LECTURE XI

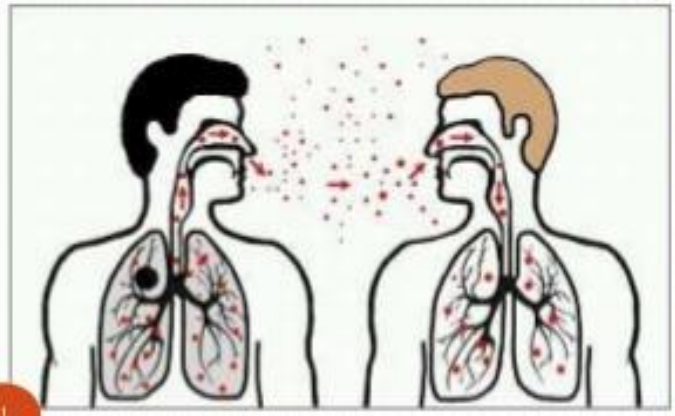
Pathogenic bacteria of Mycobacterium and Actinomyces genera.Pathogenic spirochetes, rickettsiae, chlamydia and mycoplasma.

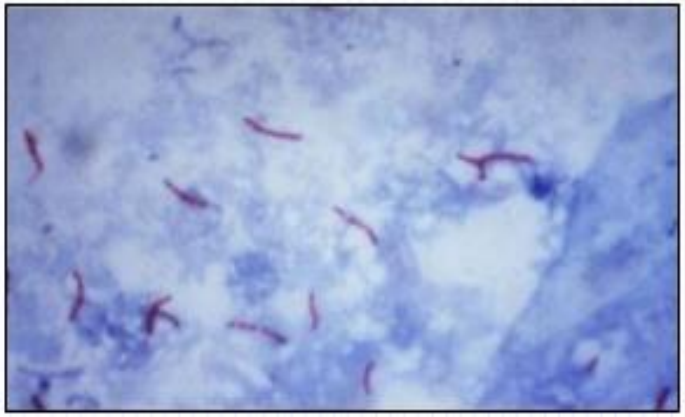
INTRODUCTION

- Tuberculosis is a worldwide public health problem

PULMONARY TUBERCULOSIS

MYCOBACTERIUM TUBERCULOSIS





Classification of Mycobacteria

1. **Tubercle bacilli**
   a) **Human – MTB**
   b) Bovine – *M. bovis*
   c) Murine – *M. microti*
   d) Avian – *M. avium*
   e) Cold blooded – *M. marinum*

   MTB Complex (*M. africanum* also included)

2. **Lepra bacilli**
   a) Human – *M. leprae*
   b) Rat – *M. leprae murium*
3. **Mycobacteria causing skin ulcers**
   a) *M. ulcerans*
   b) *M. belnei*
4. **Atypical Mycobacteria (Runyon Groups)**
   a) Photochromogens
   b) Scotochromogens
   c) Nonphotochromogens
   d) Rapid growers
5. **Johne's bacillus**

   M. paratuberculosis
6. **Saprophytic mycobacteria**
   a) *M. butyricum*
   b) *M. phlei*
   c) *M. stercoralis*
   d) *M. smegmatis*
   e) Others

What are Mycobacteria?

- Obligate **aerobes** growing most successfully in tissues with a high oxygen content, such as the lungs.
- Facultative **intracellular pathogens** usually infecting **mononuclear phagocytes** (e.g. macrophages).

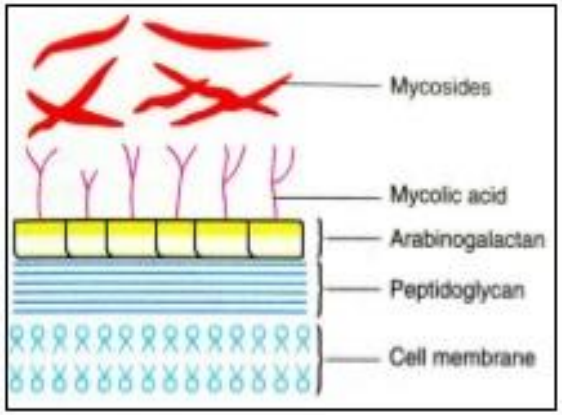
Mycobacterium differ from other routinely isolated Bacteria

- **Slow-growing** with a generation time of 14 to 15 hours (20-30 minutes for *Escherichia coli*).
- **Hydrophobic** with a high lipid content in the cell wall. As they are hydrophobic and tend to clump together, they are impermeable to the usual stains,

  e.g. **Gram's stain**

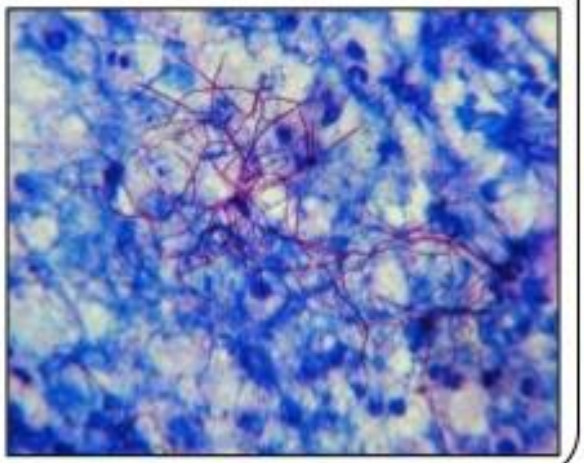
Acid fast bacilli

- Known as **"Acid-fast bacilli"** because of their lipid-rich cell walls, which are relatively impermeable to various basic dyes unless the dyes are combined with phenol.



How they are Acid fast

- Once stained, the cells resist decolourization with acidified organic solvents and are therefore called "acid-fast". (Other bacteria which also contain mycolic acids, such as *Nocardia*, can also exhibit this feature.)



Mycobacterium tuberculosis

MORPHOLOGY:-

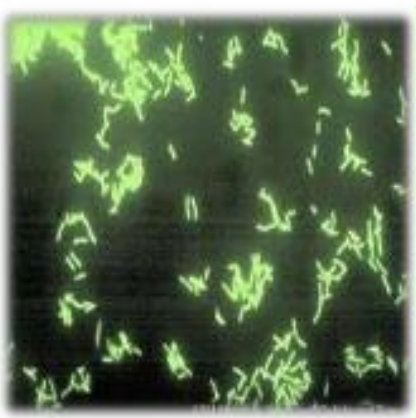
- Slender, straight or slightly curved bacilli with rounded ends, occurring singly or in pairs or in clumps.
- Non-sporing, non-capsulated and non-motile.

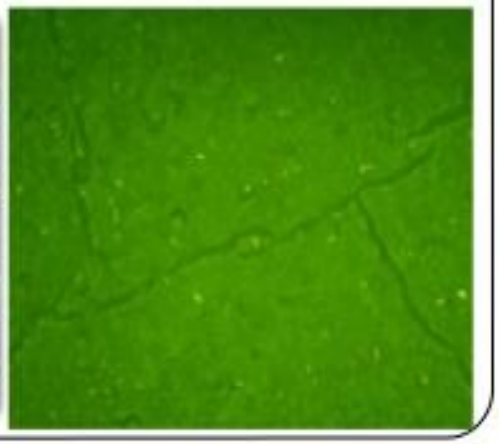
1. **Ziehl Neelsen stain** – stained by carbol fuschin; heat melts wax; resist decolourisation by 20% sulphuric acid . Resist decolourization by absolute alcohol.

   (Acid fast and alcohol fast)

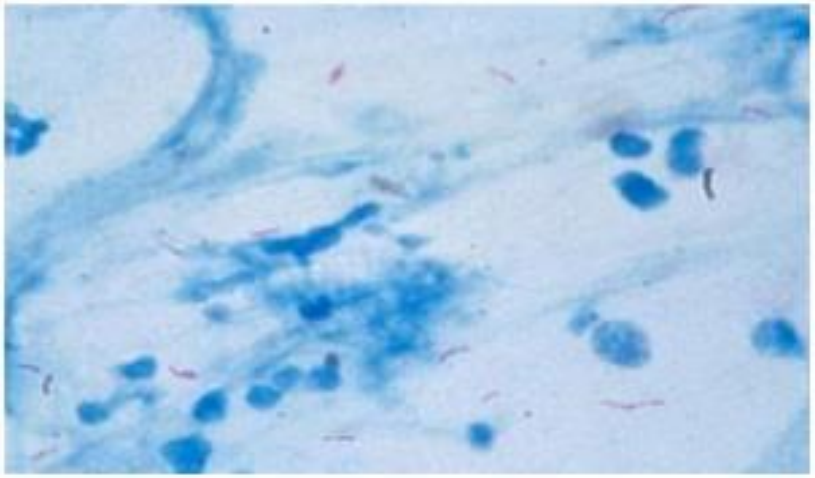
2. **Auramine rhodamine stain**

   (fluorescent stain)



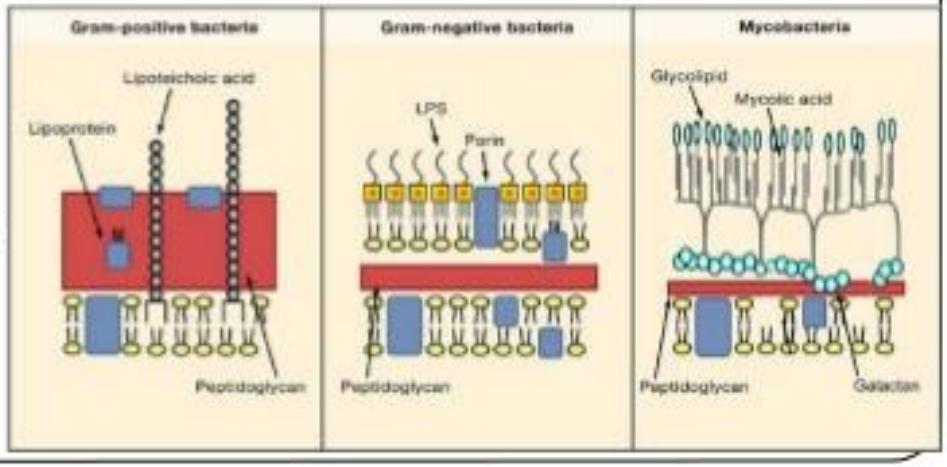


- **Acid fast bacilli**
- Straight or slightly curved.
- 1- 4 x 0.2-0.8 µm.
- Single, small clumps, pairs, long filamentous forms may be seen.
- Other (bacteria, cells – stained blue by)
- Counter stain (methylene blue)



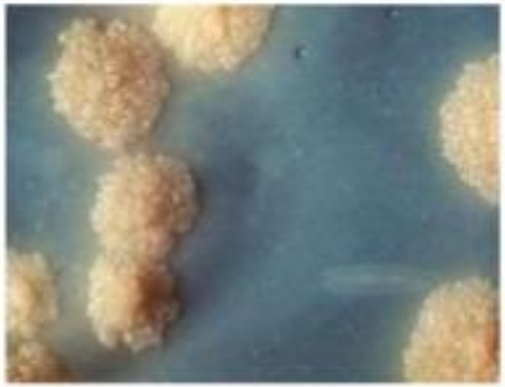
COUNTER STAINS USED:-

- Methylene blue – Blue background
- Malachite green – Green ”



CULTURAL CHARACTERS:-

- Aerobe.
- Growth stimulation by 5-10% CO_2
- Bacilli grow slowly, generation time 14- 15 hrs.
- Colonies appear in about two weeks or delayed upto 6-8 weeks.
- Optimum temp. 37°c
- Optimum pH 6.4-7.0
- Colonies - rough, tough and buff
- *M. tuberculosis* – obligate aerobe
- *M. bovis* – Microaerophilic



1. Solid media:-

i. Containing egg – **Lowenstein Jensen**, Petragnin, Dorset's egg.
ii. Containing blood – Tarshis medium.
iii. Containing potato – Pawlowsky's medium.

- Medium most commonly used is Lowenstein Jensen medium contain:-
  i. Coagulated hen's eggs (neutralise fatty acid)
  ii. Glycerol (C source)
  iii. Mineral salt solution
  iv. Asparagines (nitrogen source)
  v. Malachite green (inhibits growth of other bacteria)

2. Liquid media:-

- Dubo's, Middlebrooke's, Prouskeur & Beck's, Sula's & Sauton's.

- Liquid media useful for – sensitivity tests, for extraction of Ag & vaccines.
  i. Growth in liquid media- pellicle at surface.
  ii. Dubo's medium with tween 80 – diffuse growth

- **Virulent strain – Serpentine cords**
- **Avirulent strain – Dispersed growth.**

- Tubercle bacilli also grow in chick embryo & tissue culture.

RESISTANCE :

- Not heat resistant
- Resistant to chemical disinfectants like phenol
- Destroyed by tincture iodine -5 min
- 80% ethanol – 2-10 minutes
- Sensitive to formaldehyde and glutaraldehyde

VIABILITY :

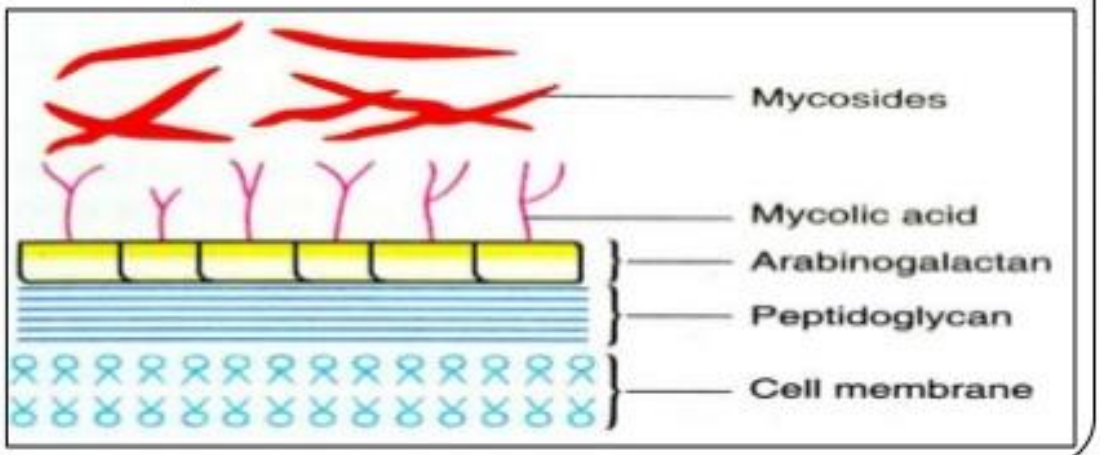
- Sputum – 20-30 hrs
- Droplets - 8-10 days
- Cultures- 6-8 months

Antigenic Structure

- **Cell Wall Antigens:**
  - Peptidoglycan layer
  - Arabinogalactan layer
  - Mycolic acid layer
  - Mycosides

- **Cytoplasmic Antigens (Protein antigens)**

- **Mycolic Acid**
  - Difficult to stain.
  - Difficult to phagocytose.
  - Intracellular survival.
  - Hypersensitivity.
  - Slow growth.
  - Resistant to heat and chemical disinfectants.



Virulence Factor:

- **Cord factor- Trehalose 6-6 dimycolate**, is a glycolipid molecule found in the cell wall of *Mycobacterium tuberculosis* and similar species. It is the primary lipid found on the exterior of *M. tuberculosis* cells.
  - **Serpentine growth** (filaments, cords) grows in close parallel arrangement.
  - Toxic to leukocytes
  - Role in development of granulomatous lesions

- **Sulfolipids-** Sulfated glycolipid (sulfatide) prevent phagosome- lysosome fusion which is important for intracellular survival.

IMMUNITY :

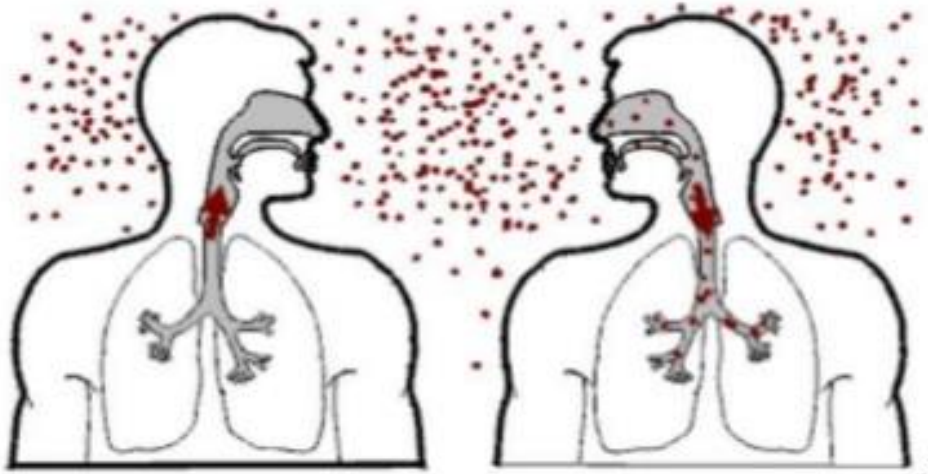
- Following injection by tubercle bacilli, delayed hypersensitivity develops against tuberculoprotein. Antibodies also develop but they don't have any diagnostic value and not relevant in immunity. Immunity in tuberculosis is mainly cell mediated by sensitized T-lymphocytes and macrophages.

- Tubercle Bacilli do not produce any toxin. Various bacterial components have biological effects.

  - **Cell wall** – Causes **Delayed Hypersensitivity**.
  - **Tuberculoprotein** – Induces D.H. Formation of cellular reaction of lymphocytes, monocytes, macrophages, epitheloid cells & giants cells.
  - **Lipids**- Accumulations of macrophages and neutrophils.

