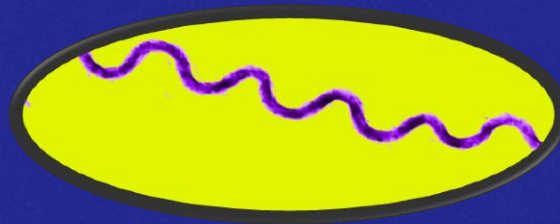


SYPHILIS

basics



Dr.T.V.Rao MD



Syphilis



Named from poem published by the Italian physician and poet Girolamo Fracastoro – shepherd from Hispaniola named Syphilis who angered Apollo and was given the disease as punishment



Historical Background



- ❧ Most accepted theory is that Christopher Columbus and his Crew brought back the bacteria which caused the disease Syphilis from the journey to the New World.
- ❧ No distinctive signs of syphilis were found in Europe before he came back.



Syphilis

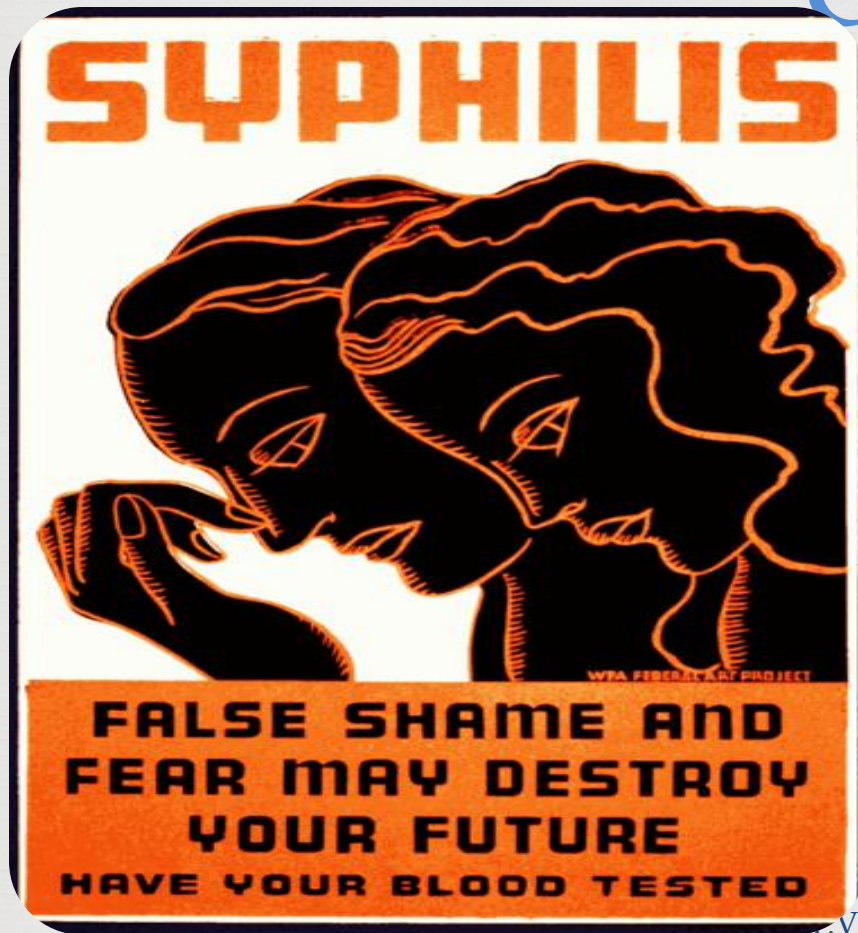


*"He who knows
syphilis, knows
medicine"*

Sir William Osler



Syphilis was a Taboo



Poster for testing of syphilis, showing a man and a woman bowing their heads in shame (ca. 1936).

Treponema pallidum



∞ Described in
1905 by
Schaudinn
and Hoffman,
Hamburg

SYPHILIS

INTRODUCTION



- ❧ Caused by *Treponema pallidum*.
- ❧ Transmission: sexual; maternal-fetal, blood transfusion and rarely by other means of both transmitting and getting infected with HIV.



Perform HIV testing in all patients with syphilis.

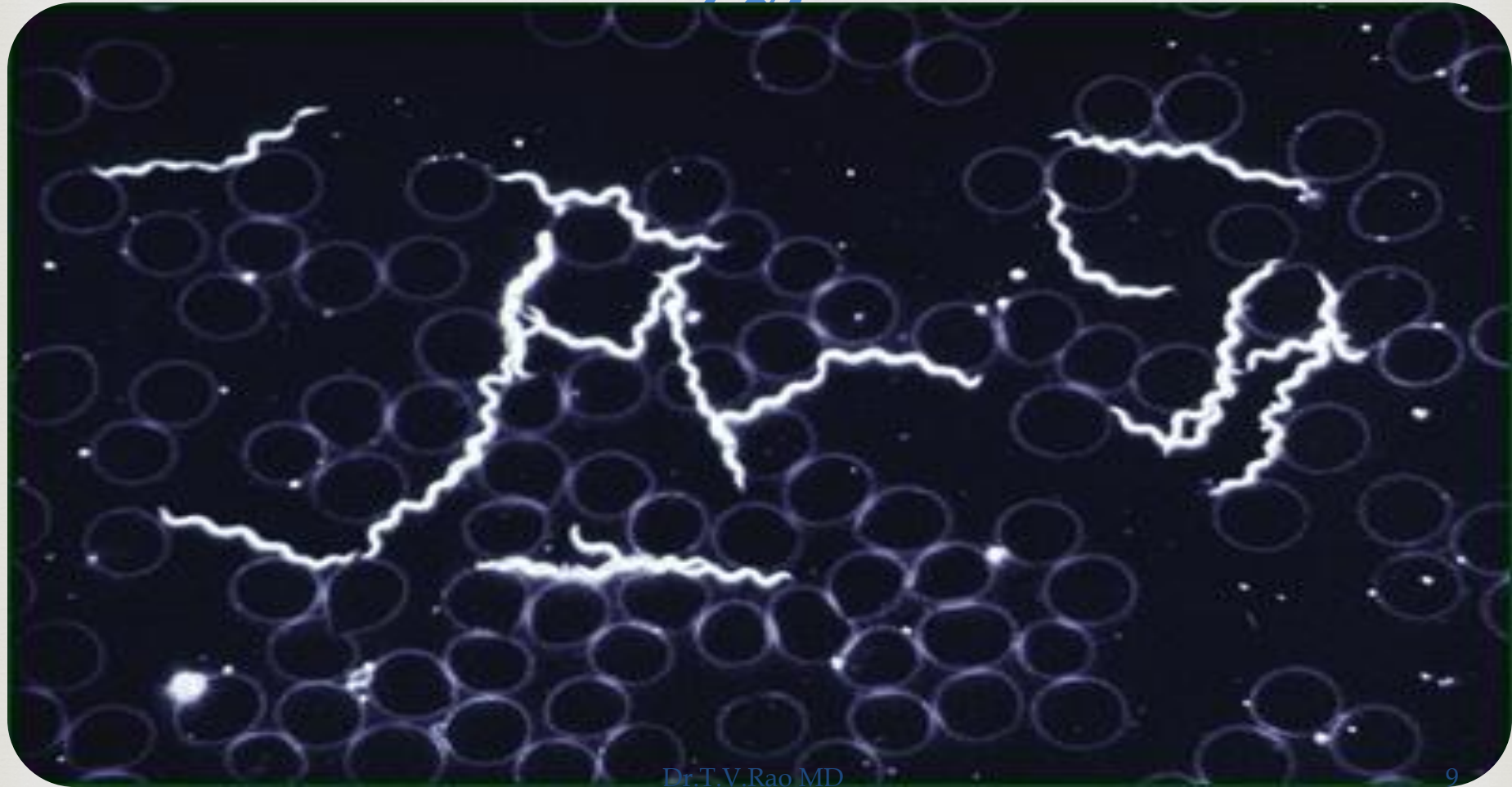
Dr. T.V.Rao MD

Introduction to Spirochetes



- ❧ Long, slender, helically tightly coiled bacteria
- ❧ Gram-negative
- ❧ Aerobic, microaerophilic or anaerobic .
- ❧ Corkscrew motility
- ❧ Can be free living or parasitic
- ❧ Best-known are those which cause disease: Syphilis and Lyme's disease

Darkfield Microscopy



Fluid From Chancre



Other Related to Treponemes

Related Treponemes cause the non-venereal treponematoses bejel, or endemic syphilis (*T. pallidum endemicum*), yaws (*T. pallidum pertenue*), and pinta (*T. carateum*).



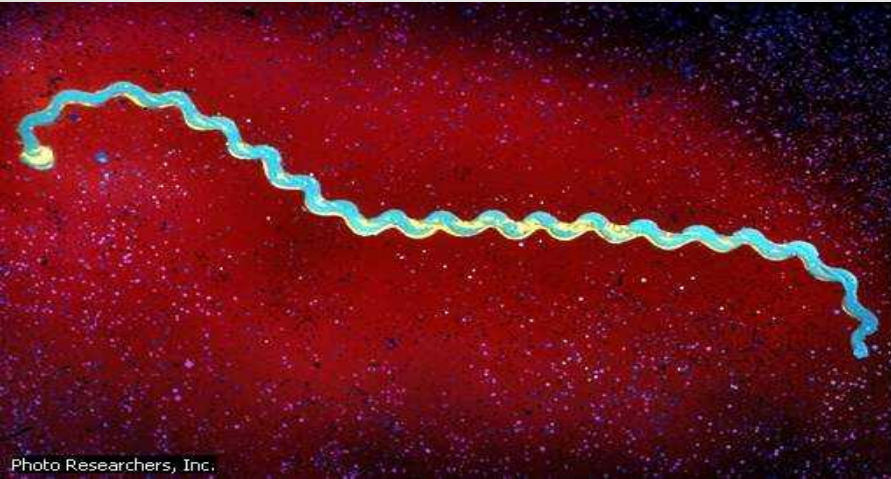
Treponema pallidum.



