasma may be used for testing, serum is **not** heated.



### **3-Unheated Serum Reagin Test - USR**

- -Modified VDRL antigen, uses choline chloride/EDTA to stabilize antigen.
- -**Microscopic** flocculation test.
- -Reagent is ready-to-use and no serum heating is required.
- -*Chelating agents* are added to neutralize the interference due to complements.
- -Several types of USR tests are available.

These tests show a high incidence (8-10%) of false negatives due to the prozone phenomenon, for this reason its preferable to run the tests at two dilutions

### **3-Reagin Screen Test - RST**

- -Modified VDRL antigen with Sudan Black to make flocculation reaction **macroscopically** visible.
- -The sensitivity and specificity of the RST are essentially the same as those of the VDRL test.
- -The specimen of serum does not have to be inactivated by heat.
- -The RST antigen is ready to use and it is stable for at least two years.

### **B-Specific Treponemal Tests**

- -Performed to confirm a positive non-specific reagin test.
- 1-Treponema Pallidum Immobilization

- 2-Treponema pallidum Hemagglutination
- 3-Fluorescent Treponemal antibody absorption test
- 4-ELISA

#### 1-Treponema Pallidum Immobilization - TPI

- -An antibody present in the serum of a syphilitic patient, in the presence of complement, causes the immobilization of actively motile *Treponema pallidum* obtained from testes of a rabbit infected with syphilis.
- Cumbersome and expensive, no longer used in US

#### 2-Treponema pallidum hemagglutination (TPHA)

- -Adapted to microtechniques (MHA-TP)
- -Tanned sheep RBCs are coated with *T. pallidum* antigen from Nichol's strain.
- -Agglutination of the RBCs is a positive result.
- -Based on agglutination of RBCs sensitized with *T. pallidum* antigen.
- -Patient sera incubated with sensitized RBCs in microtiter wells and unsensitized RBCs in control wells.
- -Patient sera containing specific antibodies will react only with the antigen to form a smooth mat of agglutinated RBCs (positive).
- -A compact button formed by the settling of the non-agglutinated RBCs in the microtiter wells containing sensitized RBCs indicates lack of specific antibody in patient sera (negative).
- If agglutination is seen with both sensitized and unsensitized RBCs, nonspecific agglutination is indicated

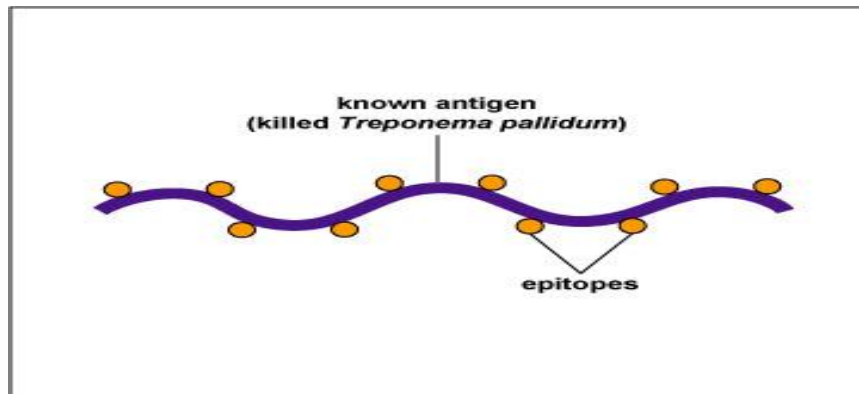
#### 3-Fluorescent Treponemal Antibody Absorption Test (FTA-ABS)

- -Reiter's strain of *T. pallidum* fixed to slide.
- -Diluted, heat inactivated serum added to Reiter's strain of *T. pallidum* to remove cross reactivity due to other *Treponema*.
- -Harvest absorbed serum.
- -Add absorbed patient serum to slides coated with Nichol's strain of *T. pallidum* and incubate.

- -Slides are washed, and incubated with anti-antibody bound to a fluorescent tag.
- -After washing the slides are examined for fluorescence.
- -Requires experienced personnel to read.