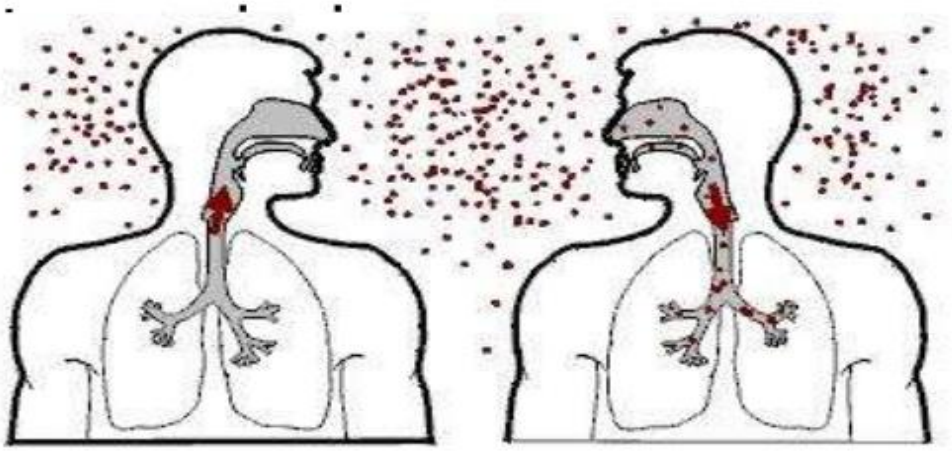
How tuberculosis spreads

- Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel bacilli into the air. A person needs only to inhale a small number of these to be infected.



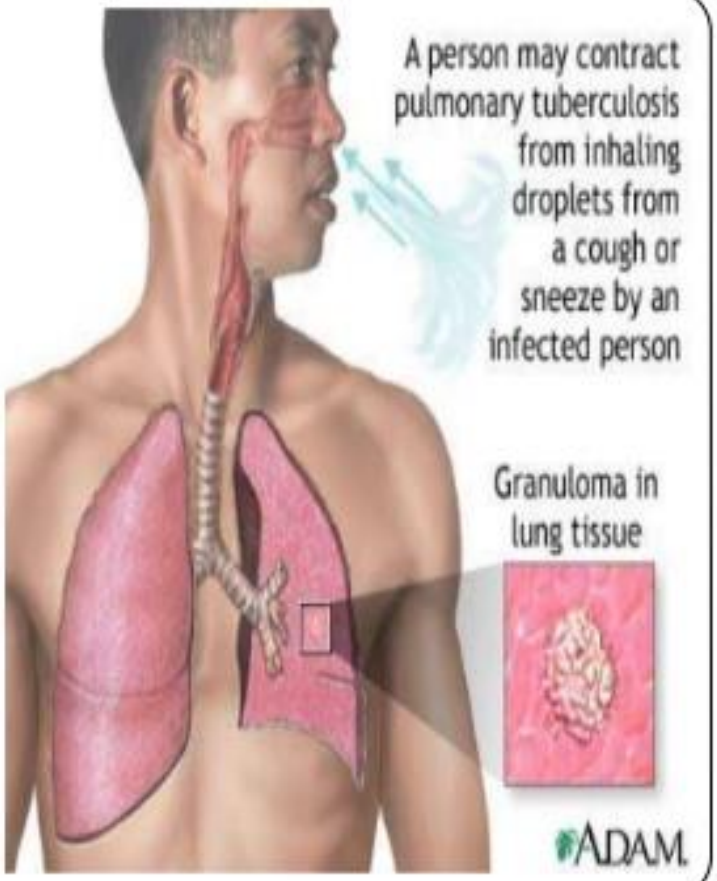
TRANSMISSION

- TB spreads from person to person by airborne



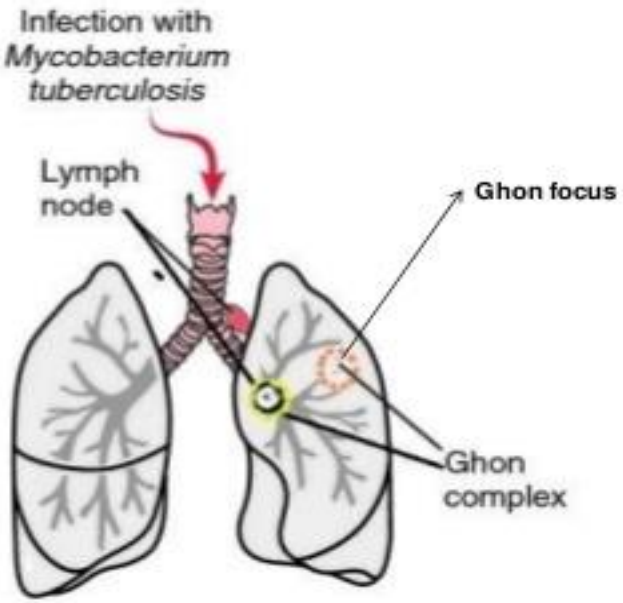
PATHOGENICITY:-

- *M. tuberculosis* can infect any organ or tissue but most commonly lungs are infected; intestines, kidneys, bones, soft tissues, brain etc.
- Infection acquired by inhalation of infected droplets.
- Engulfed by macrophages but survive and multiply.
- Lyses host cell and infect other macrophages.



Primary Tuberculosis:

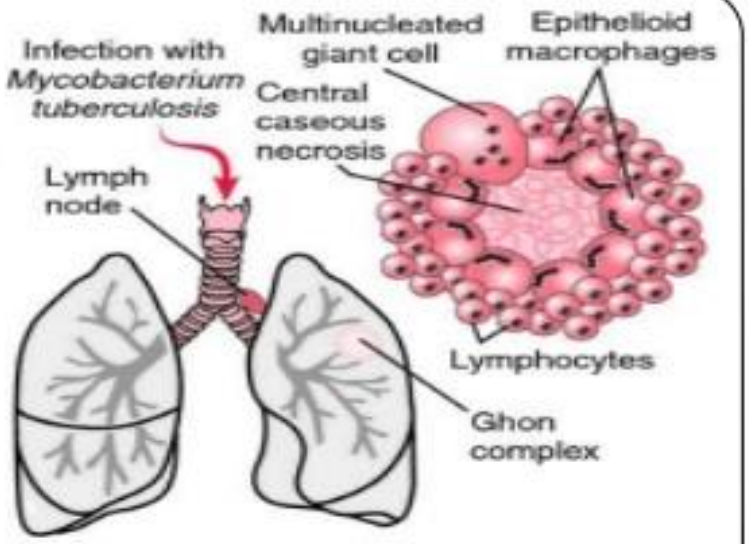
- Mostly asymptomatic.
- Some may have flu like symptoms; chest pain, mild fever and lack of appetite.
- Within 3 weeks, cell mediated immunity checks the bacilli.
- Engulfed bacilli in alveoli forms a lesion called **Ghon focus** in lower lobe. (Anton Ghon, Austrian pathologist)

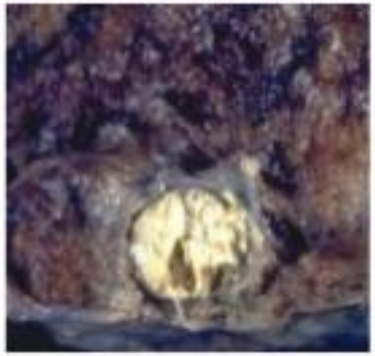


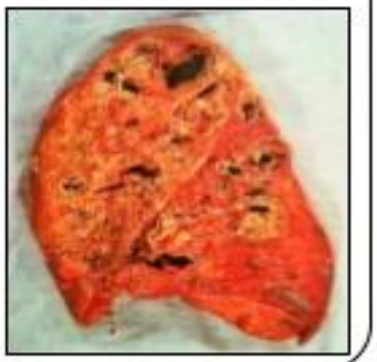
- Some bacilli are transported **to hilar lymph nodes**.
- Ghon focus together with the enlarged hilar lymph nodes is called **Primary Complex (Ghon Complex)**. (Karl Ernst Ranke, German pulmonologist)

Secondary Tuberculosis:

- Caused by reactivation (**immunosuppression**) of the primary lesion.
- Spreads to upper lobes.
- **Granuloma** occurs in apex of lungs.
- Memory T cells releases cytokines.
- Causes tissue destruction and necrosis called **tuberculomas (caeseous necrosis)**.
- Cavities may rupture into blood vessels, spreading bacilli throughout body and in sputum. Causing systemic **Miliary tuberculosis**.

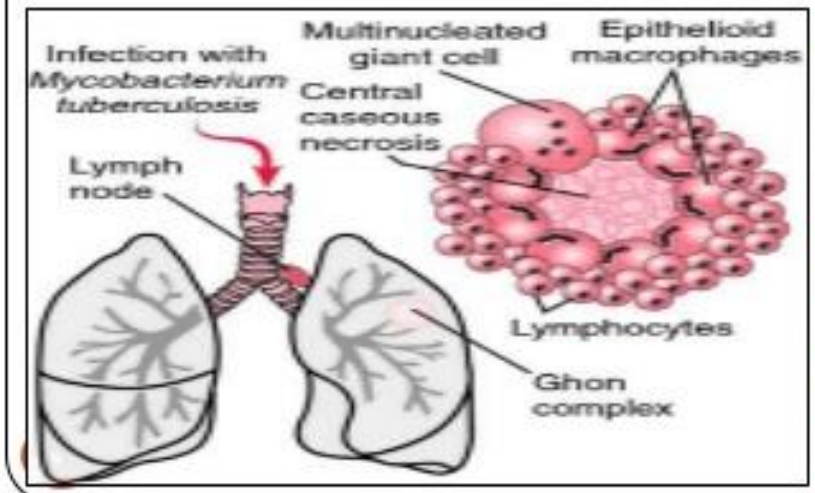


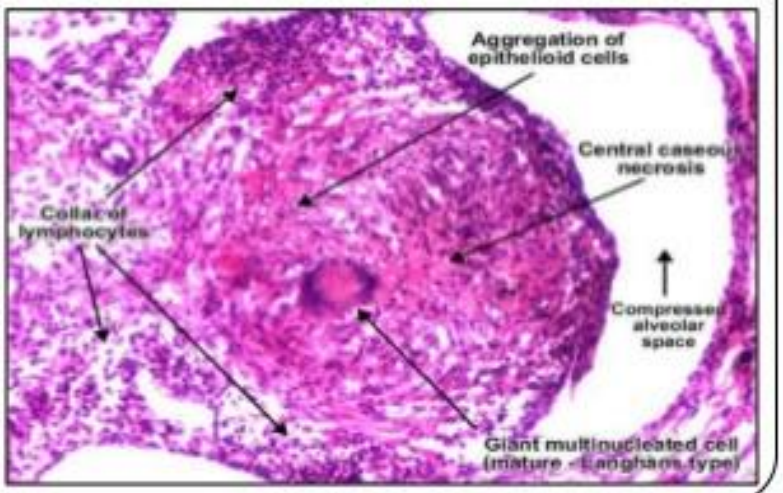




Secondary Tuberculosis: (in 10% cases caused by)

- HIV infection
- Alcoholism and liver cirrhosis
- Malnutrition
- Diabetes
- Steroid and immunosuppressive therapy
- Old age

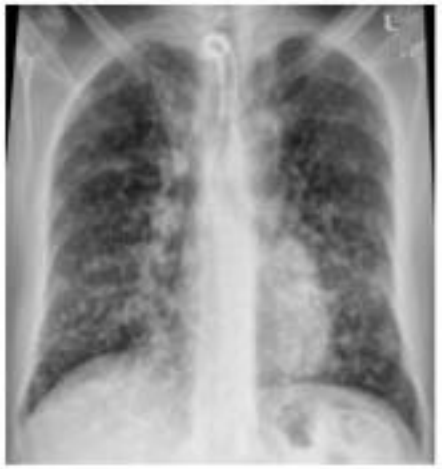


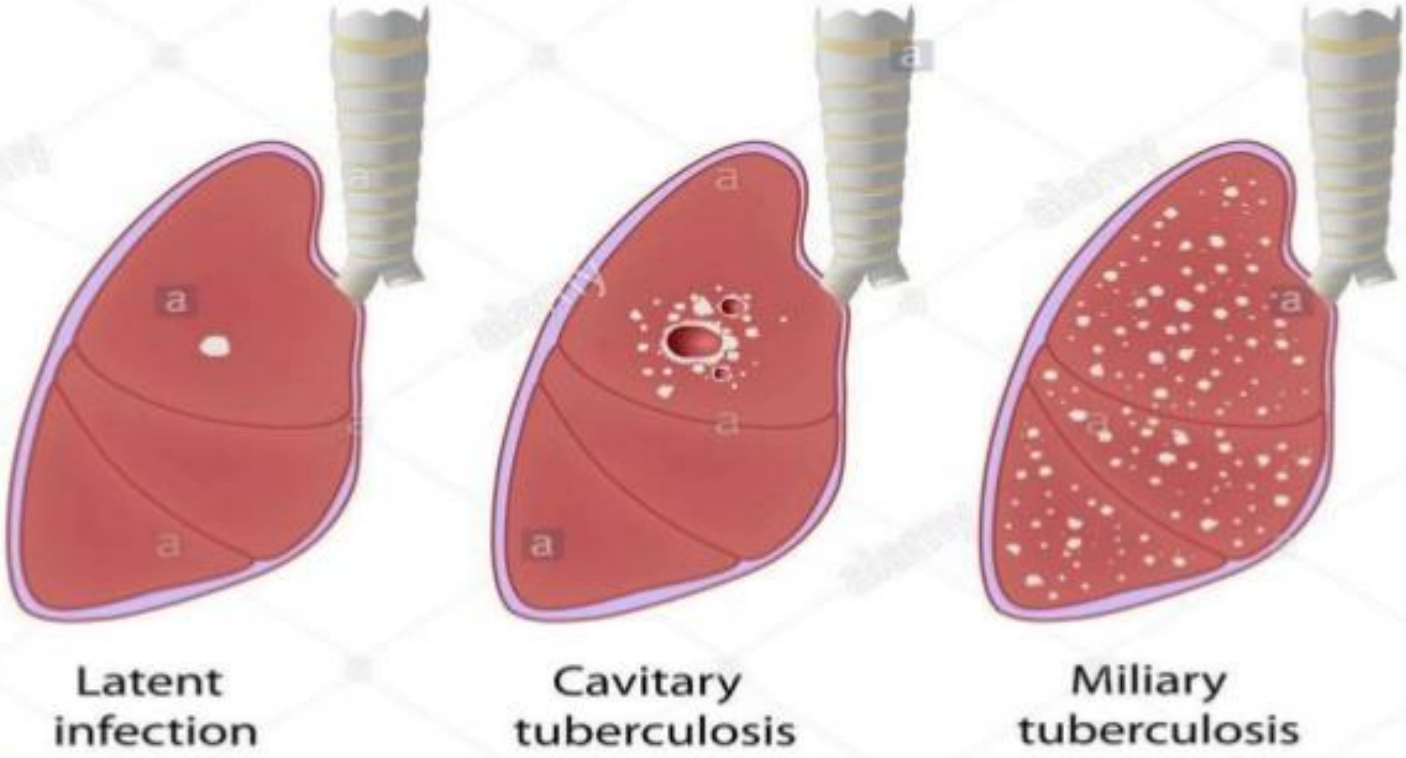


Secondary Tuberculosis:

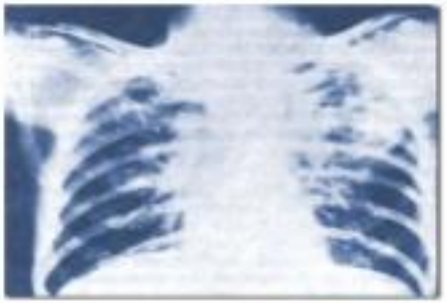
- Miliary tuberculosis may develop in any organ of the body.
- Certain tissues like heart, striated muscles, thyroid and pancreas are resistant.
- Localization sites are the bone marrow, eye, lymph nodes, liver, spleen, kidneys, adrenal, prostate, seminal vesicles, fallopian tubes, endometrium and meninges.

- **Clinical signs:**
  - Temperature elevation usually in mid-afternoon, night sweats, weakness, fatigability, loss of appetite and weight.
  - Productive cough, blood streaked sputum (hemoptysis)

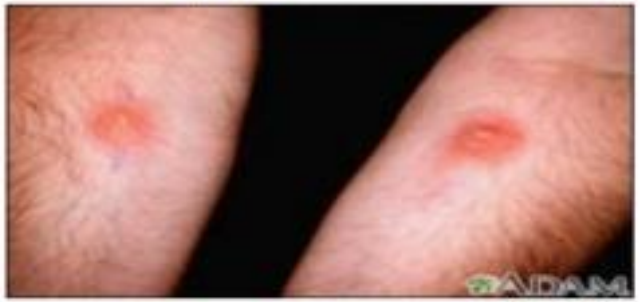



Latent infection
Cavitary tuberculosis
Miliary tuberculosis

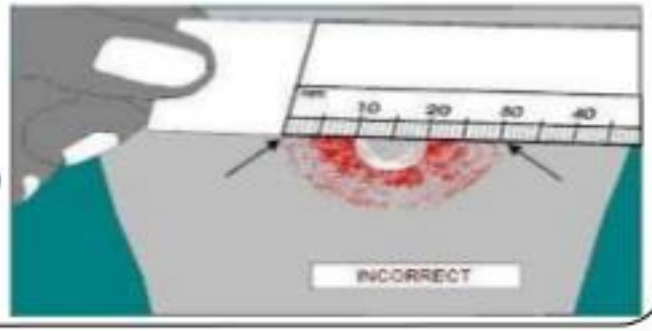
OTHER METHODS OF DIAGNOSIS OF TUBERCULOSIS:-



1. **X-ray chest**
2. **Blood exam** – lymphocytosis, increased ESR
3. **Mantoux test** – Tuberculin test.