☞ Spiral spirochete that is mobile of spirals varies from 4 to 14 Length 5 to 20 microns and very thin 0.1 to 0.5 microns. Can be seen on fresh primary or secondary lesions by **darkfield microscopy or fluorescent antibody techniques**



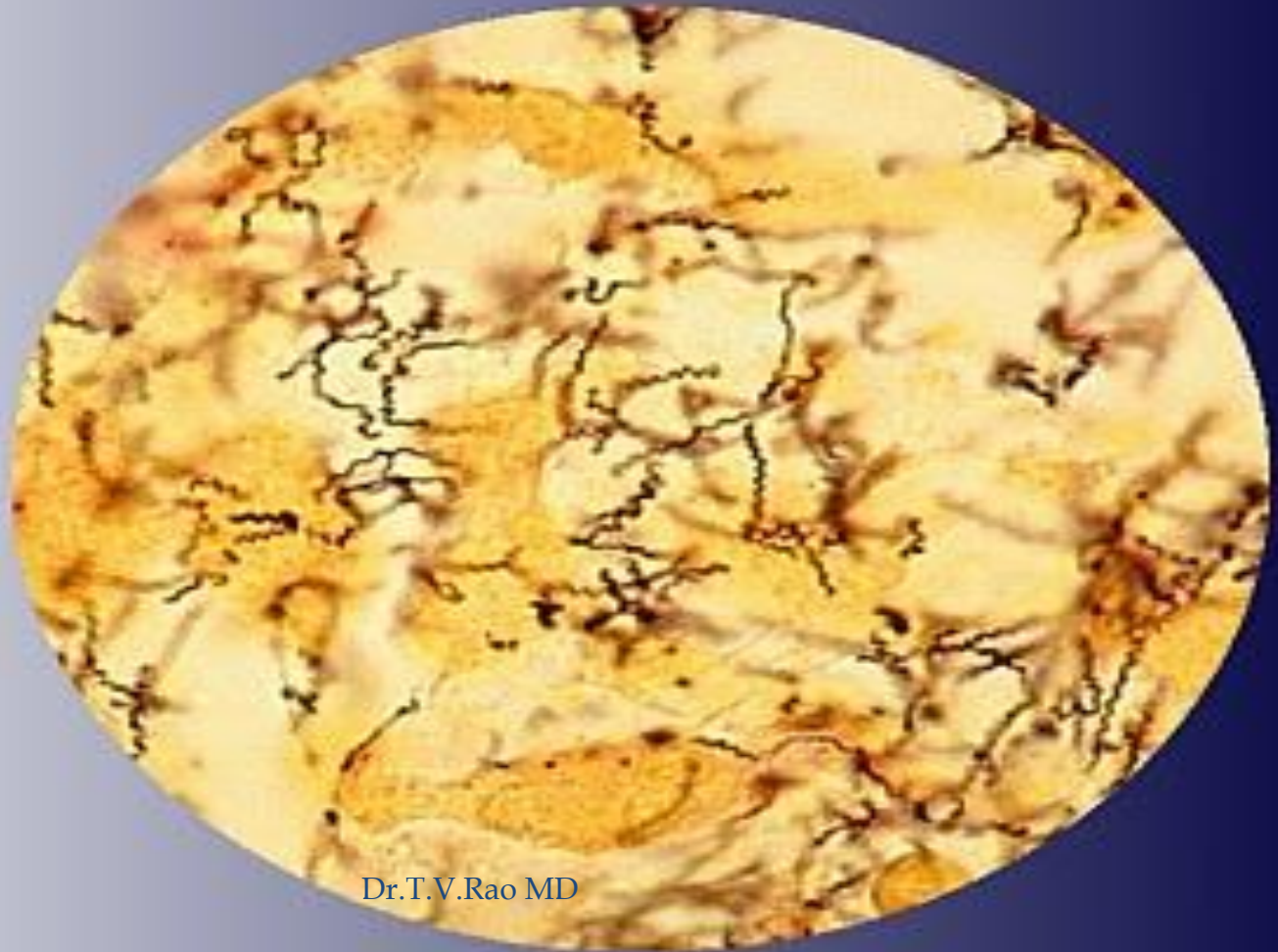
Morphology



- ☞ Have **axial filaments**, which are otherwise similar to bacterial flagella
- ☞ Filaments enable movement of bacterium by rotating in place

Special Staining Methods

Fontana's Method



*Dedicated from dr. Kamal Fahmy
to Department of Microbiology and Immunology
Faculty of Medicine - Zagazig University*

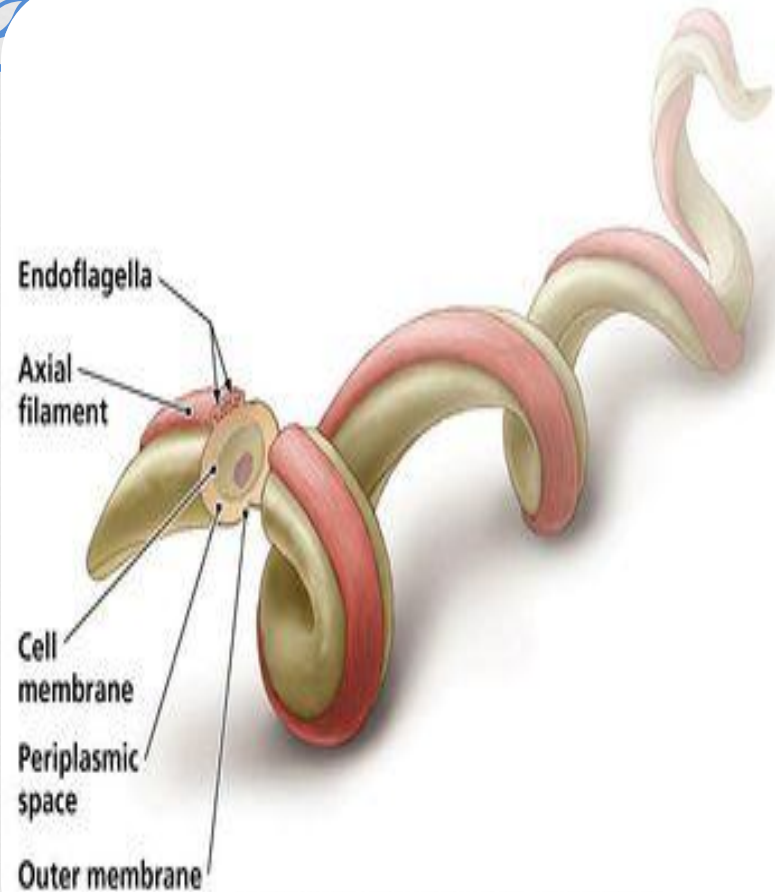
TREPONEMA PALLIDUM SSP. PALLIDUM (T. PALLIDUM)



☞ *T. pallidum*, the causative agent of syphilis, was first isolated from syphilitic lesions in 1905. Infection is usually acquired by sexual contact with infected individuals and is commonest in the most sexually active age group of 15-30-year-olds. ***Congenital syphilis usually occurs following vertical transmission of *T. pallidum* from the infected mother to the fetus in utero***, but neonates may also be infected during passage through the infected birth canal at delivery.

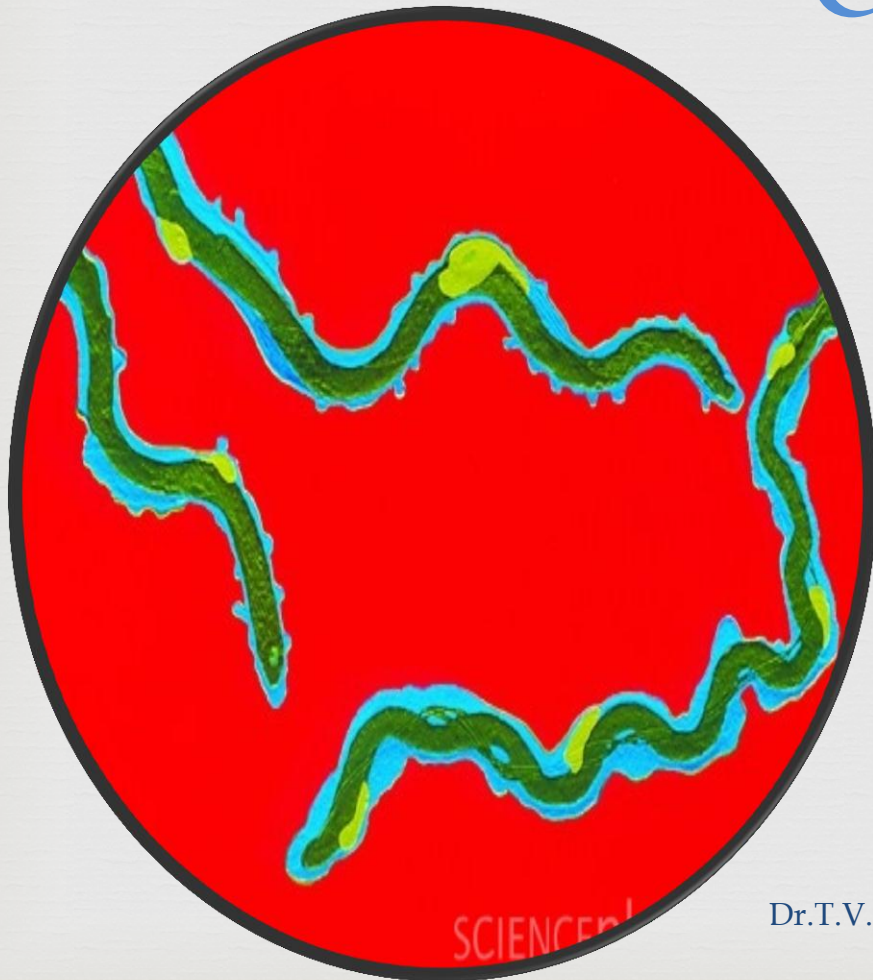
Structure of Spirochetes

❧ Spirochetes are slender unicellular helical or spiral rods with a number of distinctive ultra structural features used in the differentiation of the genera. The cytoplasm is surrounded by a cytoplasmic membrane, and a peptidoglycan layer contributes to cell rigidity and shape. **In *Treponema* species, fine cytoplasmic filaments are visible in the bacterial cytoplasm**



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Structure of Spirochetes



- Several flagella are attached at each pole of the cell and wrap around the bacterial cell body. In contrast to other motile bacteria, these flagella do not protrude into the surrounding medium but are enclosed within the bacterial outer membrane.