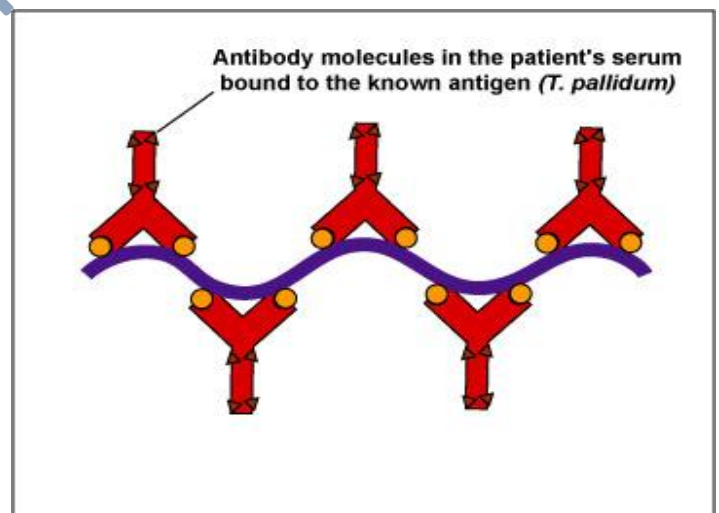
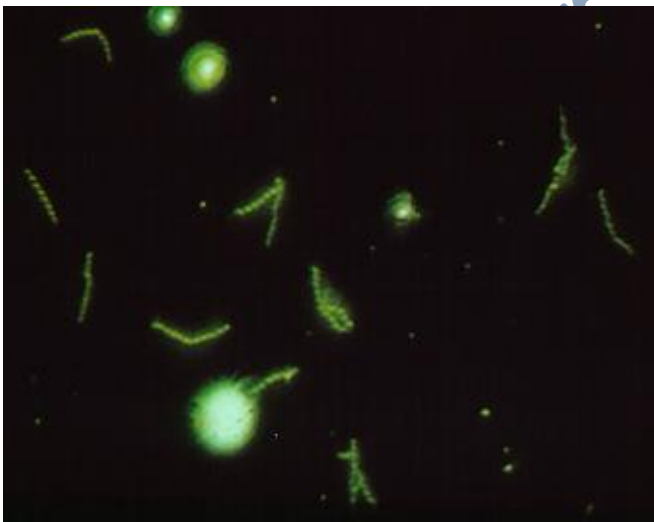
-Highly sensitive and specific, but time consuming to perform

- ***Treponema pallidum***, the known antigen, is fixed to a microscope slide.



- If there are antibodies against *Treponema pallidum* in the patient's serum, they will bind to the spirochete.

All other antibodies are washed from the slide



#### **4-ELISA**

- -Microtitration wells coated with *T.pallidum* antigens are exposed to test specimens which may contain specific antibodies.
- -After an incubation period, unbound components in the test sample are washed away.
- -Specifically-bound IgG reacts with an anti-human IgG antibody bound with horseradish peroxidase during a second incubation period.



- -Following a second wash cycle, specifically-bound enzyme conjugate is detected by reaction with hydrogen peroxide and the chromogen.
- -The color reaction is measured spectrophotometrically to indicate the presence or absence of IgG Treponemal antibodies.

### **C-Newer Technologies in Testing**

- -Enzyme immunoassay
- -Polymerase Chain Reaction

### **1-Enzyme immunoassay**

- -Screen large numbers
- -Slightly higher false positive rate than RPR
- -Can detect either IgM or IgG antibodies
- -EIA positive, RPR positive, considered positive

### **2-PCR**

- -Isolate and amplify specific region of DNA
- -Electrophoresis
- -Extremely sensitive
- -Specimens
  - Whole blood, CSF, Amniotic fluid

### **Conditions Associated with False Positive Serological Tests for Syphilis**

#### **Nontreponemal Tests**

Viral infection  
Rheumatoid arthritis  
Systemic lupus erythematosus  
Acute or chronic illness  
Pregnancy  
Recent immunization  
Drug addiction  
Leprosy  
Malaria

#### **Treponemal Tests**

Pyoderma  
Skin neoplasm  
Acne vulgaris  
Mycoses  
Crural ulceration  
Rheumatoid arthritis  
Psoriasis  
Systemic lupus erythematosus  
Pregnancy  
Drug addiction  
Herpes genitalis