- Routinely 5TU is used. 0.1 ml of PPD is injected intradermally in forearm. The area is marked by pen do not press or wash.
- Readings taken after 48-72 hrs.
- Erythema & indurations > 10mm – positive
  < 5mm – negative (+ in HIV)
  6-9mm – equivocal



TREATMENT

FIRST LINE DRUG:-

- Rifampicin(R) & Pyrizinamide (Z) – kill bacilli in lesions
- Isoniazid (H) – kills replicating bacilli
- Streptomycin (S) – kills extracellular bacilli
- Ethambutol (E) – bacteristatic

- **Intensive phase** – 3 times a week, 2 months – H, E, R, Z
- **Continuing phase** – 3 times a week, 4-5 months – H, R

SECOND LINE DRUG:-

- Quinolones, Aminoglycosides, Macrolides, Thiacetazone, Cycloserine, Capneomycin.

- **MDR-TB** – Resistance to Rifampicin & Isoniazid ; **DOTS** (directly observed therapy under supervision) important.

Actinomycetes

- Fungus-like characteristics
  - Branching filaments in tissues / culture
  - looks like mycelia
- Filaments frequently segmented
  - Pleomorphic forms (Diphtheroid & club shaped)
- Cell wall and the internal structures are typical of bacteria.
- Aaerobic OR Anaerobic.
- Slow growers

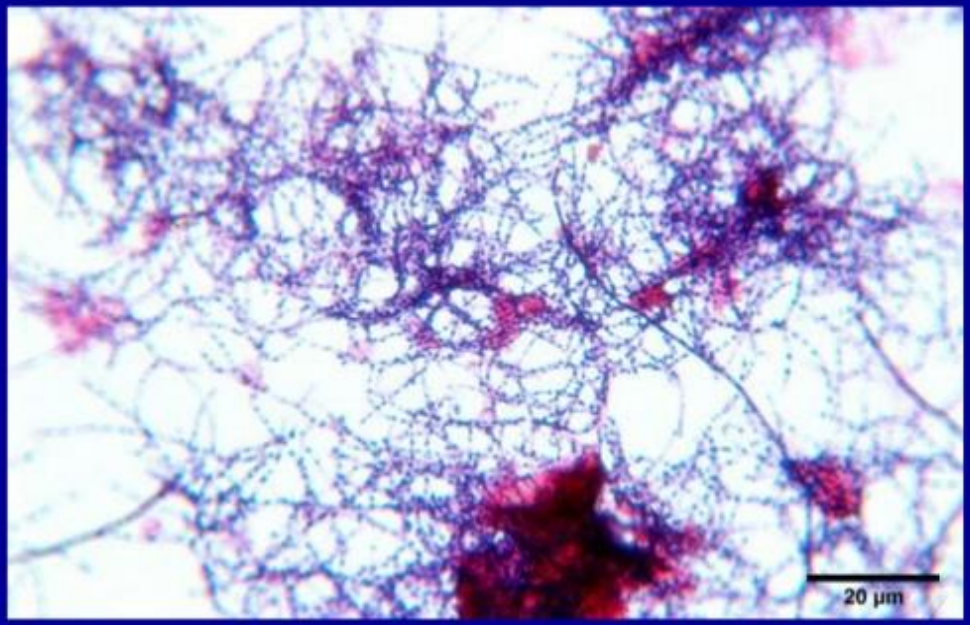
Actinomycetes

- Classification
  - Anaerobic
    - Actinomyces spp
  - Aerobic
    - Nocardia spp
    - Actinomadura spp
    - Streptomyces spp

Actinomyces

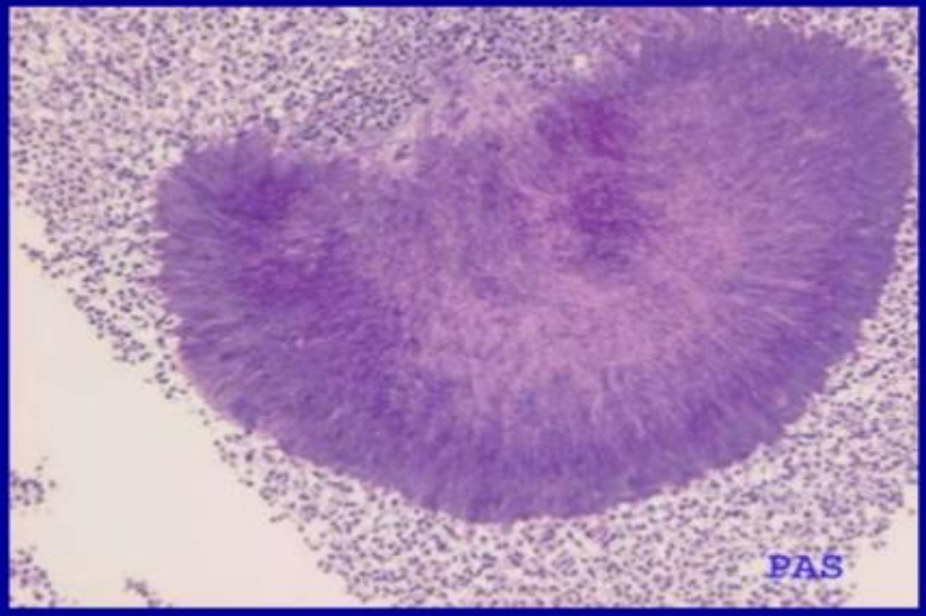
- Anaerobic Actinomycetes
  - Morphology and cultural characteristics
    - Gram positive branching, or diphtheroid-like bacilli
    - Anaerobic and require CO_2 for growth
    - Non-sporing
    - Grows well on Blood Agar.

Actinomyces – Gram stain

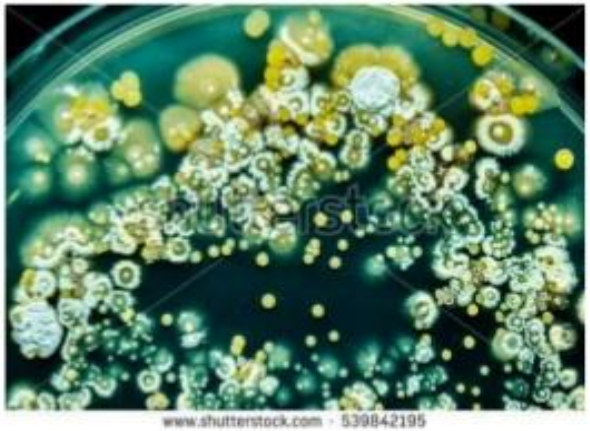


Actinomycetes - Gram staining

Ray fungus



Acrtinomycetes - culture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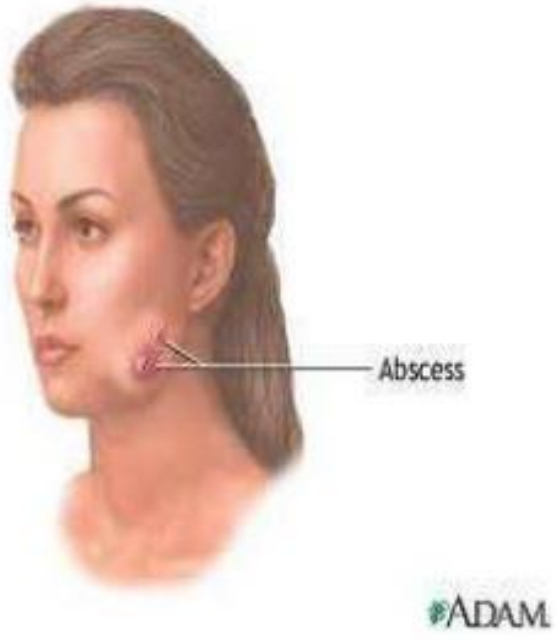
Actinomyces

- Clinical significance
  - Part of the normal oral bacterial flora in humans and animals.
  - Three clinical types
  - Cervico facial actinomycosis or "lumpy jaw"
    - » occur following tooth extractions or dental surgery
    - » rare today because of prophylactic antibiotic therapy
  - Thoraco Lumbar actinomycosis
  - Abdominal actinomycosis
  - Meningitis, endocarditis, or genital infections

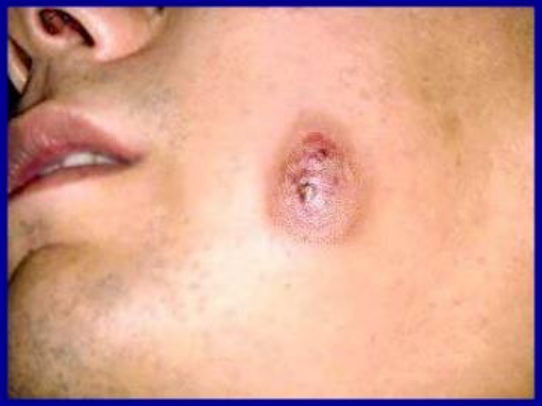
Actinomycosis

- Characterized by draining sinuses,
- containing characteristic granules
  - which are micro colonies of bacteria
  - look like dense rosettes of club-shaped filaments in radial arrangement
- Ray fungus

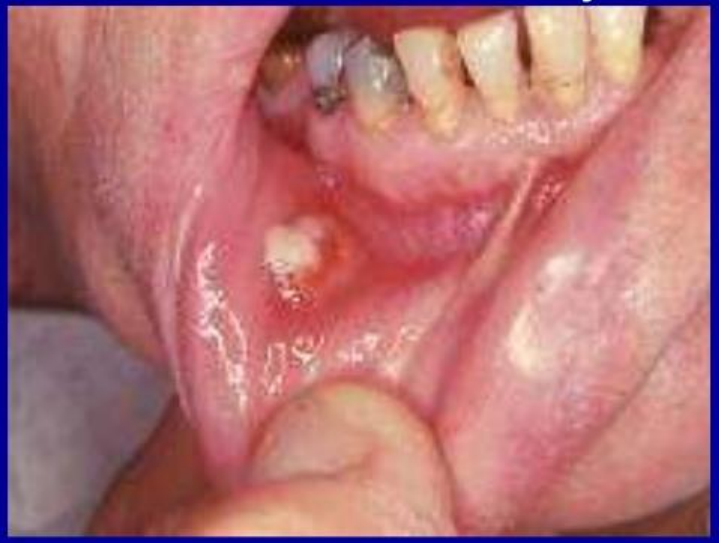
Clinical presentation



Cervicofacial Actinomycosis



Cervicofacial Actinomycetes



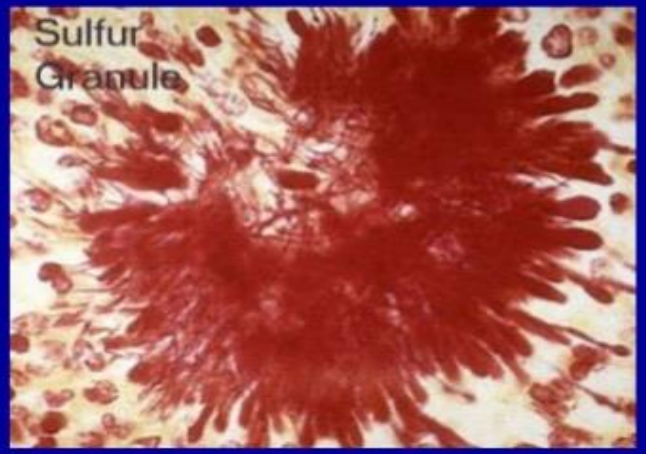
Actinomycosis – Lab Diagnosis

- Macroscopic examination of Granules
- Microscopy
  - Gram stain
- Isolation / Anaerobic Culture
- Serology – Not useful
- Molecular diagnostic tests - PCR

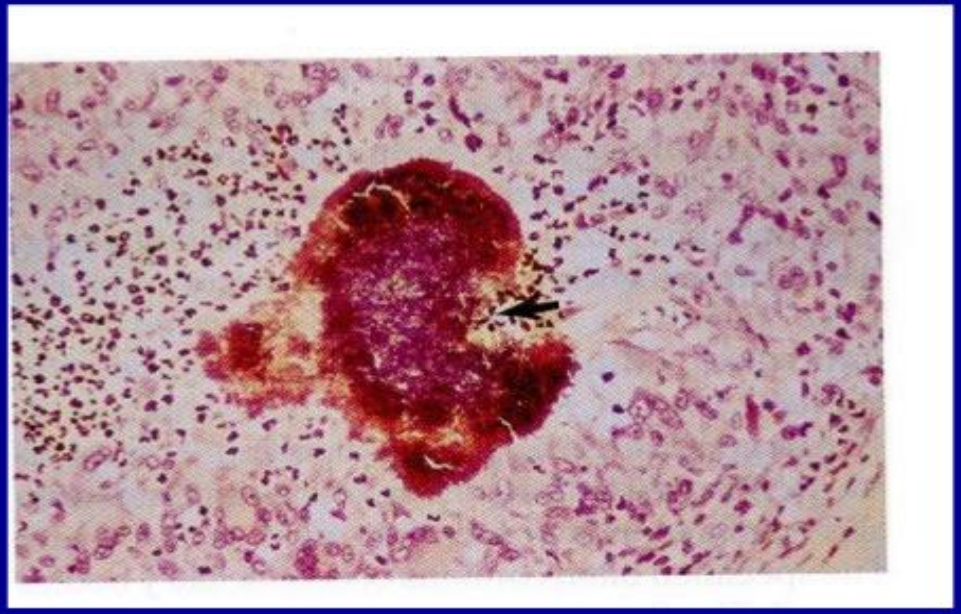
Macroscopic examination of Granules

- Yellow in colour (Hence the name Sulur granules)
- But may be white / brown
- Firm and round
- Size : 0.5 - 5mm in diameter

Sulfur granule



Granules



Treatment - Actinomycosis

- Penicillin

Taxonomy

Order: Spirochaetales

Family: *Spirochaetaceae*
Genus: *Trepanoma*
Borrelia

Family: *Leptospiraceae*
Genus: *Leptospira*

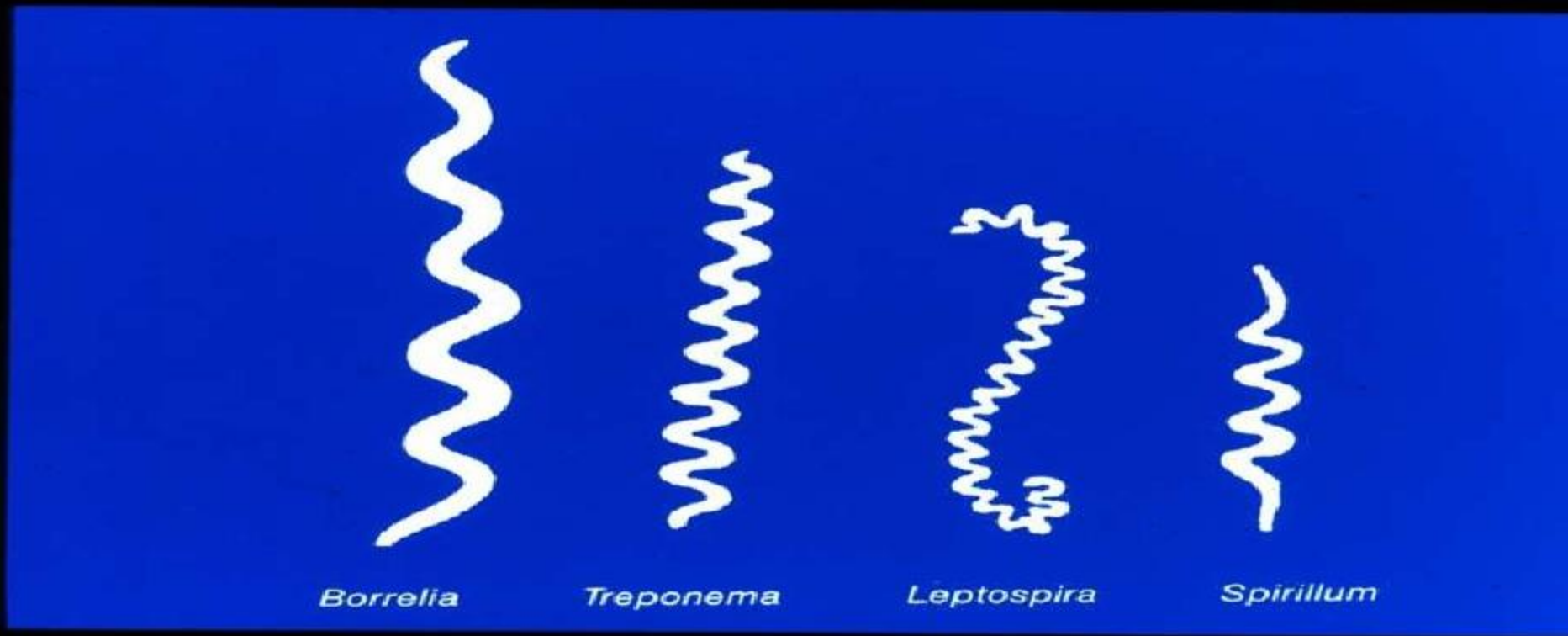
Human pathogen

A. Genera Trepanoma

B. Borreilia

C. Leptospira

How they appear



General Overview of Spirochaetales

A. Gram-negative spirochetes
B. Spirochete from Greek for "coiled hair"
C. Extremely thin and can be very long
D. Tightly coiled helical cells with tapered ends
E. Motile by Periplasmic flagella (a.k.a., axial fibrils or endoflegalla)