Treponema cannot be cultivated in Culture Media



∞ The inability to grow most pathogenic Treponemes in vitro, coupled with the transitory nature of many of the lesions, makes diagnosis of Treponemal infection impossible by routine bacteriological methods



Cultivation of .. ?



- Although the Treponemes are distantly related to Gram-negative bacteria, they do not stain by Gram's method, and modified staining procedures are used. Moreover, the pathogenic **Treponemes cannot be cultivated in laboratory media and are maintained by subculture in susceptible animals.**



Pathology



Penetration:

- ☞ *T. pallidum* enters the body via skin and mucous membranes through abrasions during sexual contact
- ☞ Also transmitted transplacentally

Dissemination:

- ☞ Travels via the lymphatic system to regional lymph nodes and then throughout the body via the blood stream
- ☞ Invasion of the CNS can occur during any stage of syphilis

Pathology



∞ The bacteria rapidly enter the lymphatic's, are widely disseminated via the bloodstream and may lodge in any organ. The exact infectious dose for man is not known, but in experimental animals fewer than ten organisms are sufficient to initiate infection. The bacteria multiply at the initial entry site forming **a chancre**, a lesion characteristic of primary syphilis, after an average incubation period of 3 weeks

Basic lesion of syphilis



☞ The **chancre** is painless and most frequently on the external genitalia, but it may occur on the cervix, perianal area, in the mouth or anal canal. Chancres usually occur singly, but in immunocompromised individuals, such as those infected with the human immunodeficiency virus (HIV), multiple or persistent chancres may develop.

Chancre



∞ The chancre usually heals spontaneously within 3-6 weeks, and **2-12 weeks later the symptoms of secondary syphilis develop.** These are highly variable and widespread but most commonly involve the skin where macular or pustular lesions develop, particularly on the trunk and extremities. The lesions of secondary syphilis are highly infectious.

Progress of Disease



- Relapse of the lesions of secondary syphilis is common, and latent syphilis is classified as early (high likelihood of relapse) or late (recurrence unlikely). Individuals with late latent syphilis are not generally considered infectious, but may still transmit infection to the fetus during pregnancy and their blood may remain infectious.

Pathology



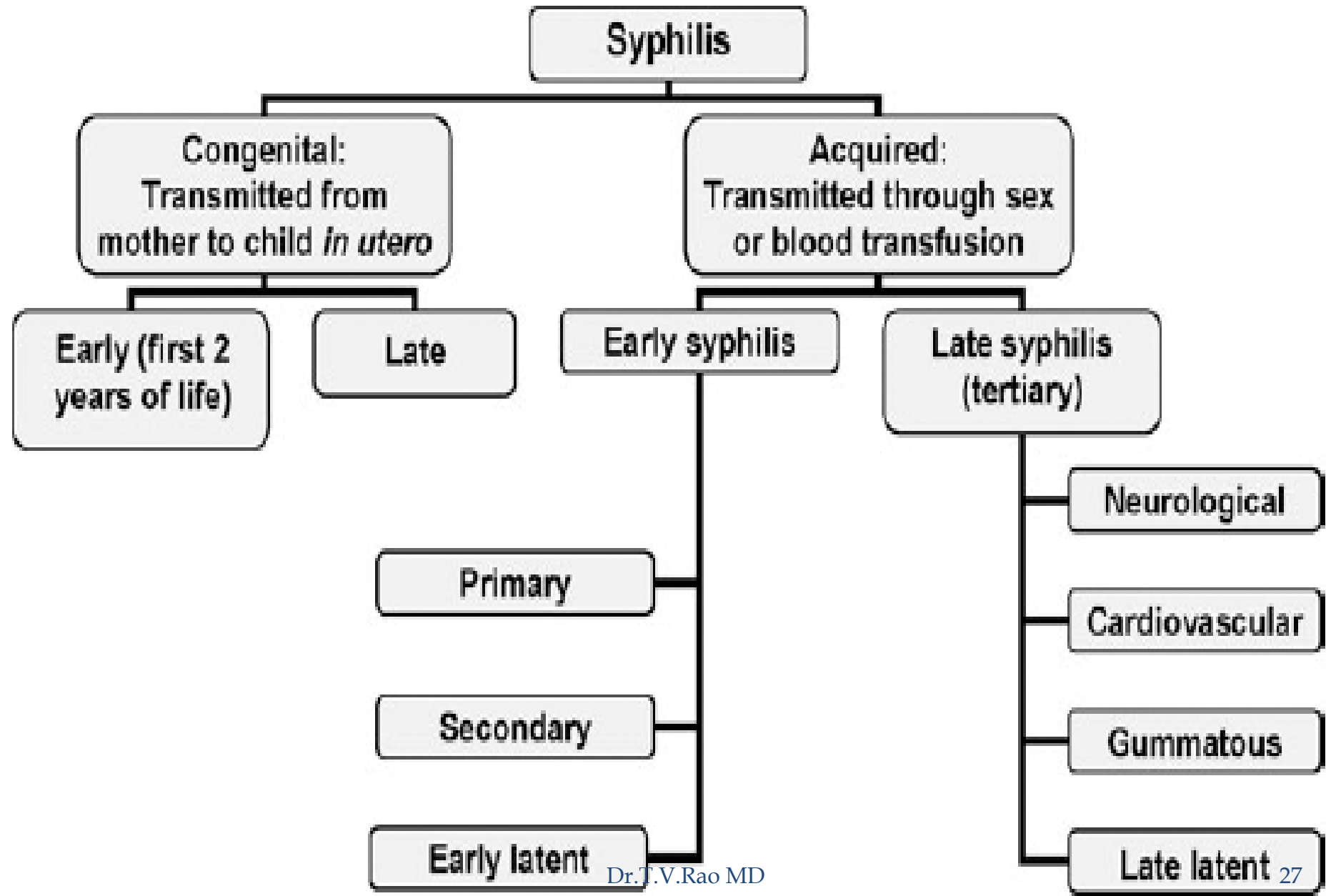
∞ The chancre is painless and most frequently on the external genitalia, but it may occur on the cervix, peri-anal area, in the mouth or anal canal. Chancres usually occur singly, but in immunocompromised individuals,



What is Syphilis



- ❧ Syphilis is a systemic, sexually transmitted disease (STD) caused by the *Treponema pallidum* bacterium. The three means of syphilis transmission are:
- ❧ Person to person via vaginal, anal, or oral sex through direct contact with a syphilis chancre.
- ❧ Person to person during foreplay, even when there is no penetrative sex (much less common).
- ❧ Pregnant mother with syphilis to fetus.



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STAGES OF SYPHILIS



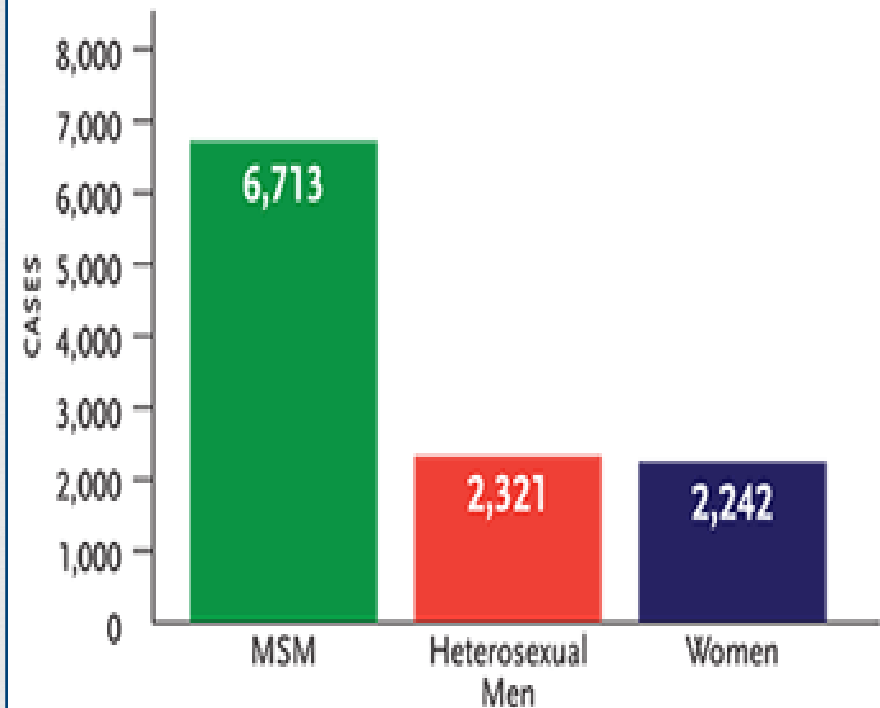
1. Primary
2. Secondary
3. Latent
 - ∞ Early latent
 - ∞ Late latent
4. Late or tertiary
 - ∞ May involve any organ, but main parts are:
 - ∞ **Neurosyphilis**
 - ∞ **Cardiovascular syphilis**
 - ∞ **Late benign (gumma)**

Stages of syphilis

Untreated syphilis may be a progressive disease with *primary, secondary, latent* and *tertiary* stages. *T. pallidum* enters tissues by penetration of intact mucosae or through abraded skin.