Spirochaetales *Associated Human Diseases*

Genus	Species	Disease
Treponema	*pallidum* ssp. *pallidum* *pallidum* ssp. *endemicum* *pallidum ssp. pertenue* *carateum*	Syphilis Bejel Yaws Pinta
Borrelia	*burgdorferi* *recurrentis* Many species	Lyme disease (borreliosis) Epidemic relapsing fever Endemic relapsing fever
Leptospira	*interrogans*	Leptospirosis (Weil's Disease)

What are Trepanoma

Trepos – Turn

Nema Meaning thread

Relatively short and slender

With fine spirals pointed and round ends

May be pathogenic or commensals in the mouth

Trepanoma pallidum

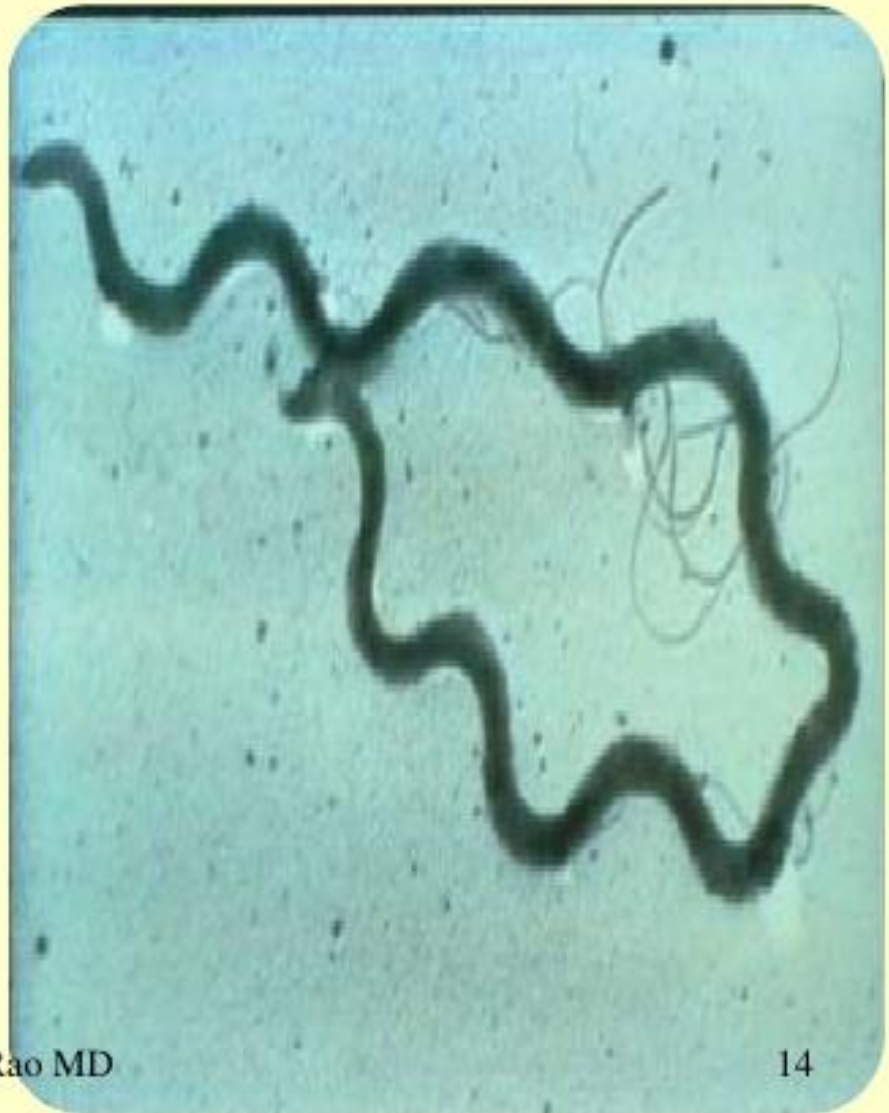
Greek words trepo "turning" & nema "head"

A. Morphology

1. Motile, sluggish in viscous environments
2. Size: 5 to 20 µm in length & 0.09 to 0.5 µm in diameter, with tapered ends
3. Structure
   - Multilayer cytoplasmic membrane
   - Flagella-like fibrils
   - Cell wall
   - Outer sheath (outer cell envelope)
   - Capsule-like outer coat

Treponema pallidum.

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

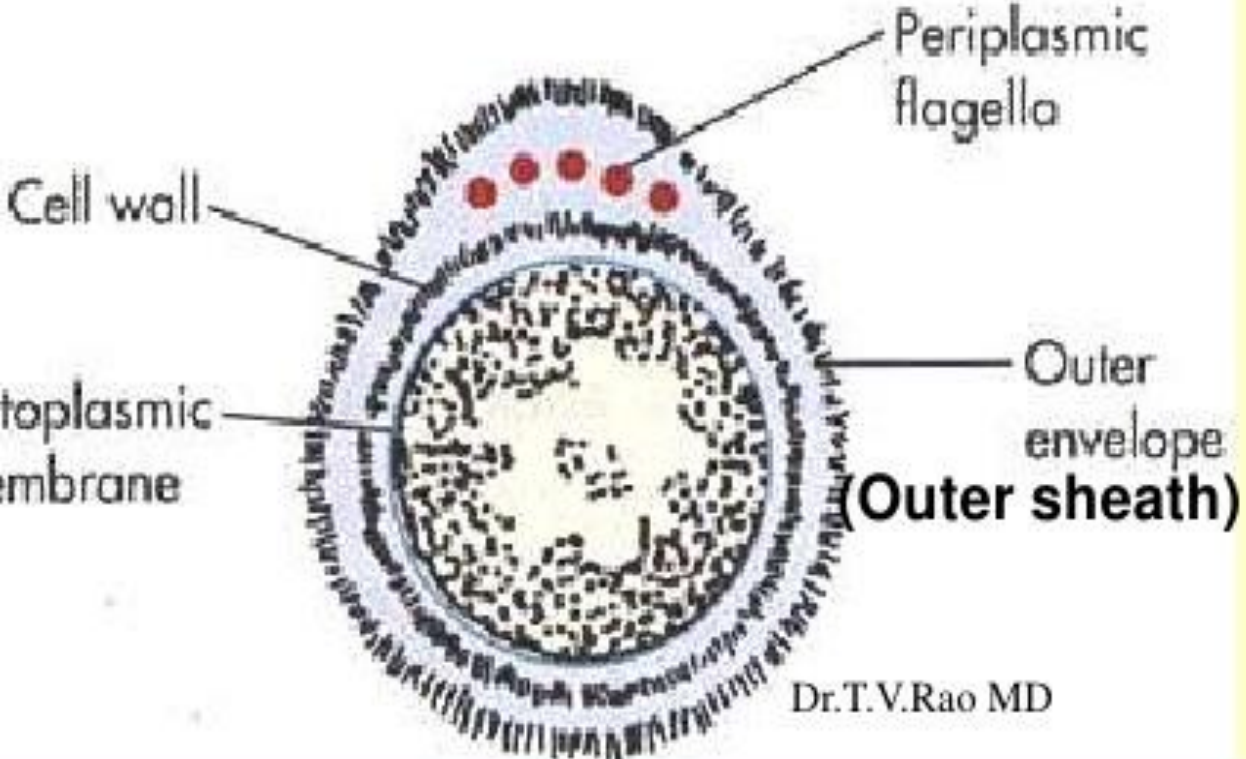


Trepanoma palladium

B. Physiology

- Difficult to culture
  - **Maintained in anaerobic** medium with albumin, sodium bicarbonate, pyruvate, cysteine
  - Microaerophilic

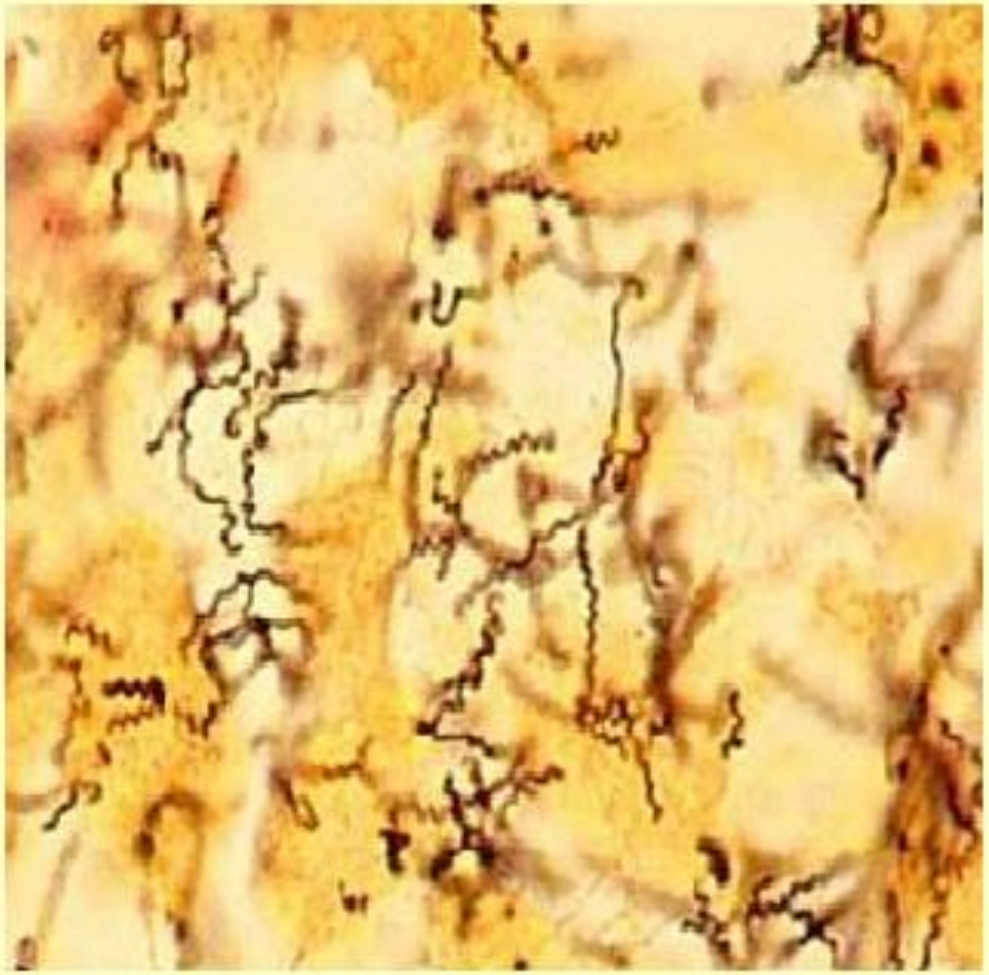
# of Spirochete with Periplasmic Flagella



NOTE: a.k.a., **endoflegalla**, **axial fibrils** or **axial filaments.**

Staining with special stains

Staining by Giemsa and Fontana



Antigenic structure

A. The Antigens are complex
B. Infection with Treponema will induce 3 types of Antigens
C. **Reagin Antibodies – STS**
D. Detected by **Standard tests for Syphilis**
E. 1 Wasserman Test, 2 Kahn Test
F. VDRL Test

Beef Heart Extracts - Antigen

Lipid Hapten – Cardiolipin

Chemically Dipphostidyl glycerol

Cardiolipin present in the Trenonems ?

Or a product of tissue Damage ?

Second Group Antigen
T.pallidum

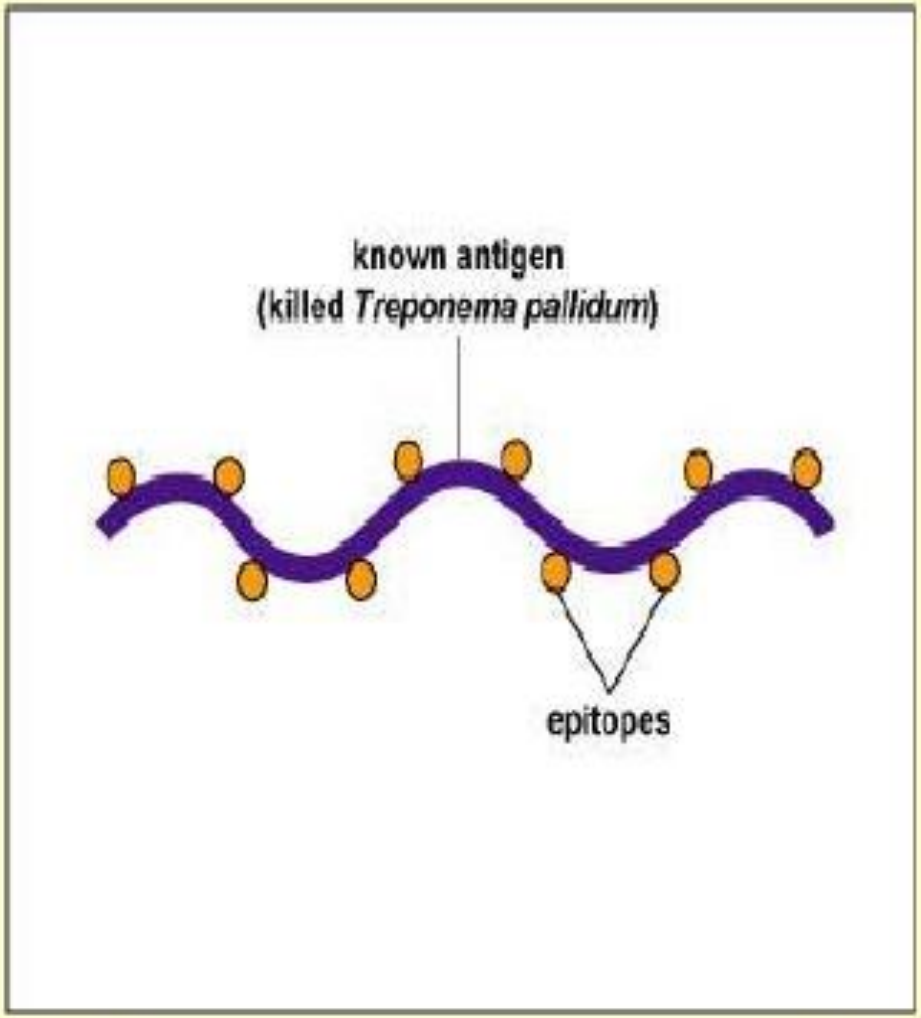
A. Present in T.pallidum and Non pathogenic cultivable treponemes

B. Reiter's Trenonems

Third Antigen

Polysaccharide species specific

Positive only in sera of patients infected with pathogenic Treponema



Venereal Syphilis

Venereal Syphilis caused by

T.pallidum Endemic syphilis

T. pallidum

Yaws T.pertune

Pinta T.carateum

STAGES OF SYPHILIS

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

Trepanoma pallidum

D. Clinical Infection: Syphilis

3. Clinical Manifestations

i. Primary Disease

- **Chancre: single lesion, non-tender & firm with a clean surface, raised border & reddish color**
- **Usually on the cervix, vaginal wall, anal canal**
- **Draining lymph nodes enlarged & non-tender**

Primary syphilis

a) One or more painless chancres (indurated raise edges & clear bases) that erupt in the genitalia, anus, nipples, tonsils or eyelids.

b) Starts as papule and then erode

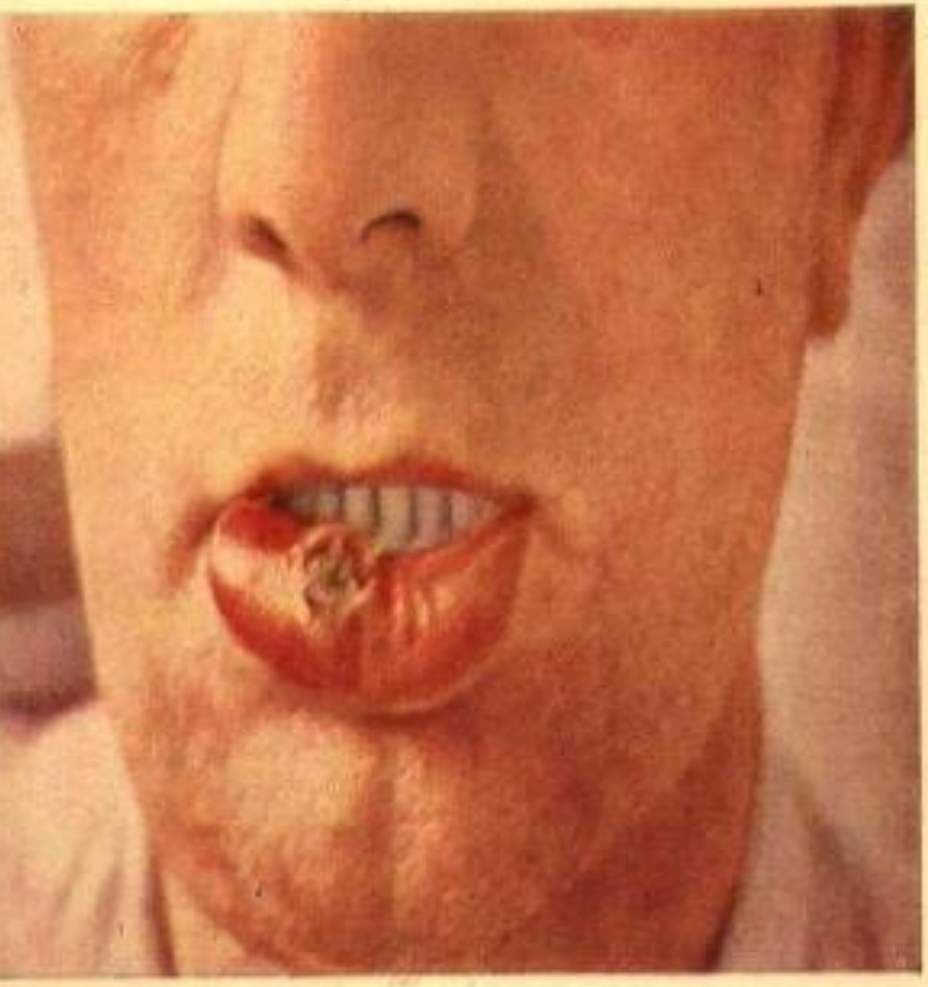
c) Disappear after three to six weeks even without treatment.

d) Lymphadenopathy that is either unilateral or bilateral

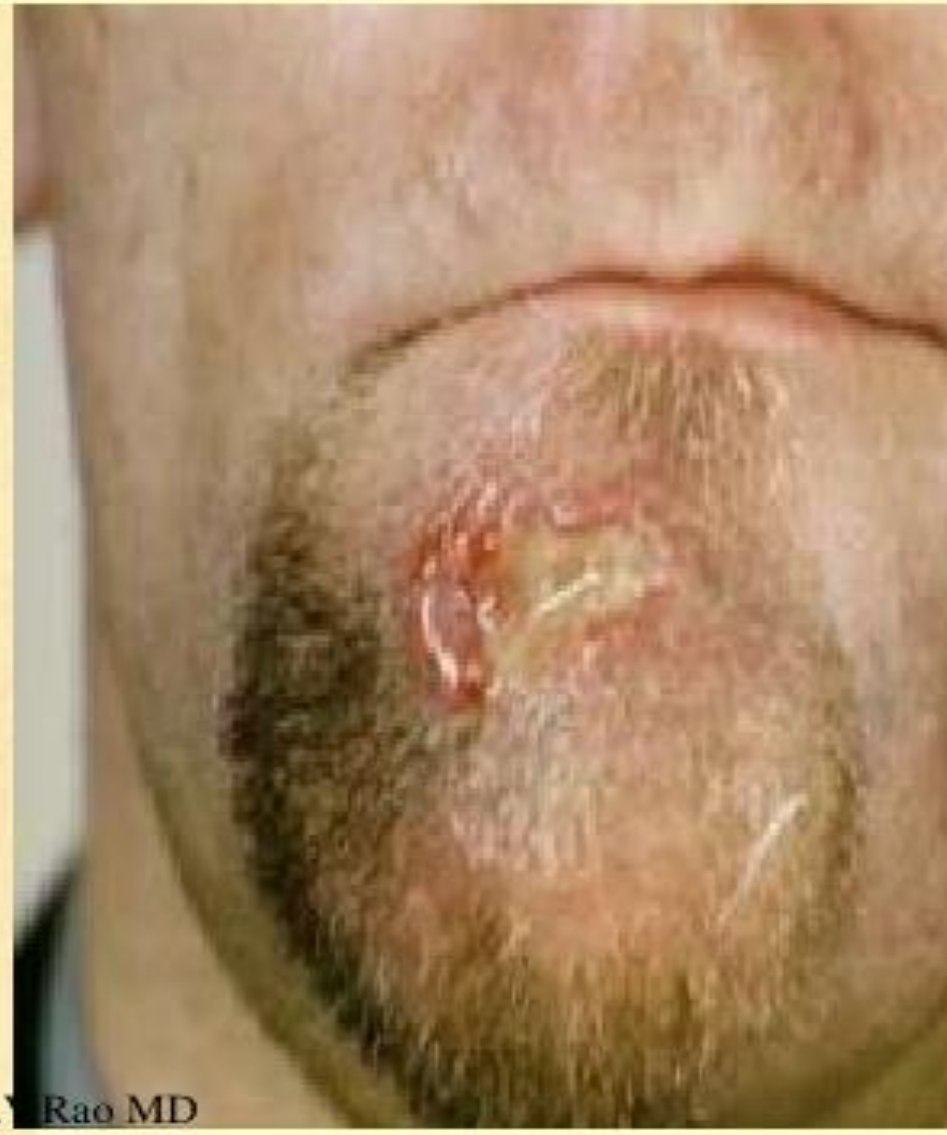
Chancre

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.

Primary Syphilis - *Chanc*

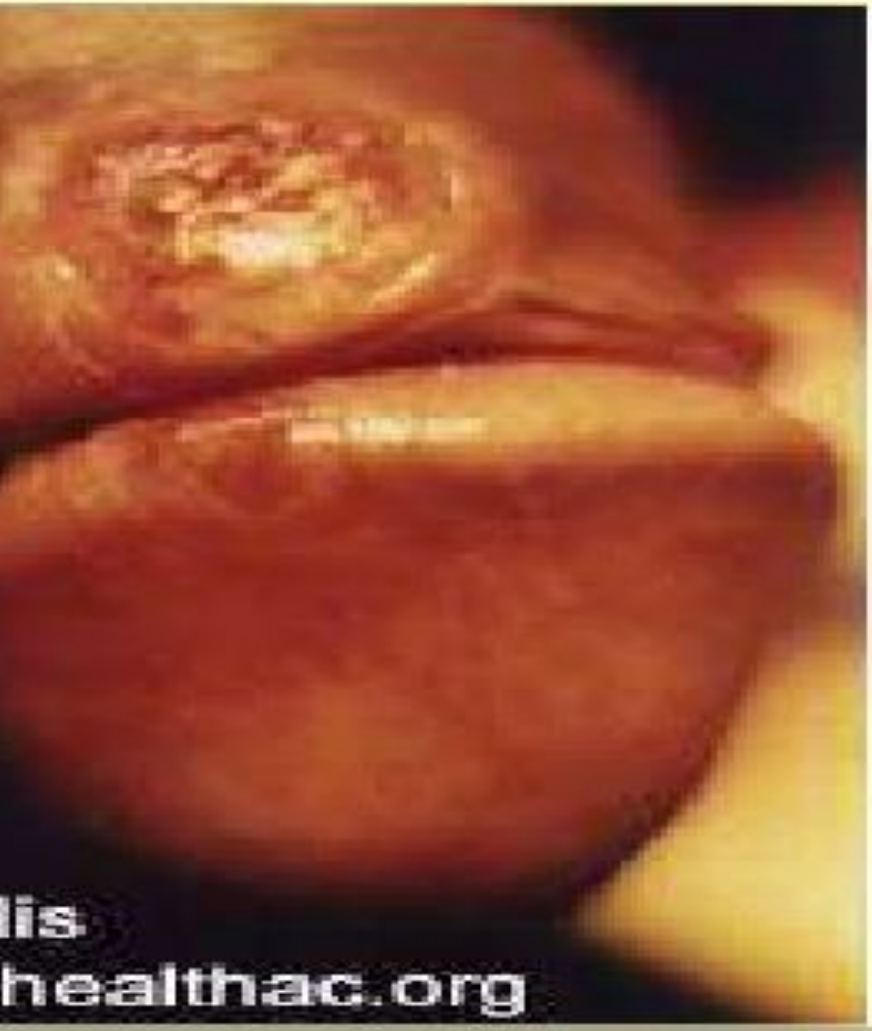


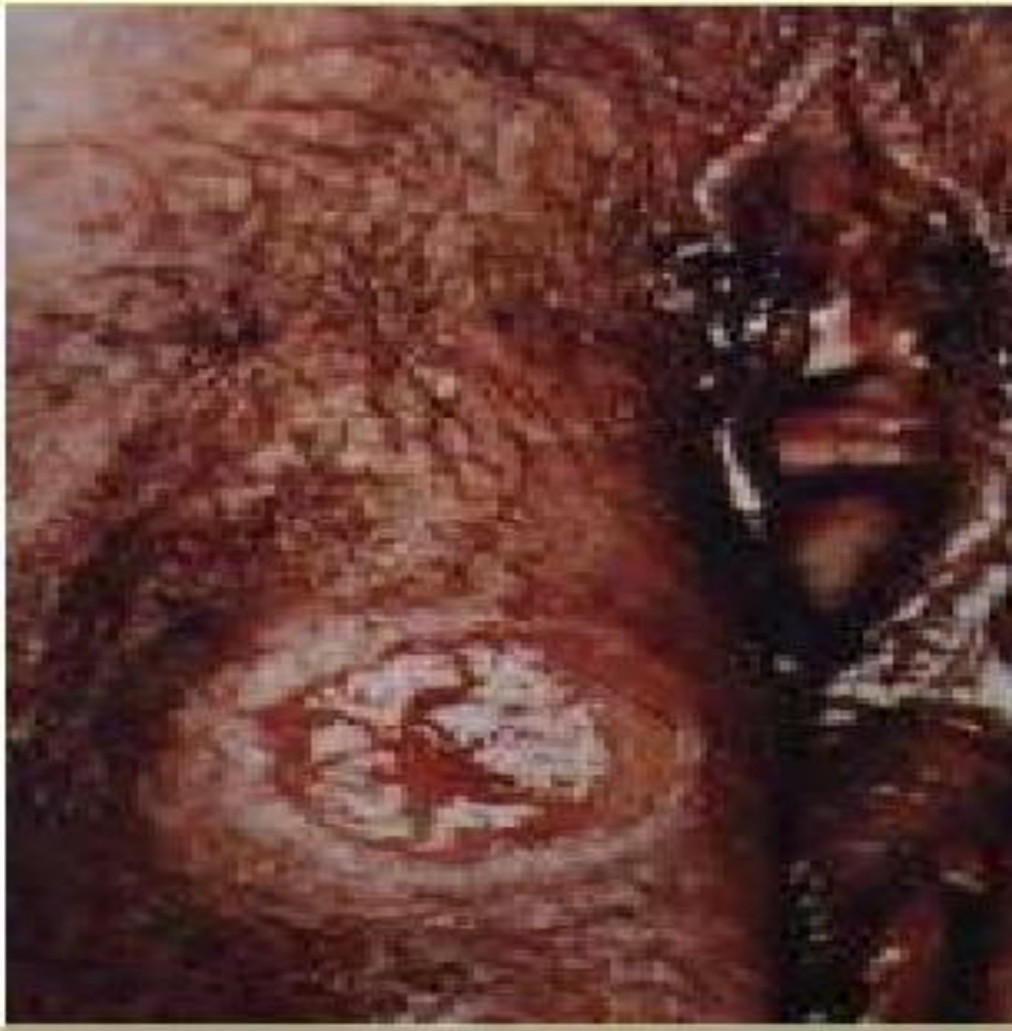
ig. 171. **Primary Syphilis of the Lower Lip.** A chancre appearing on the lower lip the same clinical appearance as one appearing on the genital mucosa. This lesion simulate squamous cell carcinoma.



Dr.T.V. Rao MD

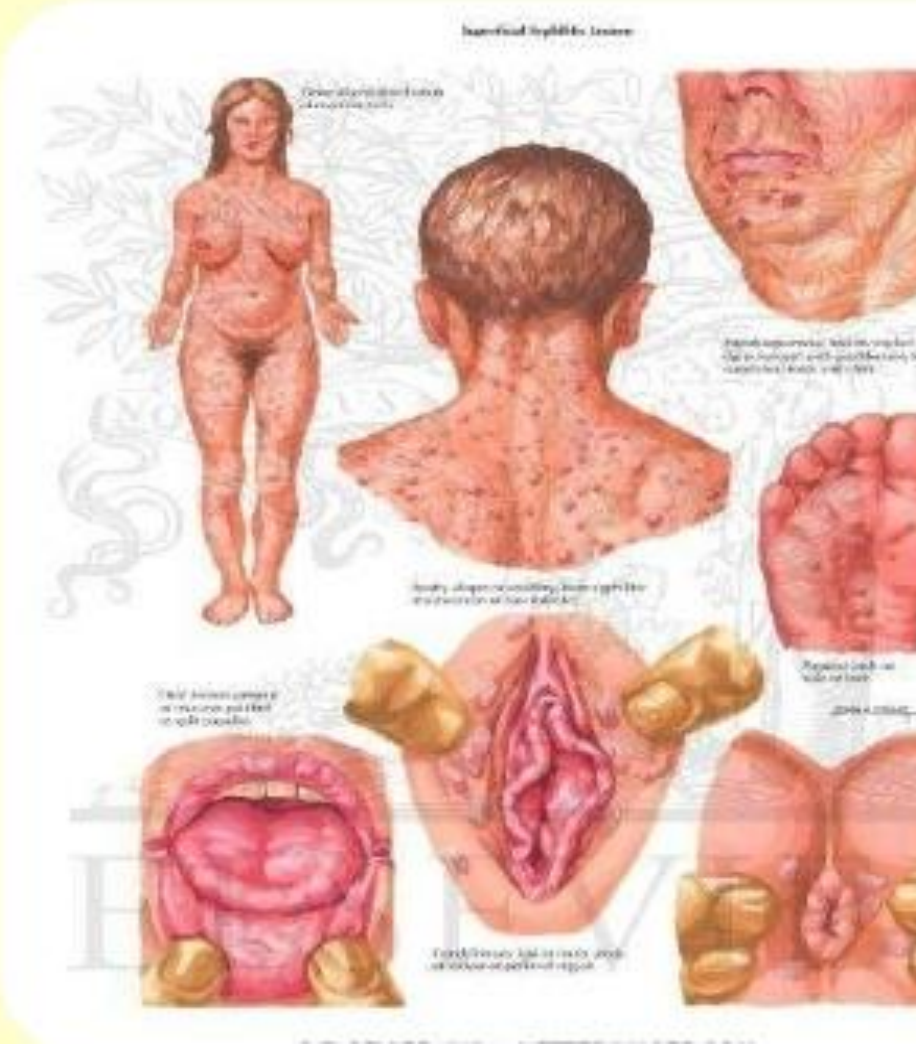
Primary Syphilis



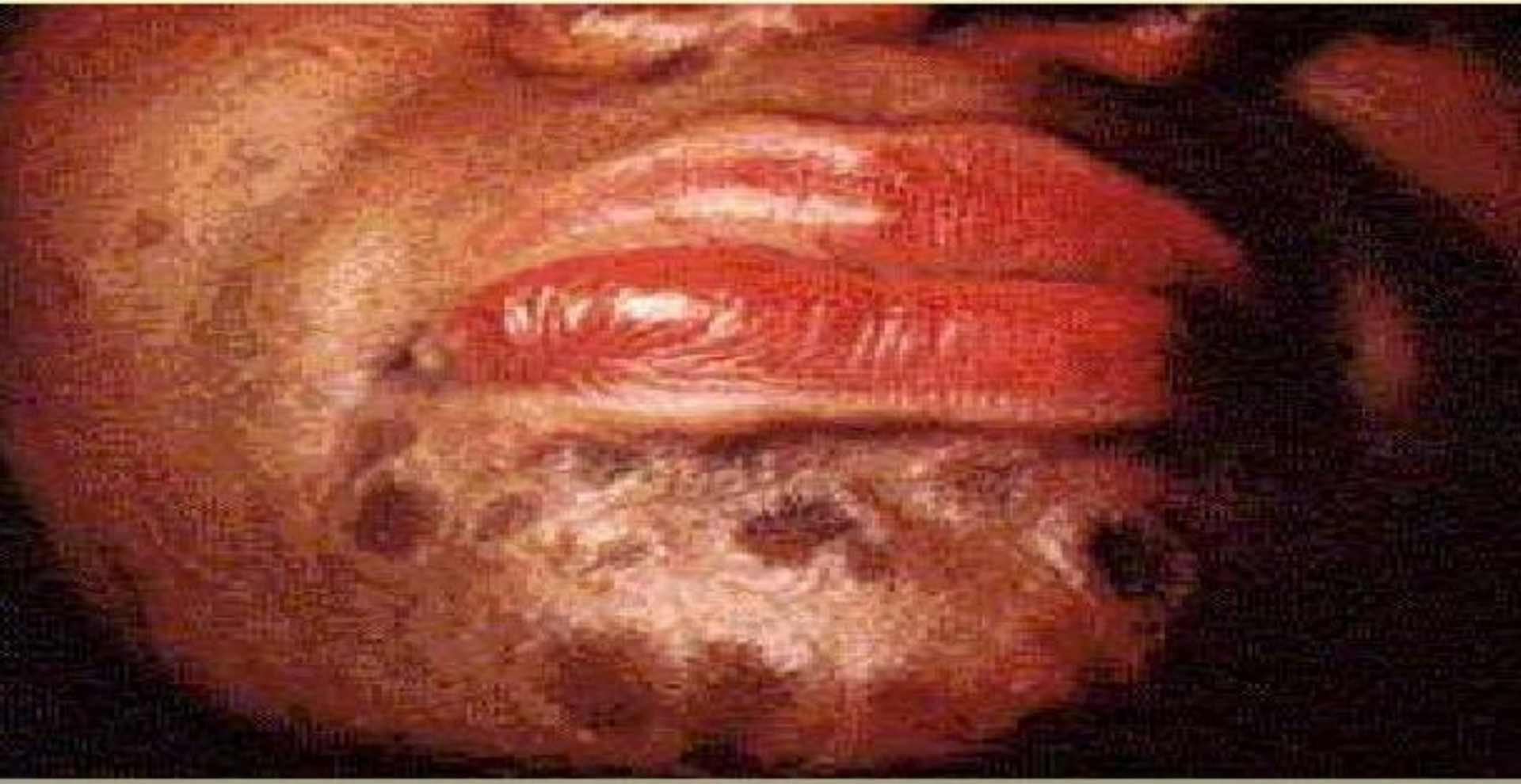


Secondary Syphilis

A. Secondary syphilis at 6-8 weeks – diffuse symptoms:
   1. Fever
   2. Headache
   3. Skin pustules
B. Usually disappears even without