Primary and Secondary Syphilis—Reported Cases, 2008, by Sexual Orientation



Dr. T. V. Rao, MD

Note: Bars do not add up to total cases since information on gender of sex partners was not available for 20 percent of cases among men.

PRIMARY SYPHILIS (The Chancre)



- ∞ Incubation period 9-90 days, usually ~21 days.
- ∞ Develops at site of contact/inoculation.
- ∞ Classically: single, painless, clean-based, indurated ulcer, with firm, raised borders. Atypical presentations may occur.
- ∞ Mostly anogenital, but may occur at any site (tongue, pharynx, lips, fingers, nipples, etc...)
- ∞ Non-tender regional adenopathy
- ∞ Very infectious.
- ∞ May be darkfield positive but serologically negative.
- ∞ Untreated, heals in several weeks, leaving a faint scar.

Primary syphilis

- a) One or more painless chancres (indurated raised edges & clear bases) that erupt in the genitalia, anus, nipples, tonsils or eyelids.
- b) Starts as papule and then erode
- c) Disappear after three to six weeks even without treatment.
- d) Lymphadenopathy that is either unilateral or bilateral

Primary Syphilis



syphilis
www.healthac.org

www.healthac.org
syphilis

Primary Syphilis- Penile Chancre



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Primary Syphilis - Chancre



Primary Syphilis - *Chancre*

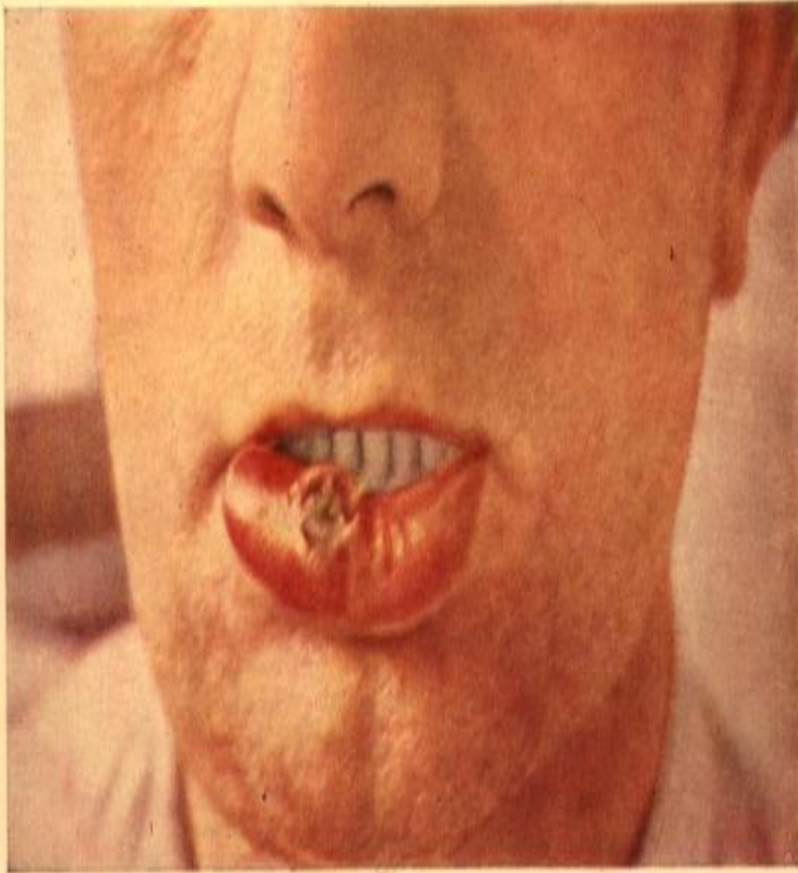


Fig. 171. **Primary Syphilis of the Lower Lip.** A chancre appearing on the lower lip has the same clinical appearance as one appearing on the genital mucosa. This lesion may simulate squamous cell carcinoma.



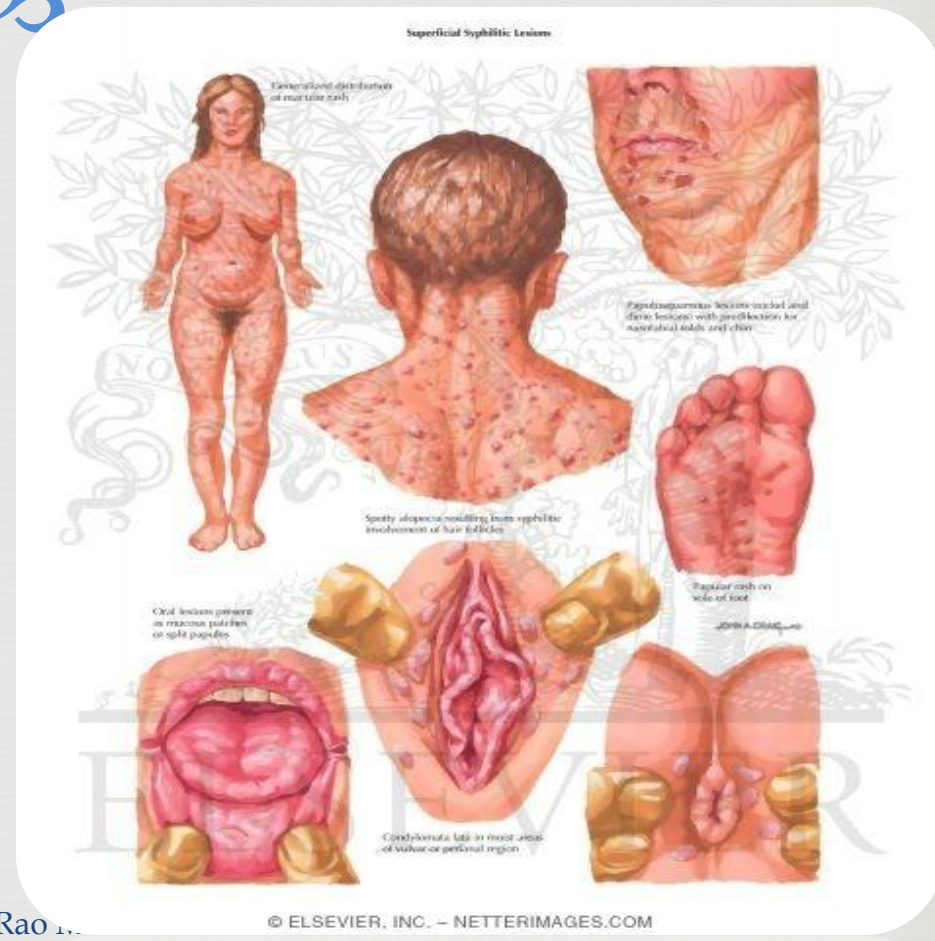
Primary Syphilis



Secondary Syphilis



- Secondary syphilis at 6-8 weeks – diffuse symptoms:
 - Fever
 - Headache
 - Skin pustules
- Usually disappears even without treatment



Secondary syphilis



- a) The rash can be macular, papular, pustule, or nodular.
- b) Lesions are of a uniform size, well-defined and generalized
- c) Macules often erupt between rolls of fat on the trunk and on the arms, palms, soles, face and scalp
- d) Lesions enlarged and erode in warm moist areas of the body (condylomata lata).
- e) Headache, anorexia, malaise, weight loss, nausea and vomiting, sore throat and slight fever.
- f) Temporary alopecia may occur