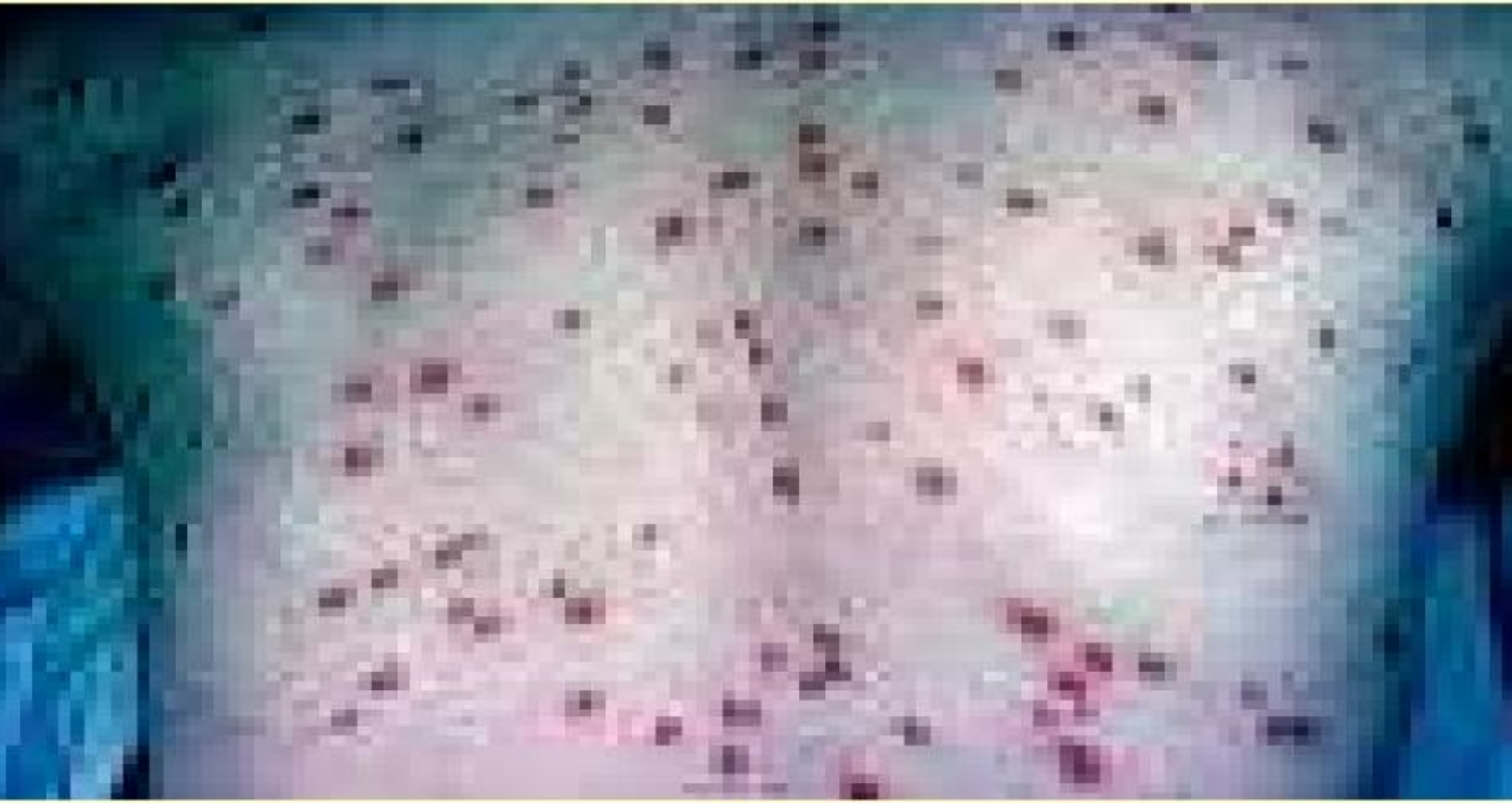


Secondary Syphilis

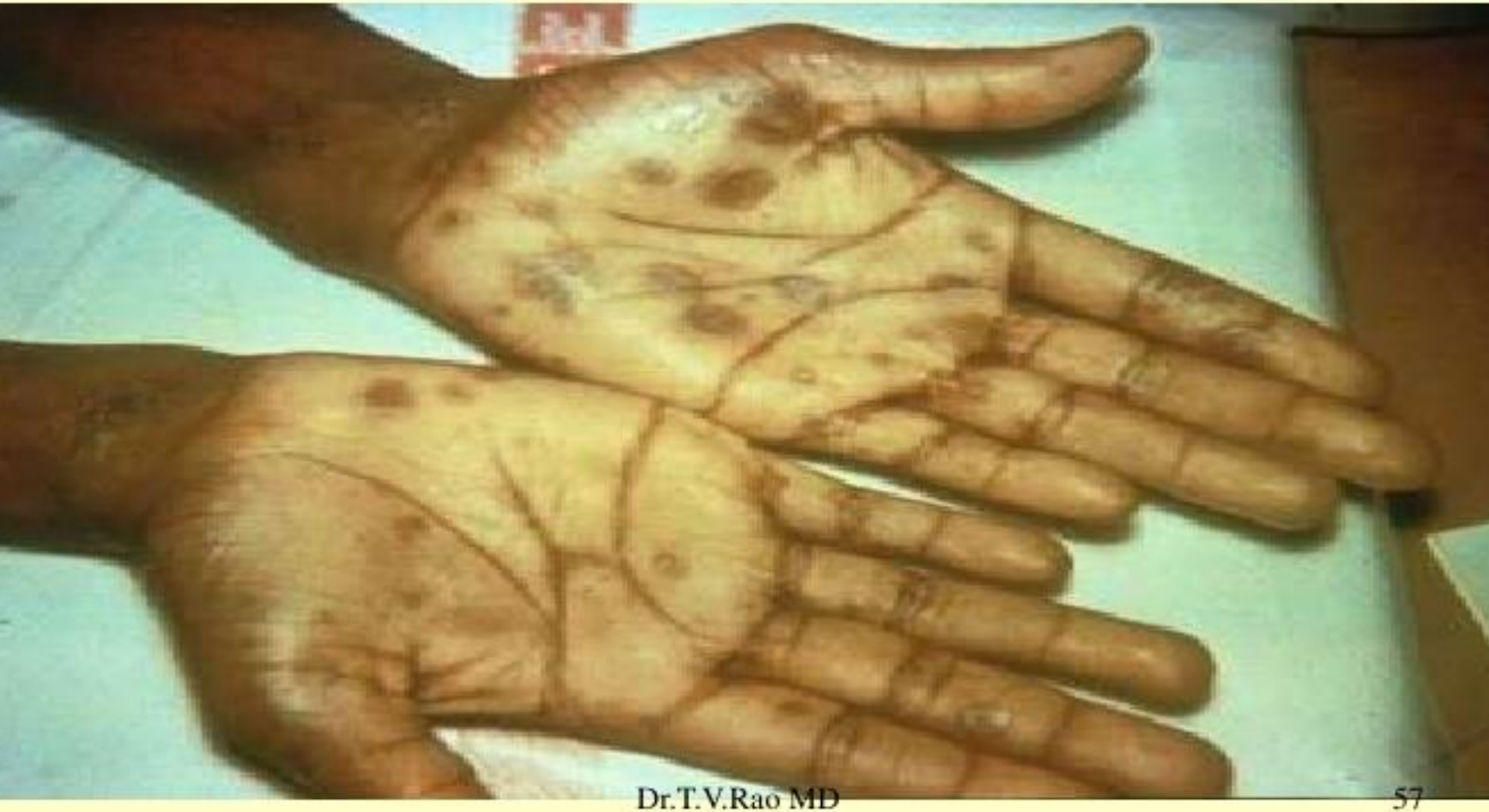


Secondary Syphilis

Secondary Syphilis



Secondary syphilis



Tertiary Syphilis

A. Affects 2/3 of untreated cases
   1. Gummata: rubbery tumors
   2. Bone deformities
   3. Blindness
   4. Loss of coordination
   5. Paralysis
   6. Insanity

Tertiary Syphilis

Tertiary syphilis characterized by **localized granulomatous dermal lesions (gummas)** in which few organisms are present

- Granulomas reflect containment by the immunologic reaction of the host to chronic infection

Pathogenesis of T. pallidum *(cont.)*

Latent Stage Syphilis

- Following secondary disease, host enters latent period
  - First 4 years = **early latent**
  - Subsequent period = **late latent**
- **About 40% of late latent patients progress to late tertiary syphilitic**

Latent Syphilis

A. Latent syphilis

a) Reactive serologic test

b) Asymptomatic until death

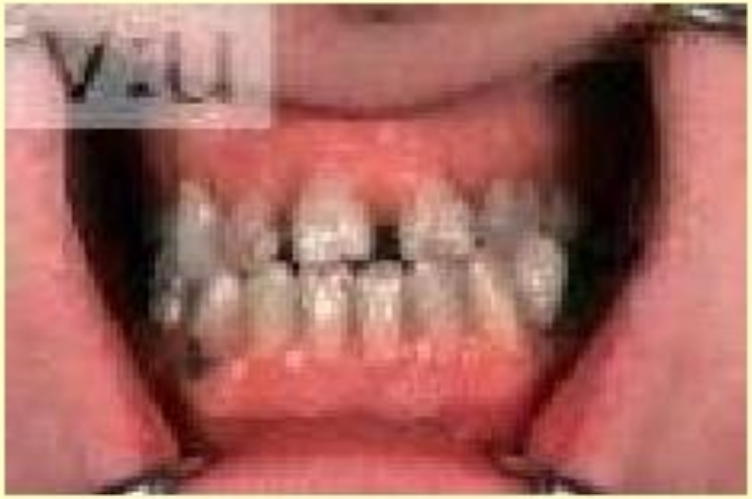
A. Late syphilis

Three subtypes of Late syphilis

1. Late, benign syphilis

*Develops between 1 to 10 years after the infection

*Presence of gumma

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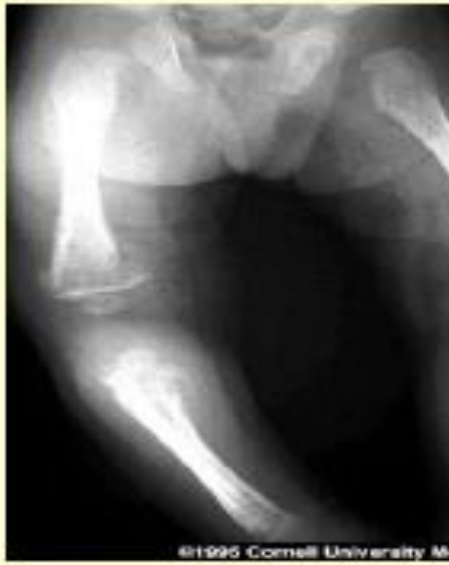
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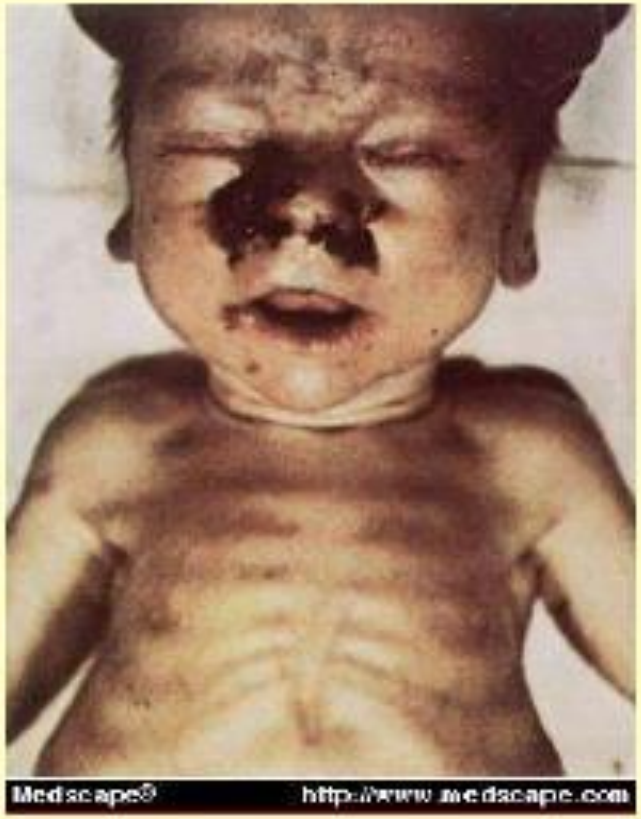
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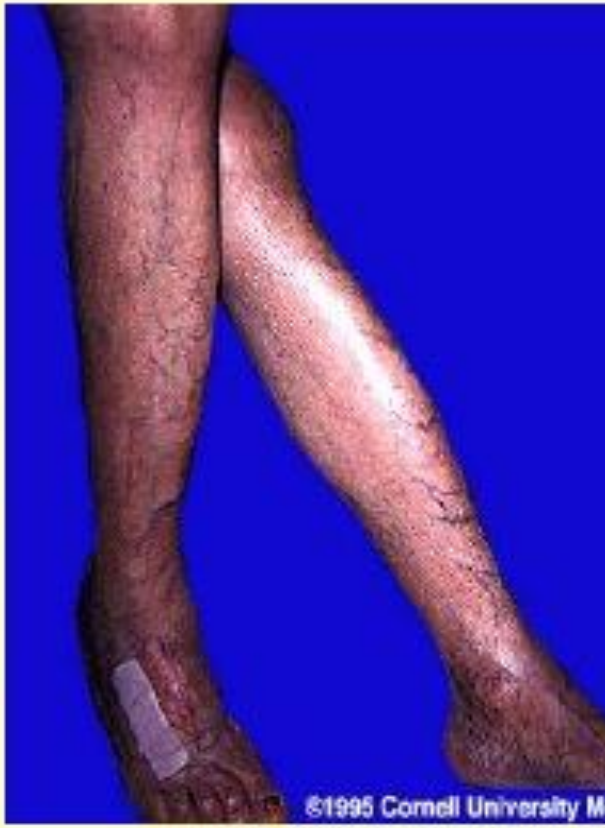
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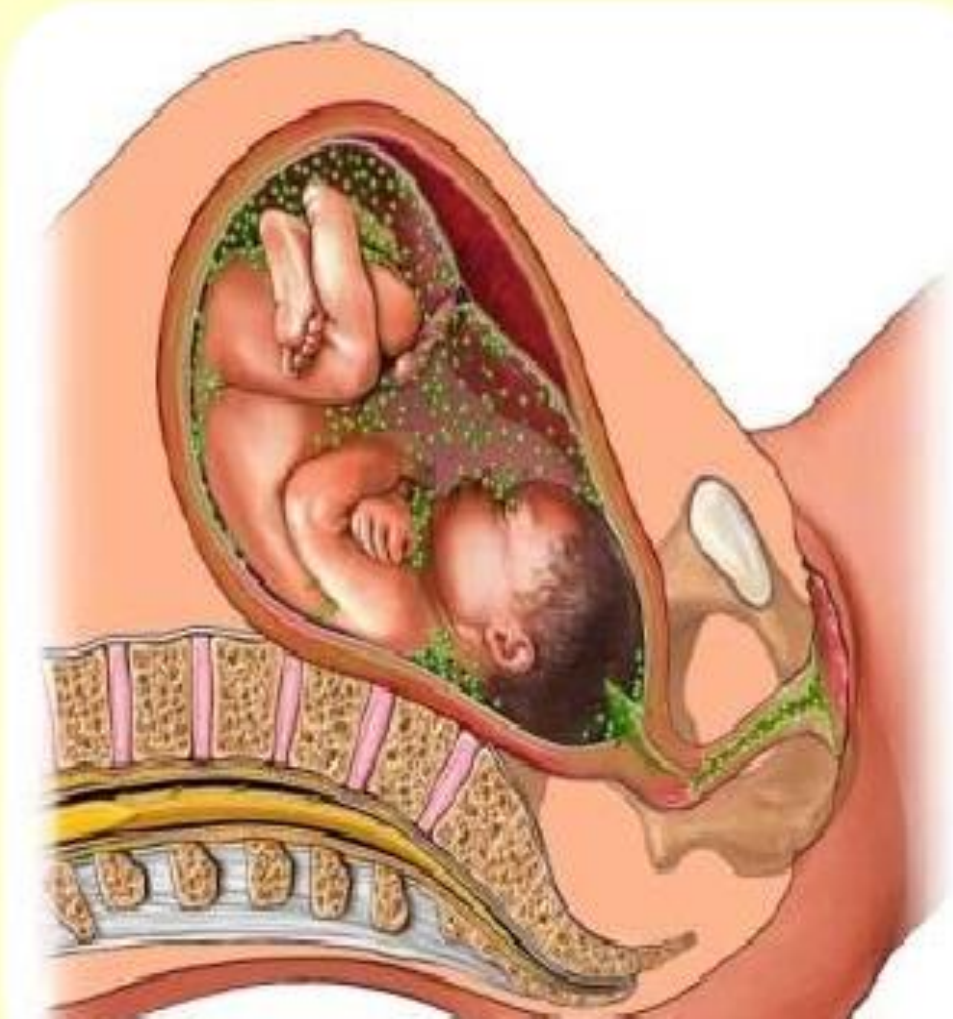
Mother to Child Transmission

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



Pathogenesis of T. pallidum (cont.