Secondary Syphilis



MD Challenger Sample Photo



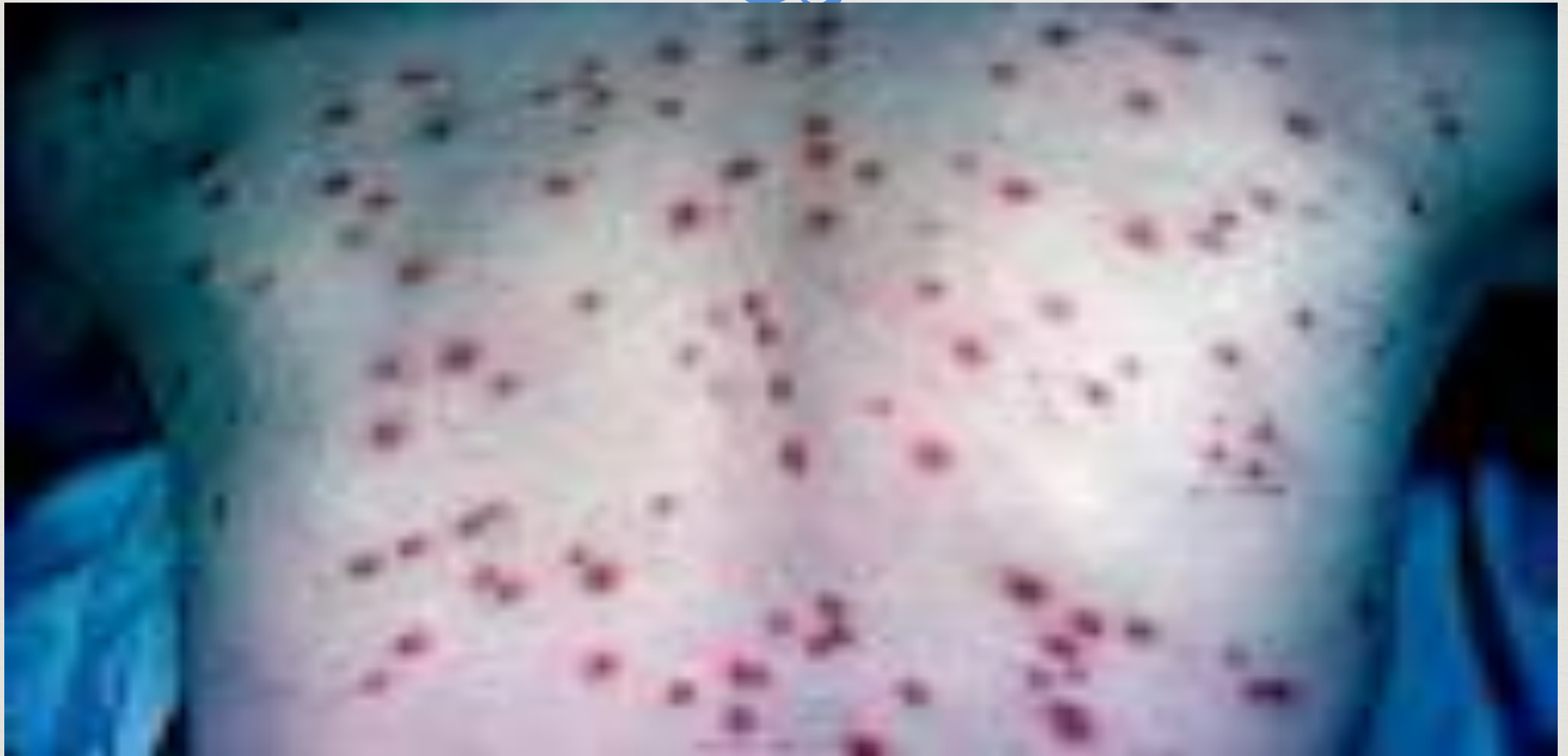
Secondary syphilis - papulo-pustular rash



Secondary Syphilis



Secondary Syphilis



Secondary syphilis

CR



Tertiary Syphilis



∞ Affects 2/3 of untreated cases

∞ Gummata: rubbery tumors

∞ Bone deformities

∞ Blindness

∞ Loss of coordination

∞ Paralysis

∞ Insanity

Tertiary Syphilis



Latent Syphilis



☞ Latent syphilis

- a) Reactive serologic test
- b) Asymptomatic until death

☞ Late syphilis

Three subtypes of Late syphilis

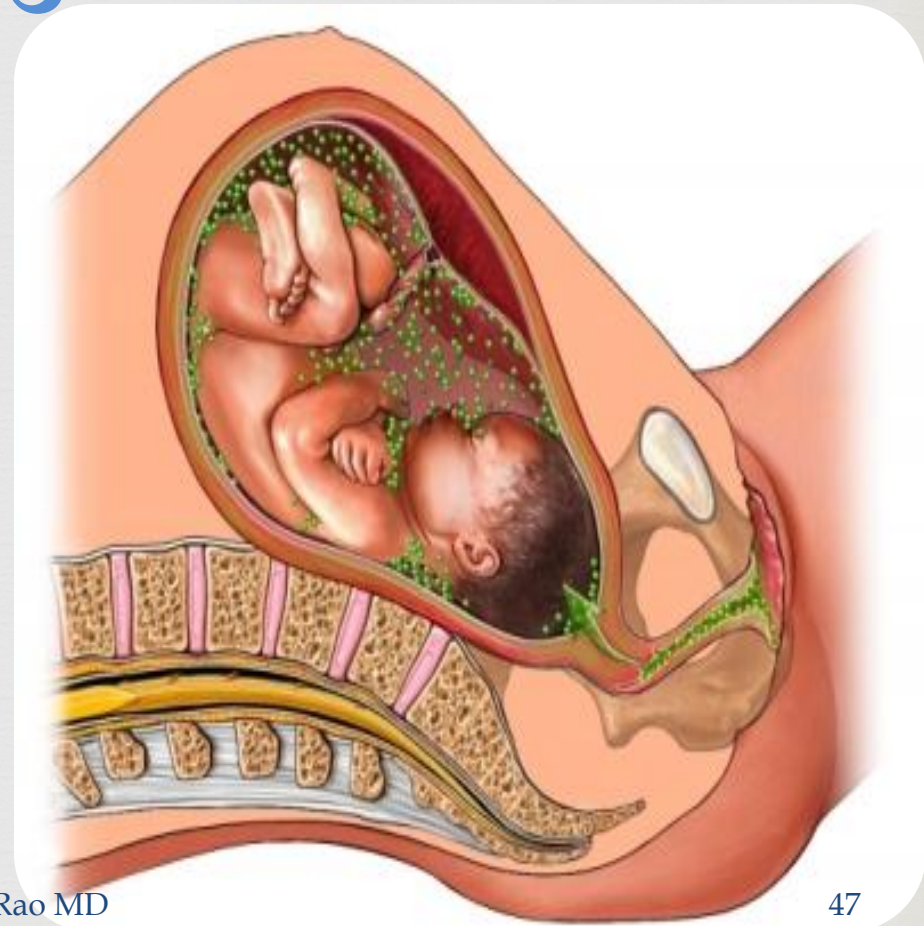
☞ Late, benign syphilis

- *Develops between 1 to 10 years after the infection
- *Presence of gumma

Mother to Child Transmission



☞ Infection in utero may have serious consequences for the fetus. Rarely, syphilis has been acquired by transfusion of infected fresh human blood.



Congenital Syphilis



- Passed from mother to fetus during pregnancy
- Abnormally shaped teeth
- Nasal septum collapses
- Skeletal abnormalities

What Congenital Syphilis Means



- ∞ If a pregnant woman has syphilis and is not treated quickly, these tiny bacteria travel with her blood to the baby's body. Syphilis infection can be a cause of fetal death and spontaneous abortion, or can result in the delivery of the dead baby, or the baby can die within several days of life. If the baby survives, there is a high risk that this baby will have copious nasal discharge (snuffles) packed with Treponemes and severe inflammatory reaction as a consequence, destroying nasal cartilages and bones. The baby will likely suffer from liver and spleen enlargement and dysfunction, meningitis or meningoencephalitis, and inflammatory skin rash – all of these are symptoms of early congenital syphilis.

Congenital syphilis - - Hutchinson's teeth



Some present in Differed Manner



Some babies will not develop signs of early congenital syphilis, but around eight years of age or older they will demonstrate symptoms of late congenital syphilis: their vision will become deteriorated due to inflammatory changes in eyes, some of their central permanent teeth will have unusual conic shape and notching, and they may become deaf with complaining of vertigo and ringing in the ears. Their bones will be deformed, resulting in the look of “saddle” nose and “saber” shins.

Tertiary Syphilis

CS

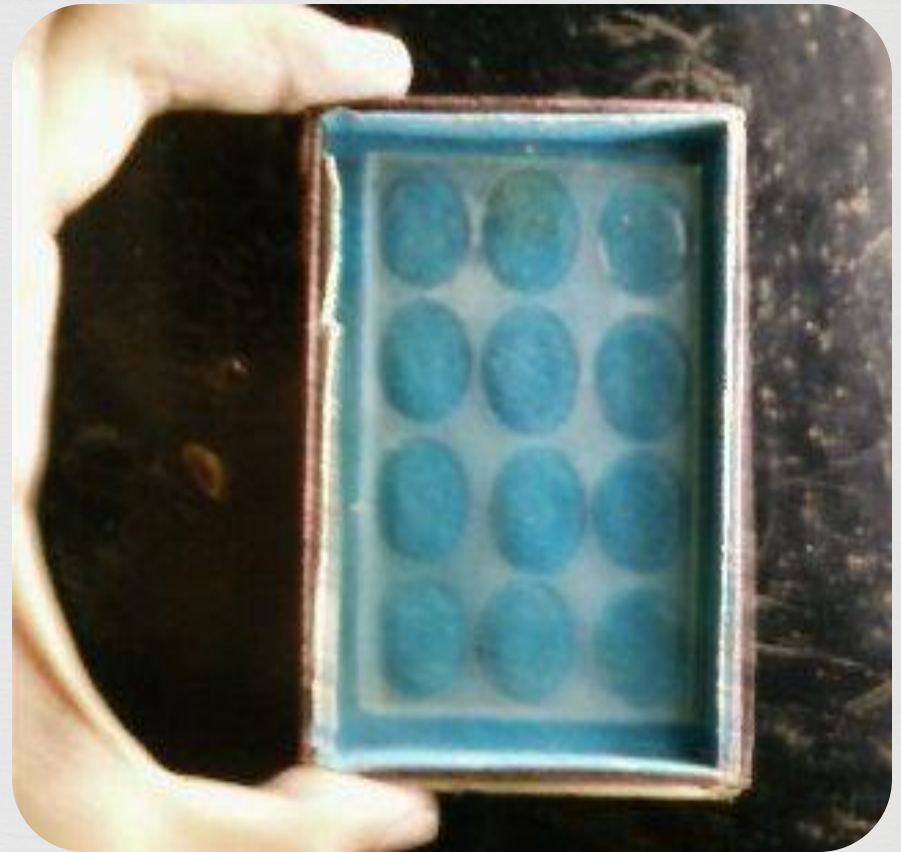


DIAGNOSIS OF SYPHILIS

-
- ❧ 1. History and clinical examination.
 - ❧ 2. Dark-field microscopy: special technique use to demonstrate the spirochete as shiny motile spiral structures with a dark background.
 - ❧ The specimen includes oozing from the lesion or sometimes L.N. aspirate. It is usually positive in the primary and secondary stages and it is most useful in the primary stage when the serological tests are still negative.

Provisional Diagnosis of Syphilis

∞ A presumptive diagnosis is possible with sequential serologic tests (e.g. VDRL, RPR), using the same testing method each time. A fourfold change in titer (e.g. from 1:8 to 1:32) is usually considered to be clinically significant. Confirmatory tests should be performed



DIAGNOSIS OF SYPHILIS



- ∞ 1. History and clinical examination.
- ∞ 2. Dark-field microscopy: special technique use to demonstrate the spirochete as shiny motile spiral structures with a dark background.
- ∞ The specimen includes oozing from the lesion or sometimes L.N. aspirate. It is usually positive in the primary and secondary stages and it is most useful in the primary stage when the serological tests are still negative.

Other Diagnostic Tests



- ❧ Serological tests of syphilis.
- ❧ 4. Biopsy rarely needed. It shows endarteritis obliterans with inflammatory reaction containing a plenty of plasma cells. Granuloma may found in tertiary syphilis.



Laboratory Testing

- ❧ Direct examination of clinical specimen by **dark-field microscopy** or fluorescent antibody testing of sample.
- ❧ **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.

SERLOGICAL TESTS OF SYPHILIS: STS



- ❧ 1. **Non-specific or lipoidal tests**
- ❧ A. **VDRL (venereal disease research laboratories)**
 - It is useful for the screening, diagnosis and follow up.
 - The results can be qualitative or qualitative
- False positive results**
- ❧ 1. Acute type: usually low titre and don't persist for more than 6 months
- ❧ 2. Chronic type: usually last for more than 6 months
- ❧ B. **Rapid plasma reagin test.**
- ❧ C. **Wasserman test not used more**

Specific serological tests of syphilis



- ∞ A. Reiter protein complement fixation test.
- ∞ B. Fluorescent Treponemal antibody/absorption test, FTA/ABS. the most specific and most sensitive .
- ∞ C. **Treponema pallidum haemagglutination test- TPHA-** D. Treponema pallidum immobilization test- TPI

Diagnosis of Syphilis

- ∞ Evaluation based on three factors:
 - ∞ Clinical findings.
 - ∞ Demonstration of spirochetes in clinical specimen.
 - ∞ Present of antibodies in blood or cerebrospinal fluid.
- ∞ More than one test should be performed.
- ∞ No serological test can distinguish between other Treponemal infections.

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.
- ❧ Calibrated syringe utilized to dispense antigen **must deliver 60 drops/mL +/- 2drops.**

VDRL



☞ Performing the test:

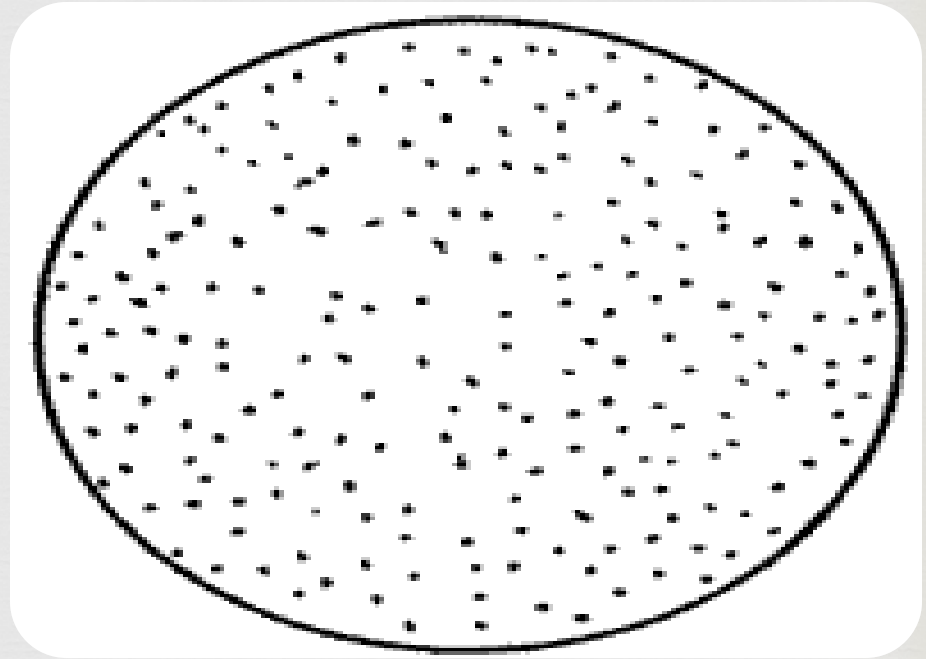
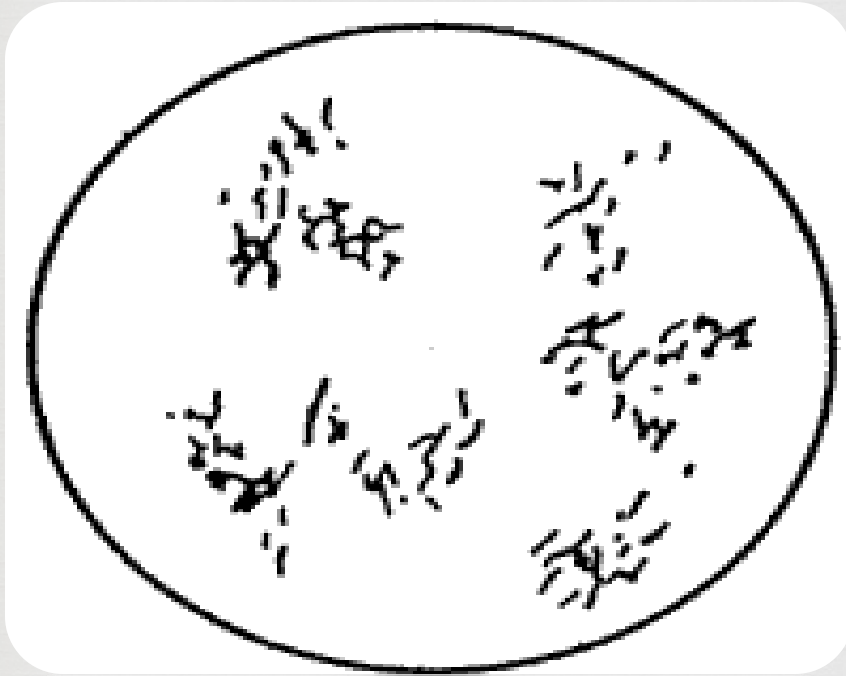
- ☞ 0.05 mL of serum added to circle on ceramic slide and spread.
- ☞ Add one calibrated drop of antigen to each circle.
- ☞ Rotate at **180 rpms** for 4 minutes.
- ☞ **Read microscopically** at 100x and grade reaction if positive.
- ☞ Perform titer on positive samples, report out titer.

☞ Quality control:

- ☞ Run three levels of control: Non-reactive, weakly reactive and reactive.
- ☞ Glass syringe with 18g delivery needle must be checked daily to ensure delivery of **60 drops/mL**.
- ☞ Rotator rpms must be checked to ensure 180 rpms.
- ☞ Room temperature must be 23-29 C.

☞ VDRL used primarily to screen cerebral spinal fluid.

VDRL



- ☞ Each preparation of antigen suspension should first be examined by testing with known positive or negative serum controls.
- ☞ The antigen particles appear as short rod forms at magnification of about 100x. Aggregation of these particles into large or small clumps is interpreted as degrees of positivity
- ☞ Reactive on left, non-reactive on right

Rapid Plasma Reagin Test - RPR

- ❧ General screening test, can be adapted to automation.
- ❧ **CANNOT** be performed on CSF.
- ❧ 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.