Congenital Syphilis

- Congenital syphilis results from **trans placental infection**
- *T. pallidum* **septicemia in the developing fetus and widespread dissemination**
- **Abortion, neonatal mortality, and late mental or physical problems** resulting from scars from the active disease and progressio of the active disease state

Congenital Syphilis

A. Passed from mother to fetus during pregnancy
   1. Abnormally shaped teeth
   2. Nasal septum collapses
   3. Skeletal abnormalities

DIAGNOSIS OF SYPHILIS

A. 1. History and clinical examination.

B. 2. Dark-field microscopy: special technique use to demonstrate the spirochete as shiny motile spiral structures with a dark background.

C. 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.

Diagnosis of syphilis

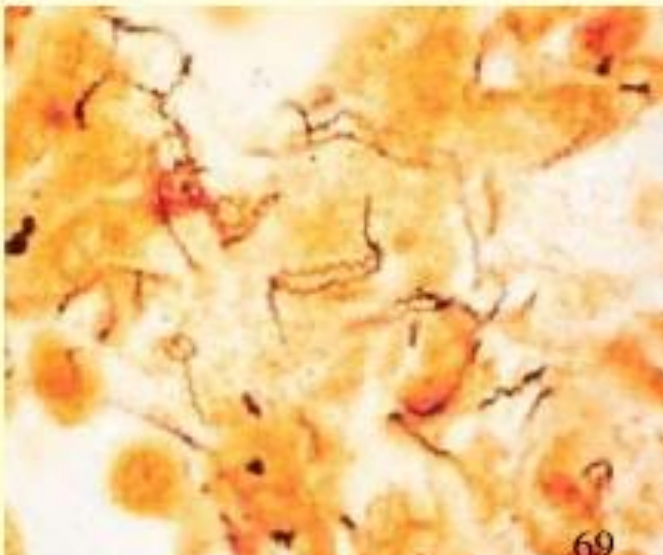
Direct detection of spirochetes :

Darkfield microscopy (motile bugs + experience + prompt examination)

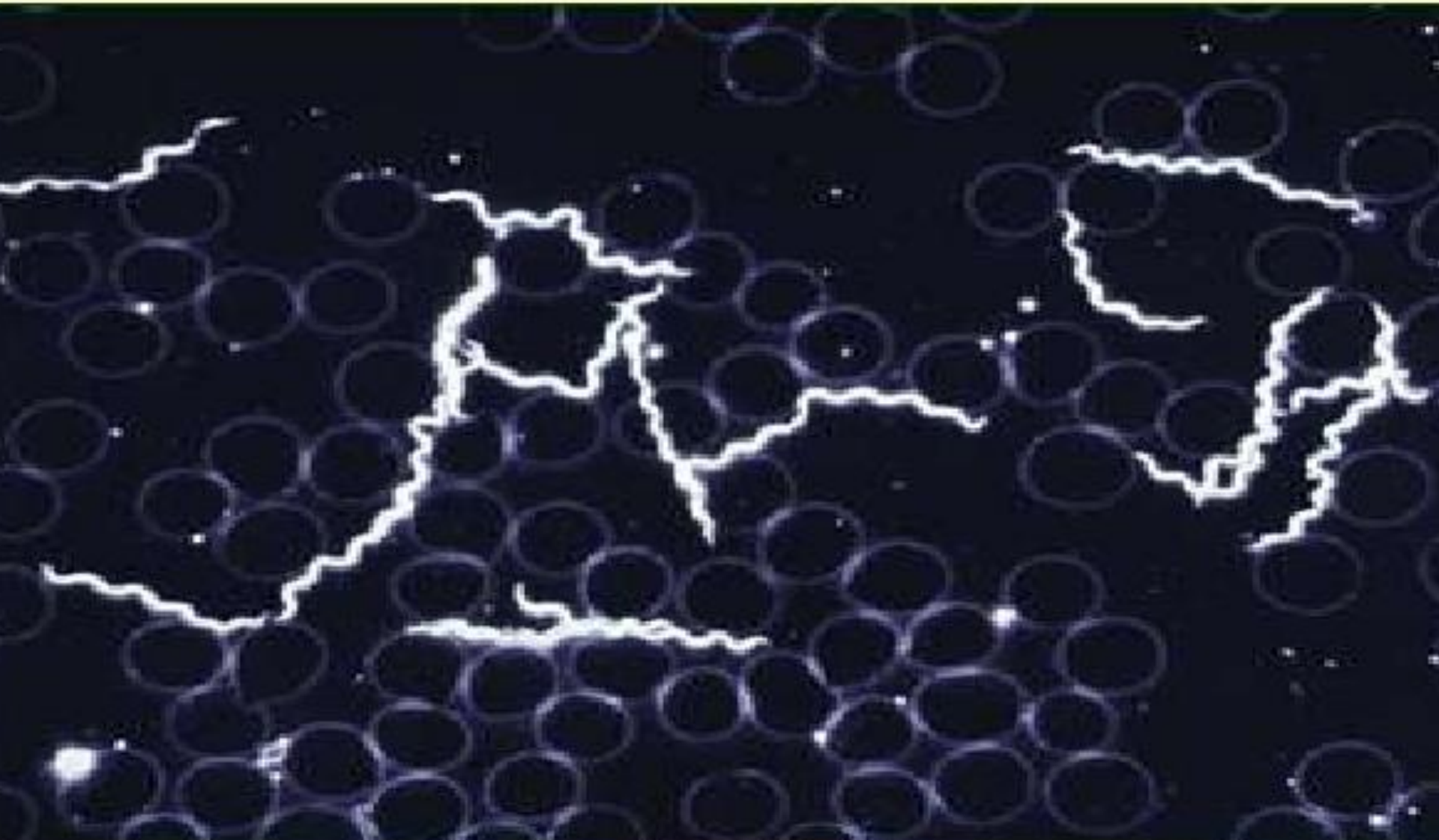
Silver stain

Culture : not used

Serology: non-specific and specific tests



Dark field Microscopy



Treponema cannot be cultivated in Culture Media

. The inability to grow most pathogenic Treponema in vitro, coupled with the transitory nature of many of the lesions, makes diagnosis of Treponema 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**.

Serologic Tests

A. Reveal patients immune status *not* whether they are currently infected
B. Use lipoidal antigens rather than *T. pallidum* or components of it; *non-treponemal antigen tests*
C. RPR; rapid plasma reagin
D. VDRL; Venereal Disease Research Laboratory

Treponema pallidum

F. Laboratory diagnosis

1. Serologic testing
   i. Nontreponemal Tests (uses Cardiolipin-lecithin as antigen)
      a. Complement-fixation tests (Wasserman & Kolmer test)
      b. Flocculation tests (Venereal Disease Research Laboratory, (VDRL), Hinton & rapid reagin tests)

Serologic Tests

A. Positive within 5 to 6 weeks after infection
B. Strongly positive in secondary phase
C. Strength of reaction is stated in dilutions
D. May become negative with treatment or over decades

Non-treponemal tests

A. Antigen: **cardiolipin** (beef heart) + lecithin + cholesterol

B. Detect nonspecific antibody **(Reagin)**: a mixture of IgM & IgG direct against some normal tissue antigens

C. VDRL (Venereal Disease Research Laboratory) test for serum and CSF samples

Venereal Disease Research Laboratory - VDRL

Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin.

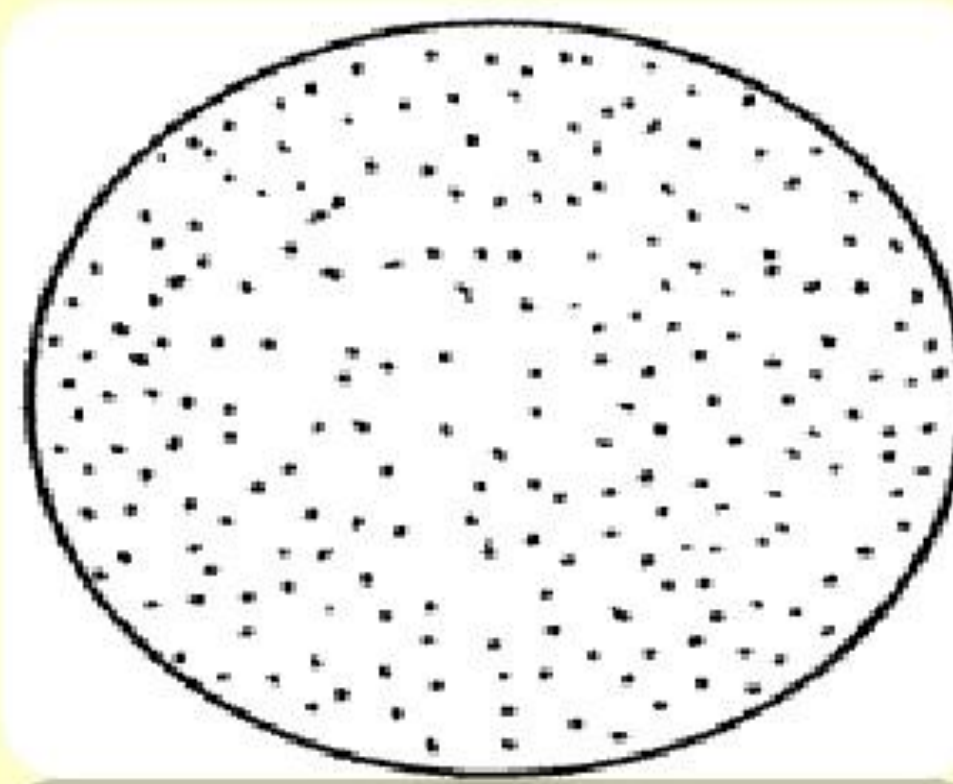
Utilizes an antigen which consists of ***cardiolipin, cholesterol and lecithin***.

1. Antigen very technique dependent.
2. 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 +/- 2**drops

VDRL



A. Each preparation of antigen suspension should first be examined by testing with known positive or negative serum controls.

B. 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

C. Reactive on left, non-reactive on right

Rapid Plasma Reagin Test - RPR

A. General screening test, can be adapted to automation.

B. CANNOT be performed on CSF.

C. Antigen
   1. VDRL cardiolipin antigen is **modified with cholin chloride** to make it more stable
   2. attached to charcoal particles to allow macroscopi reading
   3. antigen comes prepared and is very stable.

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

Specific serological tests of syphilis

A. A. Reiter protein complement fixation test.

B. B. **Fluorescent Treponemal antibody/absorption test, FTA/ABS. the most specific and most sensitive** .

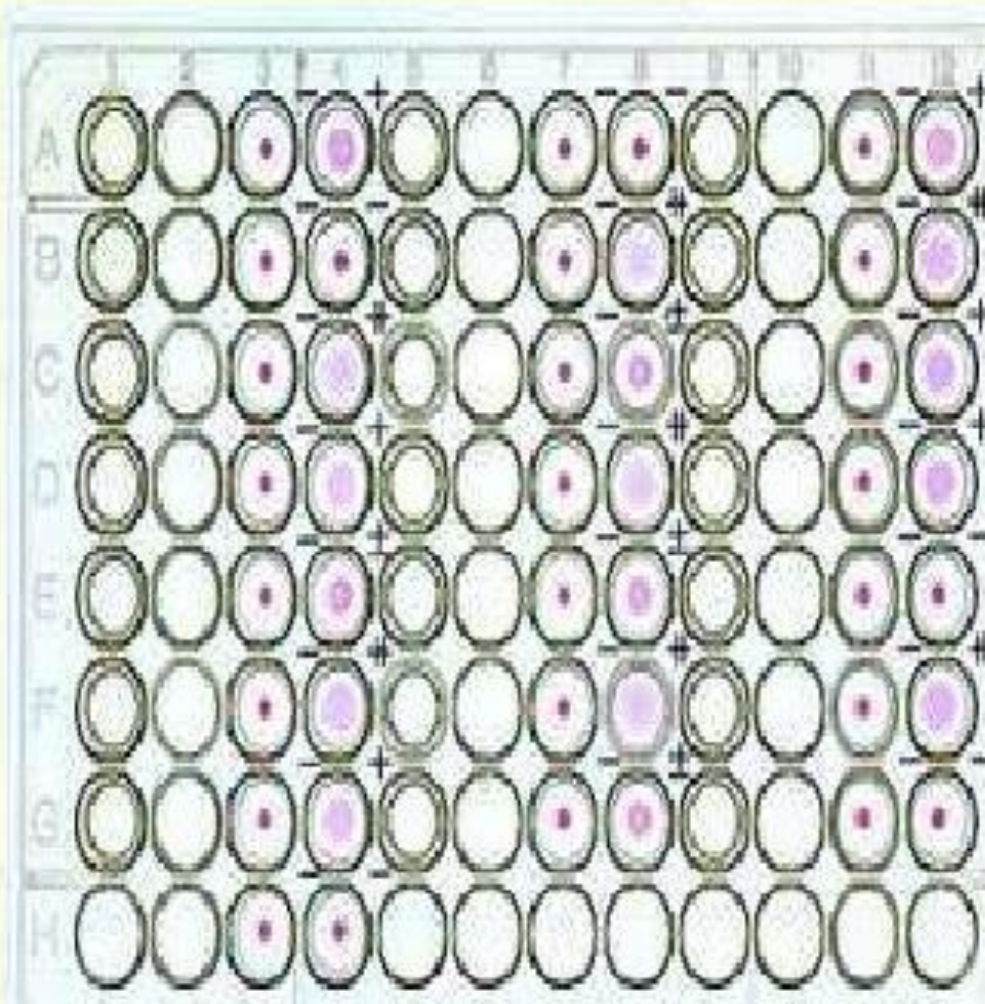
C. C. **Treponema pallidum haemagglutination test- TPHA-** D. Treponema pallidum immobilization test-

Treponema pallidum haemagglutination (TPHA)

A. Adapted to micro techniques (MHA-TP)

B. Tanned sheep RBCs are coated with T. pallidum antigen from Nichol's strain.

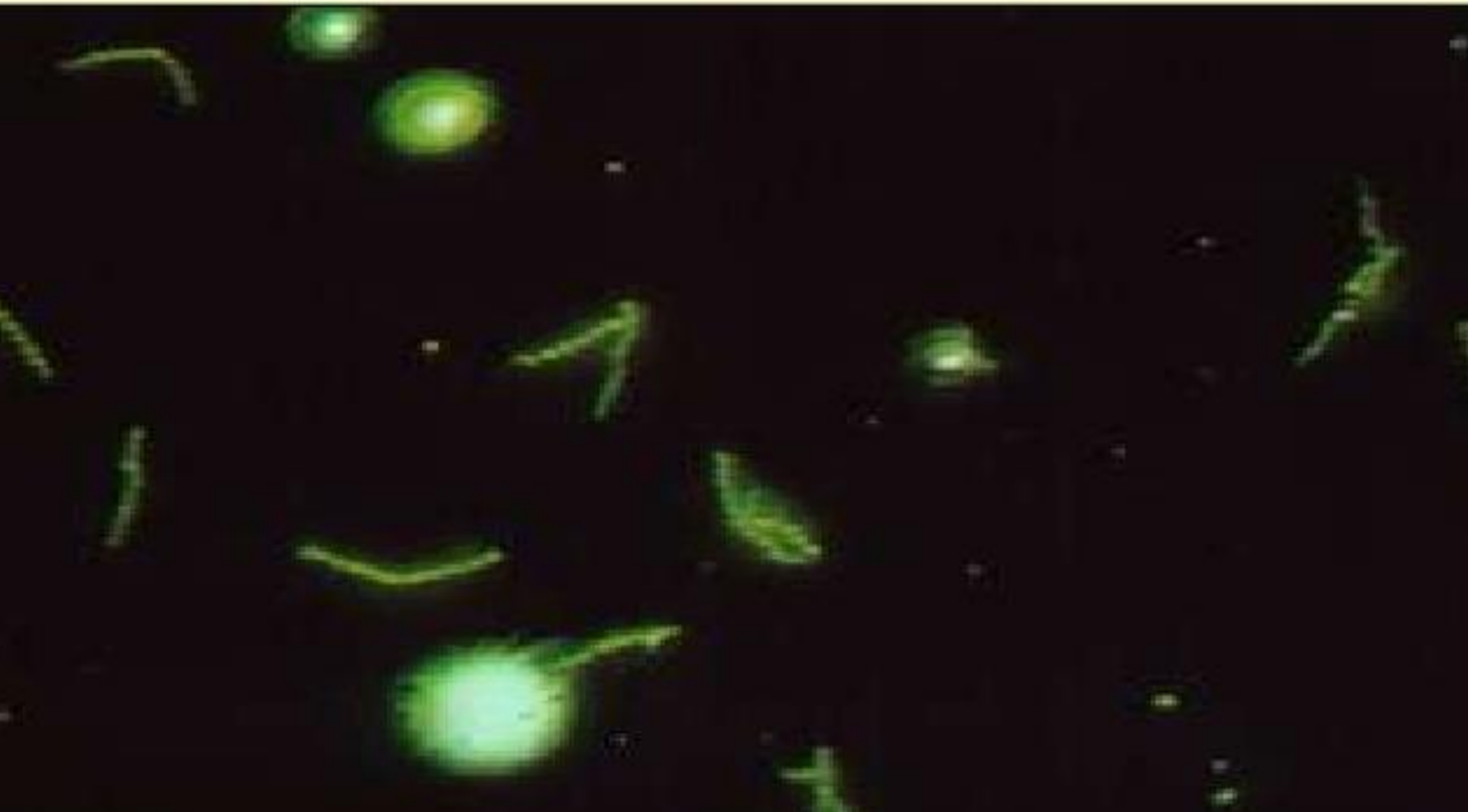
C. Agglutination of the RBCs is a positive result.