RPR

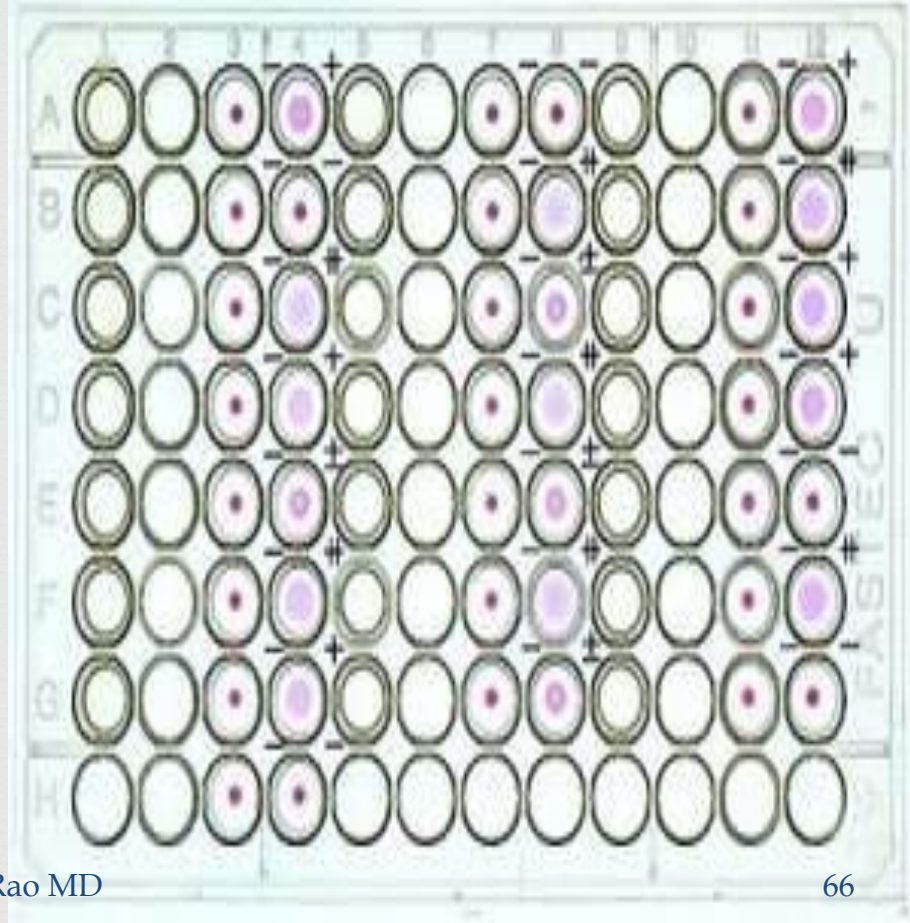


- ❧ Test Procedure:
 - ❧ Serum or plasma added to circle on card and spread.
 - ❧ One drop of antigen from a needle **capable of delivering 60 drops/mL** is added.
 - ❧ Rotate at **100 rpms/minute for 8 minutes.**
 - ❧ Results are read **macroscopically.**
- ❧ Daily quality control:
 - ❧ 20 gauge needle checked for delivery of 60 drops/mL
 - ❧ Rotator checked for 100 rpms/minute
 - ❧ Room temperature must be 23-29 C.
 - ❧ Three levels of control must be run and give appropriate results.
- ❧ RPR appears to be more sensitive than the VDRL.

Treponema pallidum *haemagglutination (TPHA)*



- Adapted to micro techniques (MHA-TP)
- Tanned sheep RBCs are coated with *T. pallidum* antigen from Nichol's strain.
- Agglutination of the RBCs is a positive result.



Treponema pallidum Haemagglutination (TPHA)

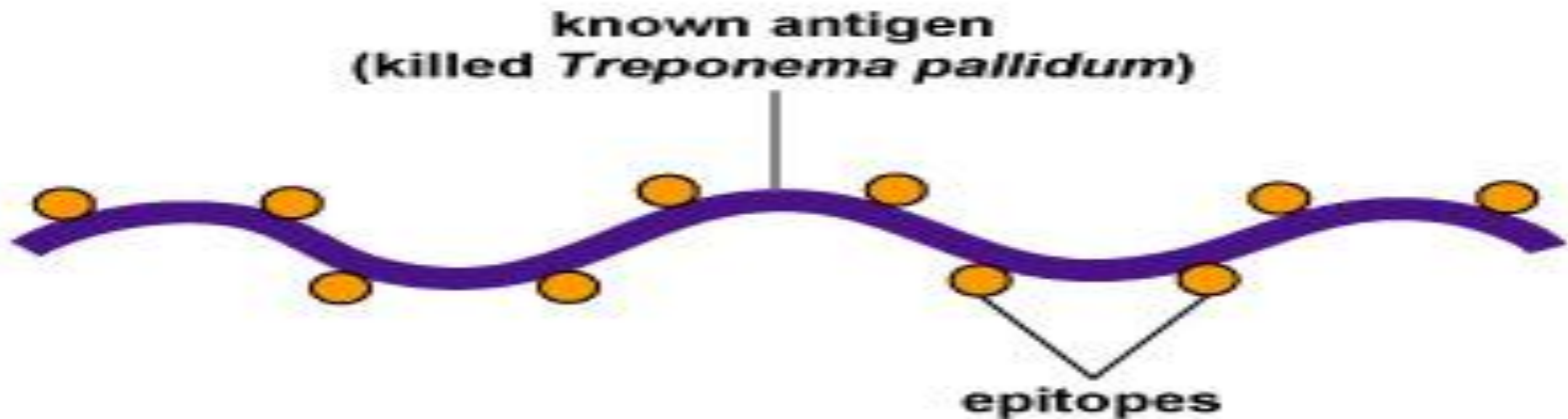
- Based on the agglutination of colored gelatin particle carriers sensitized with T. pallidum antigen.
- Patient sera incubated with sensitized particles in microliter wells and sensitized gelatin particles in control wells.
- Patient sera containing specific antibodies will react only with the antigen to form a smooth mat of agglutinated particles.
- A compact button formed by the settling of the non-agglutinated particles in the microtiter wells containing sensitized particles indicates lack of specific antibody in patient sera (-).
- If agglutination is seen with both sensitized and unsensitized particles, nonspecific agglutination is indicated.

Fluorescent Treponemal Antibody Absorption Test (FTA-ABS)

- ∞ Diluted, heat inactivated serum added to Reiter's strain of *T. pallidum* to remove cross reactivity due to other *Treponemes*.
- ∞ Slides are coated with Nichol's strain of *T. pallidum* and add absorbed patient serum.
- ∞ Slides are washed, and incubated with 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.

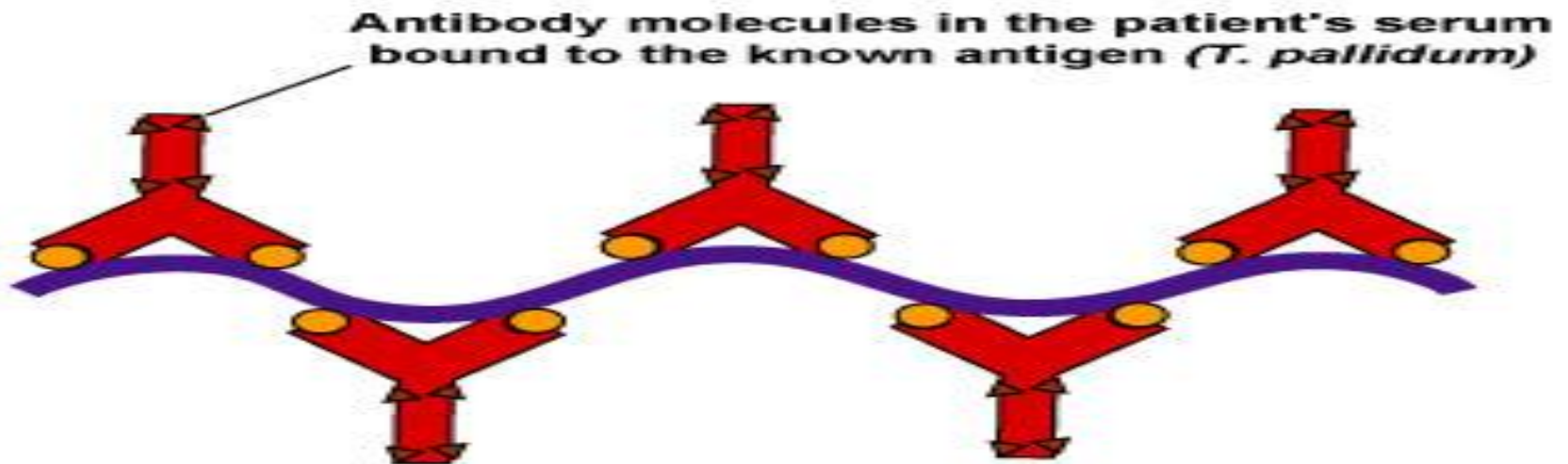
FTA-ABS Step 1

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



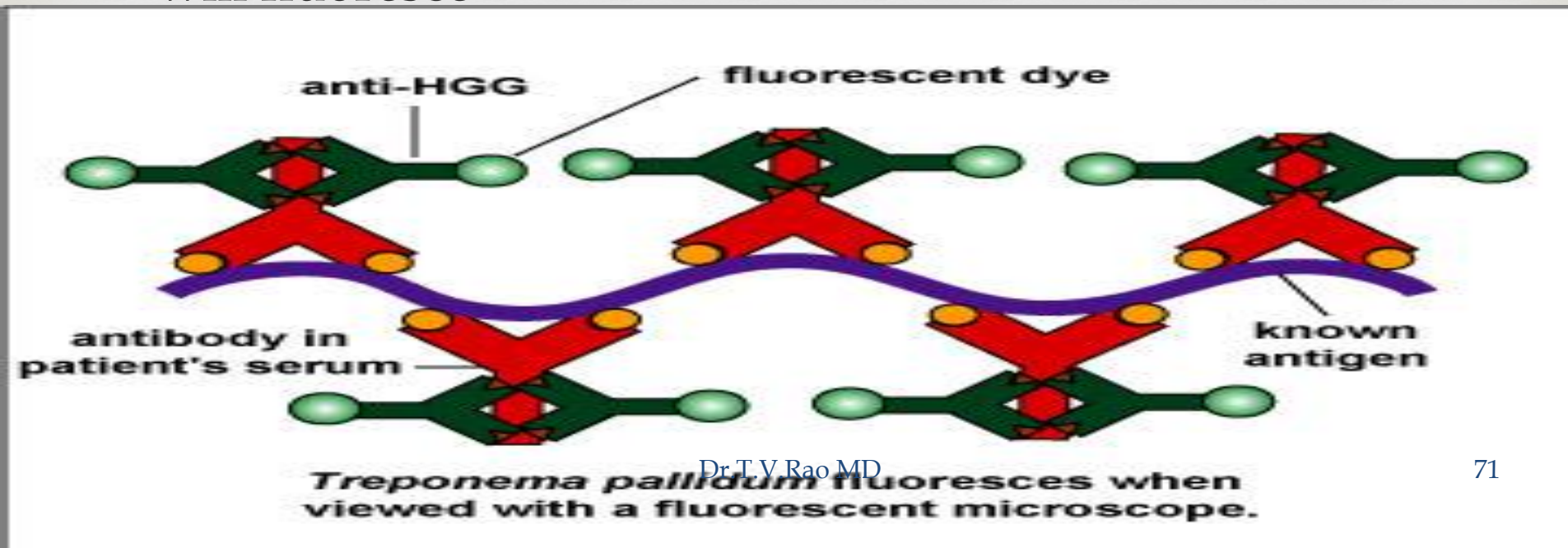
FTA-ABS – Step 2

- ☞ 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.

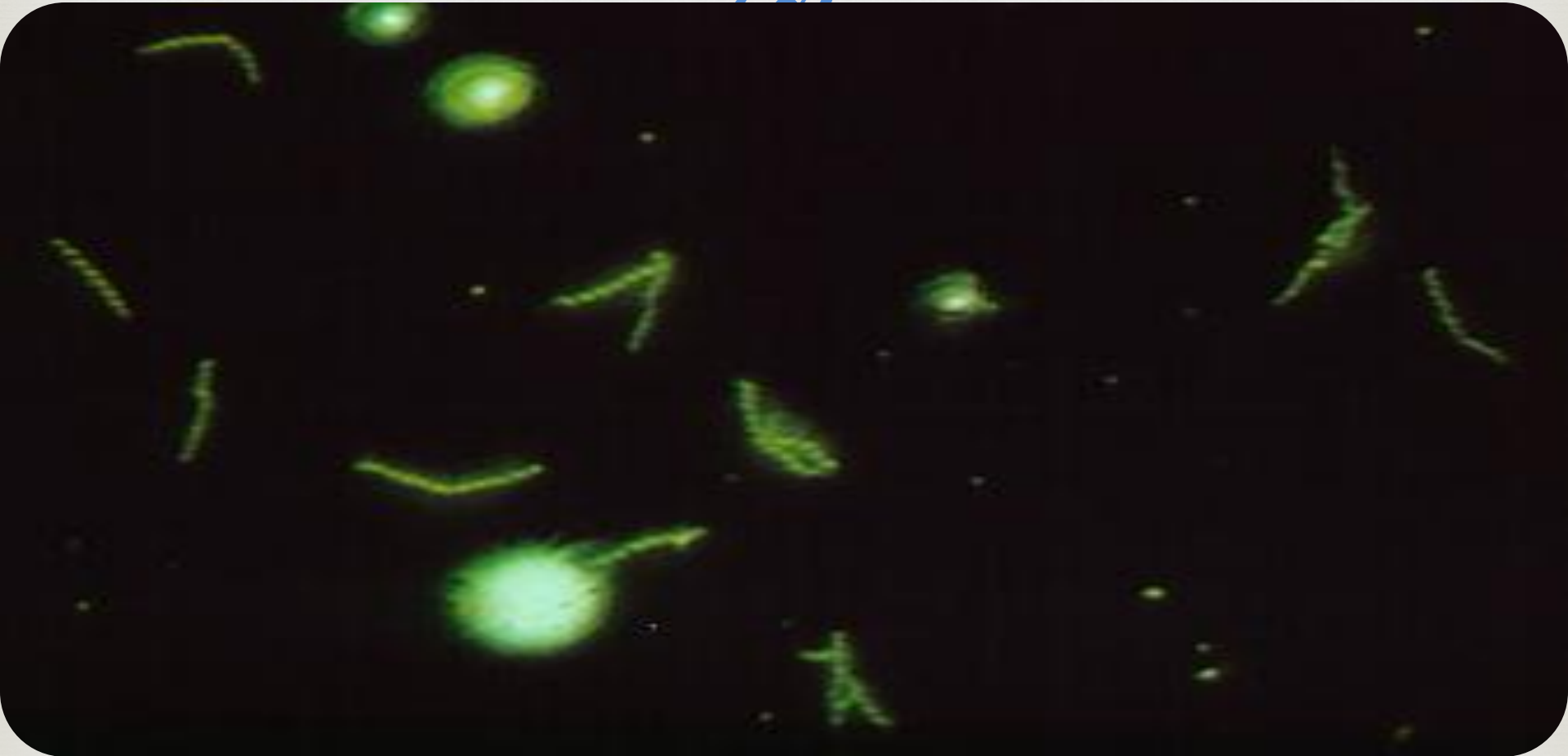


FTA-ABS- Step 3

- Fluorescent anti-human gamma globulin (anti-HGG) is added to the well.
- The anti-HGG will bind with human IgG antibodies bound to the *Treponema pallidum* on the slide.
- All unbound anti-HGG is washed from the slide.
- Viewed with a fluorescent microscope, the spirochetes will fluoresce



Positive FTA Test for Syphilis Viewed with a Fluorescent Microscope

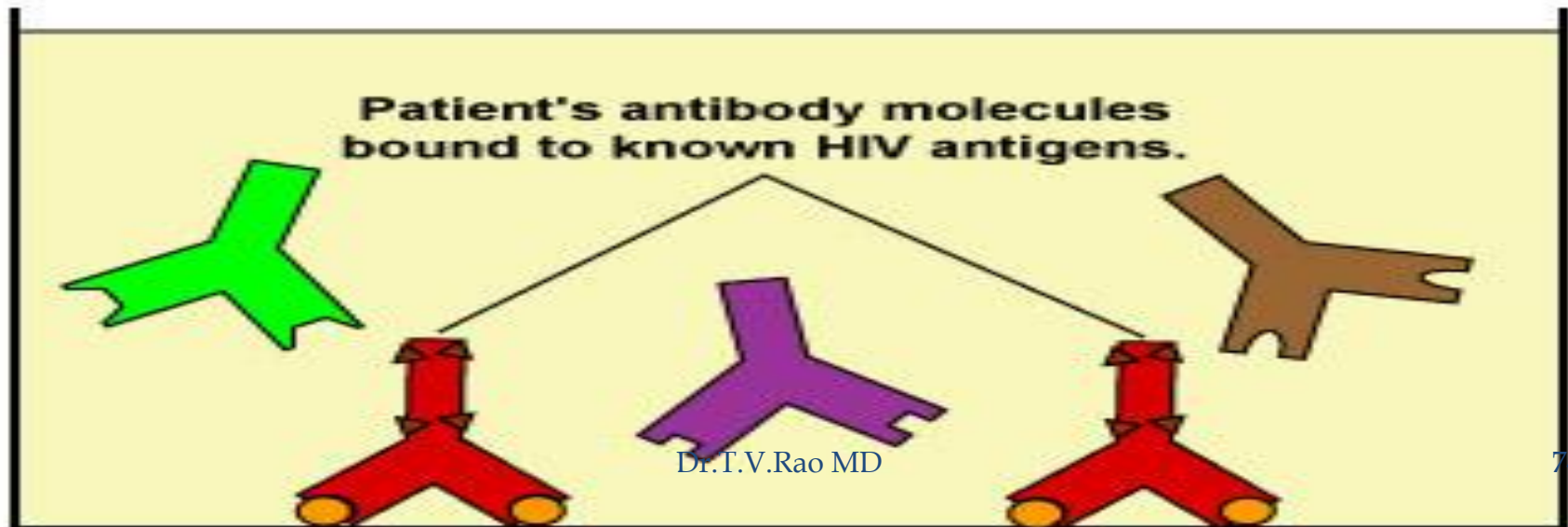


ELISA

- ❧ 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.

Enzyme Immunoassay for HIV Antibodies

- Step 2 - The patient's serum is added.
- If the serum contains antibodies against the known HIV antigens, they will bind to those antigens.
- All other antibodies are then washed from the well.



Enzyme Immunoassay for HIV Antibodies

- Step 3 - Enzyme-linked anti-human gamma globulin (anti-HGG) is added to the well.
- The anti-HGG will with any human IgG antibodies bound to the adsorbed HIV antigens.
- All unbound anti-HGG is then washed from the well.

Anti-HGG with attached enzyme binds to human antibody molecules bound to known HIV antigens.



Non Treponemal diseases too React

☞ The nontreponemal tests, VDRL and rapid plasma reagent (RPR), are antilipoidal antibodies seen in other disease states, pregnancy, and occasionally after vaccination. They are nonspecific and cannot rule in disease. These tests have sensitivities approaching 80% in patients with symptomatic primary syphilis and virtually 100% in patients with secondary syphilis

Every Pregnant women Needs Screening



Congenital Syphilis



- Passed from mother to fetus during pregnancy
- Abnormally shaped teeth
- Nasal septum collapses
- Skeletal abnormalities

TREATMENT OF SYPHILIS:



- Early syphilis:
 - benzathine penicillin 2.4 million units intramuscularly once
 - procaine penicillin 600,000 units intramuscularly daily for 10 days
 - if the patient is unable to take penicillin, then give tetracycline or erythromycin 500 mg 4 times a day by mouth - or doxycycline 100 mg x2- for 15 days.
- Ceftriaxone, 2 gm qd IM/IV for 10-14 d is a new alternative treatment

Treatment of Late Syphilis

- ❧ Late syphilis:
 - ❧ benzathine penicillin 2.4 million units intramuscularly weekly for 3 weeks.
 - ❧ procaine penicillin 1.2 million units intramuscularly daily for 21 days
 - ❧ Tetracycline or erythromycin 500 mg 4 times a day – or doxycycline 100 mg x2- by mouth for 30 days
- ❧ Jarrisch-Herxheimer reaction
- ❧ Follow-up

April is

STD

Month

Awareness



Created by Dr.T.V.Rao MD
for 'e' learning resources for
Microbiologists in the
Developing World

Email

doctortvrao@gmail.com