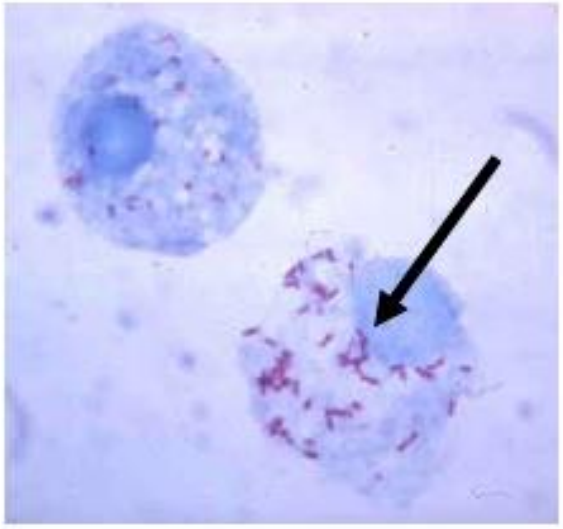
Fluorescent Treponemal Antibody Absorption Test (FTA-ABS)

A. Diluted, heat inactivated serum added to Reiter's strain of *T. pallidum* to remove cross reactivity due to other *Treponemes*.
B. Slides are coated with Nichol's strain of *T. pallidum* and add absorbed patient serum.
C. Slides are washed, and incubated with antibody bound to a fluorescent tag.
D. After washing the slides are examined for fluorescence.
E. Requires experienced personnel to read.
F. Highly sensitive and specific, but time

Positive FTA Test for Syphilis Viewed with a Fluorescent Microscope

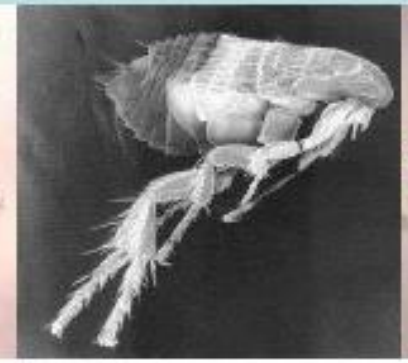


- Obligate intracellular parasite
- Gram negative pleomorphic rods
- Parasite of arthropods – fleas, lice, ticks and mites.
- No Human to human transmission.



Rickettsia inside the host cell

TICK



FLEA

LICE

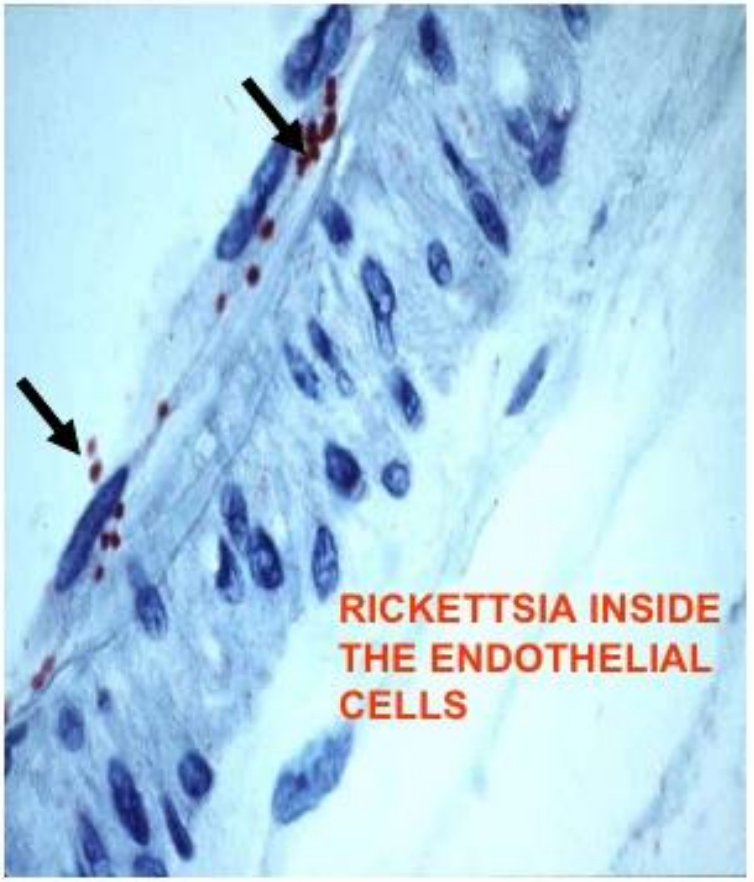
MITE

Rickettsial species and its disease

- **R. prowazekii – Epidemic typhus, Brill-Zinsser disease – Human body louse**
- **R. typhi – Endemic typhus – Rat flea**
- **R. rickettsii –**
  Rocky-Mountain spotted fever-Ticks
- **R. conori – Boutonneuse fever - Ticks**
- **R. australis – Australian tick typhus - Ticks**
- **R. siberica – Siberian tick typhus - Ticks**
- **R. akari – Rickettsial pox - Mites**

GENERAL PATHOGENESIS

- Rickettsia are transmitted to humans by the bite of infected arthropod vector.
- Multiply at the site of entry and enter the blood stream.
- Localise in the vascular endothelial cells and multiply to cause thrombosis lead to rupture & necrosis.



Rickettsial infections- classification

- **Typhus fever group**
  - **Epidemic typhus/Brill-Zinsser typhus**
  - **Endemic typhus**
- **Spotted fever group**
  - **Rocky mountain spotted fever**
  - **Siberian tick typhus**
  - **Boutonneuse fever**
  - **Australian tick typhus**
  - **Rickettsial pox**

EPIDEMIC TYPHUS (CLASSICAL TYPHUS)

Cause: Rickettsia prowazekii

Vector:

Human body louse (Pediculus humanus corporis)

Human head louse (Pediculus humanus capitis)

Incubation period – 5-21 days

Mortality rate is 20-30% in untreated cases.

LICE

SYMPTOMS

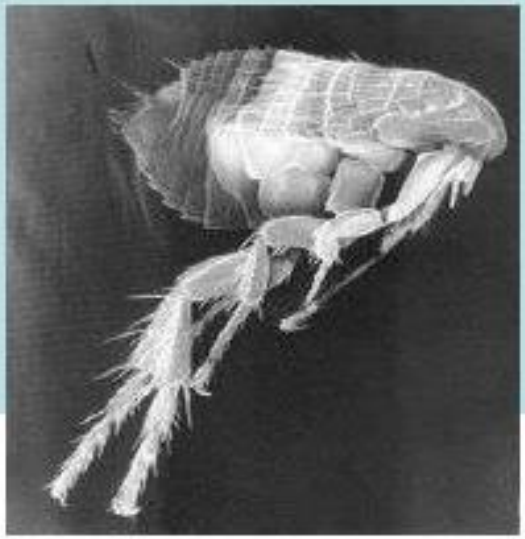
- Severe headache
- Chills
- Generalised myalgia
- High fever (39-41^{0}C)
- Vomiting
- Macular rash after 4-7 days – first on trunk and spreads to limb.
- Lacks conciousness.

Brill –Zinsser/ Recrudescent typhus

- This occur after the person recovered from epidemic typhus and reactivation of the rickettsia prowazekii which remained latent for years.
- Mild illness and low mortality rate.

ENDEMIC TYPHUS (MURINE TYPHUS)

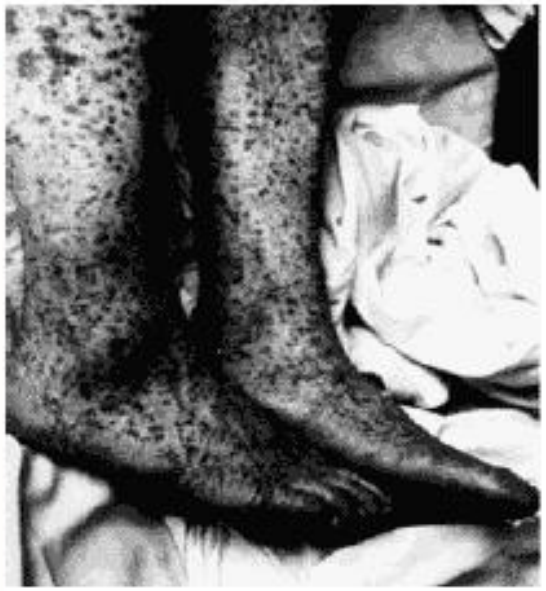
- R. typhi
- Vector: Rat flea (Xenopsylla cheopis)
- Reservoir: Rat
- Infection occurs after rat flea bite



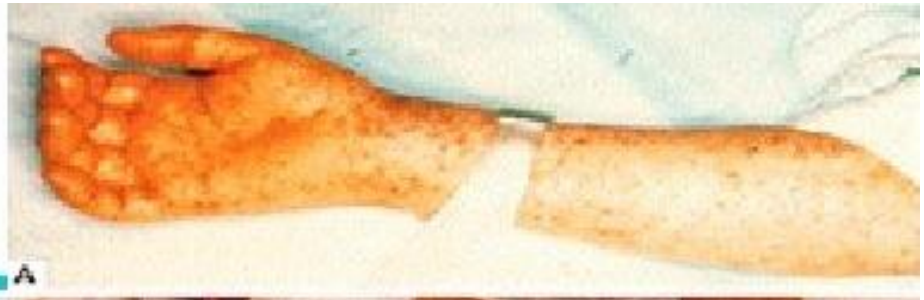
Spotted fever group

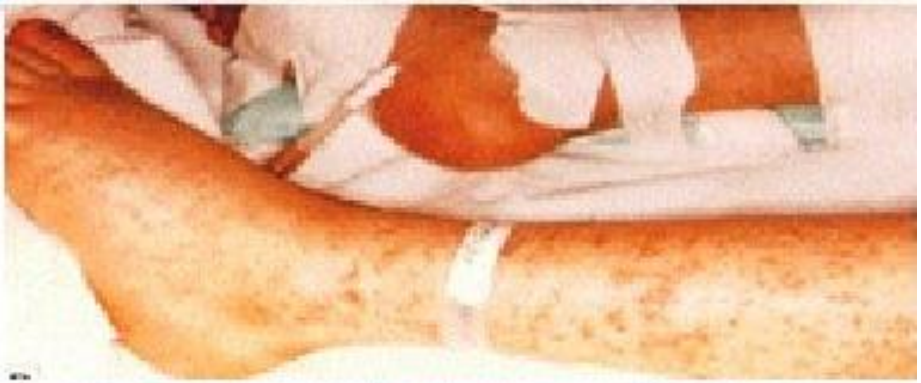
Rocky mountain spotted fever

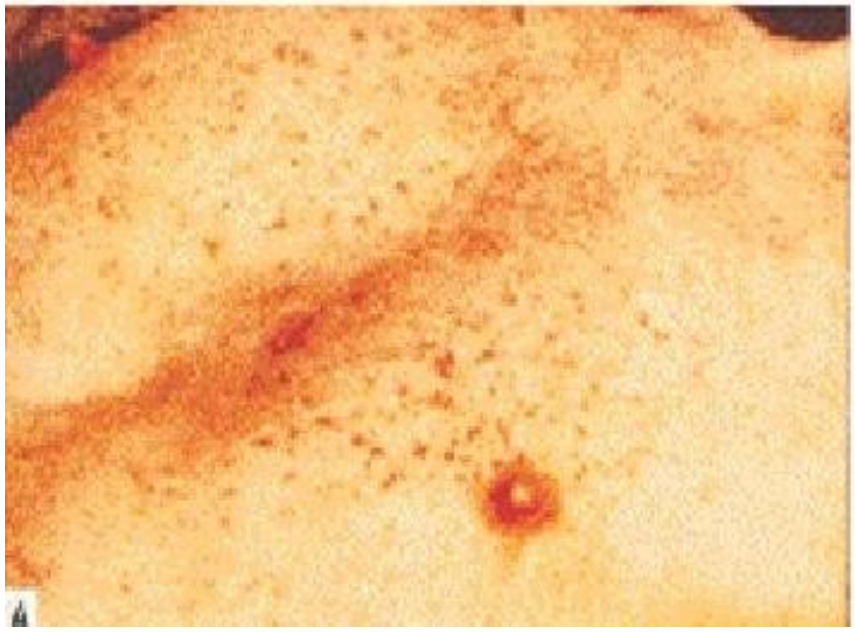
- Most serious form
- Cause – R. rickettsii
- Infection occurs after tick bite
- Incubation period – 1 week
- More similar to typhus fever but the rash appears earlier and is more prominent.

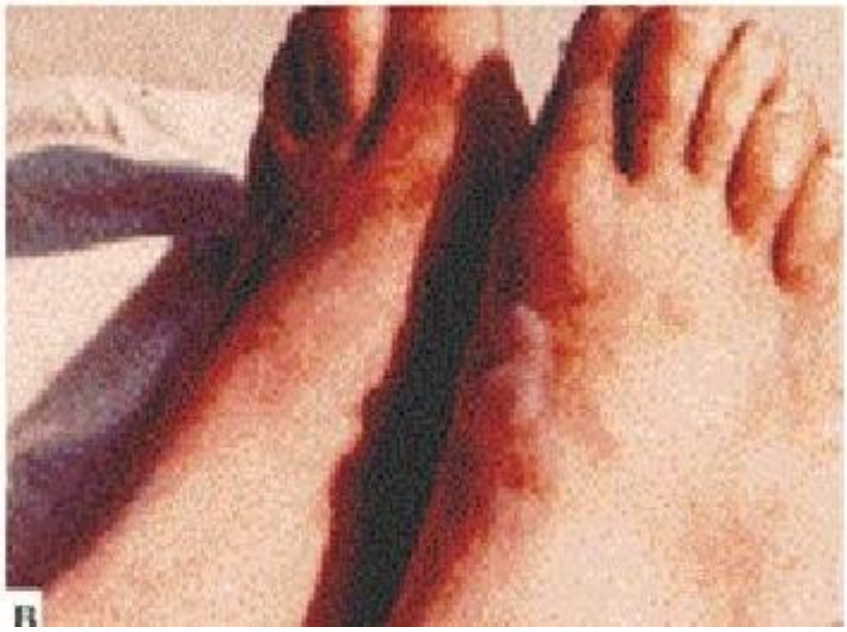


Rash

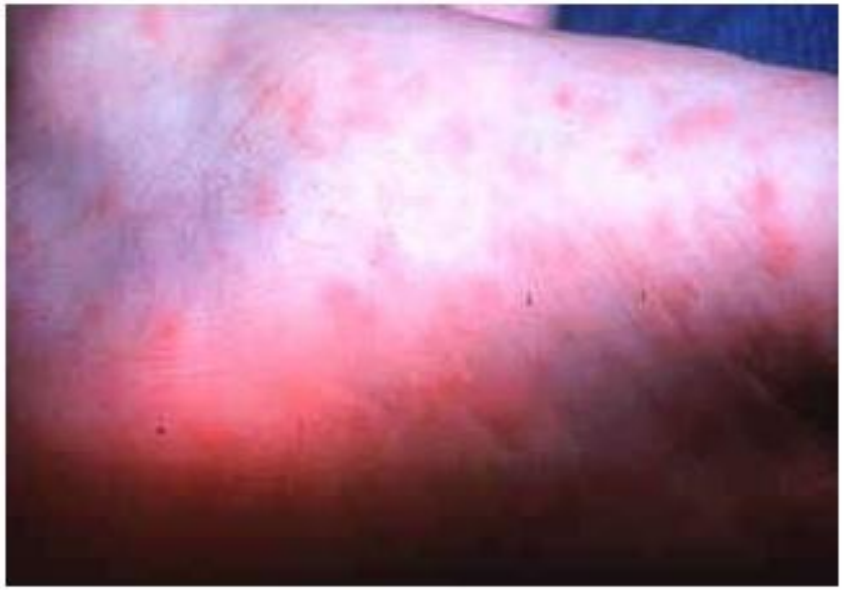




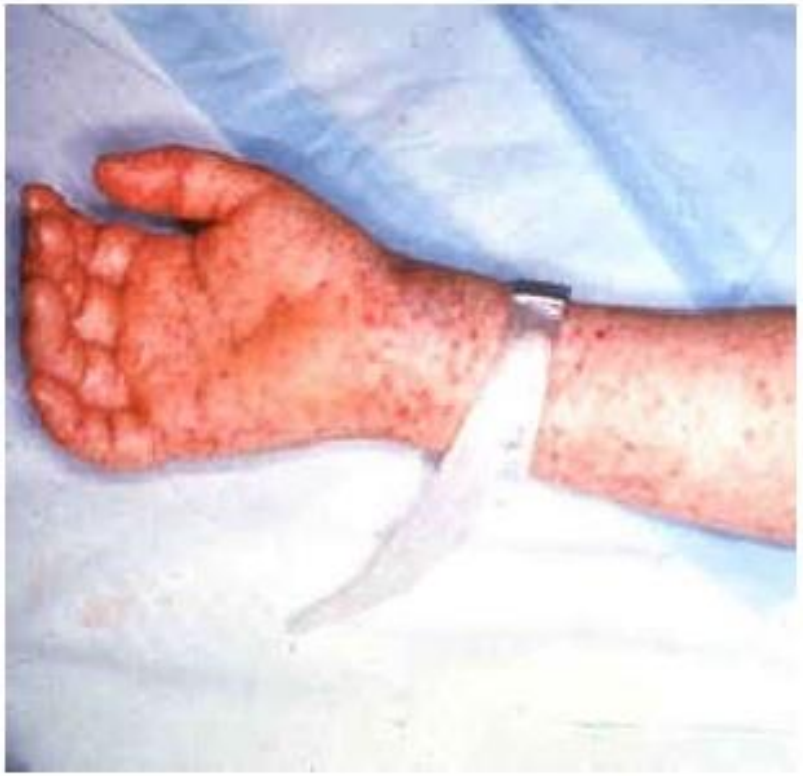




- The clinical symptoms of other spotted fevers are very similar to Rocky mountain spotted fever



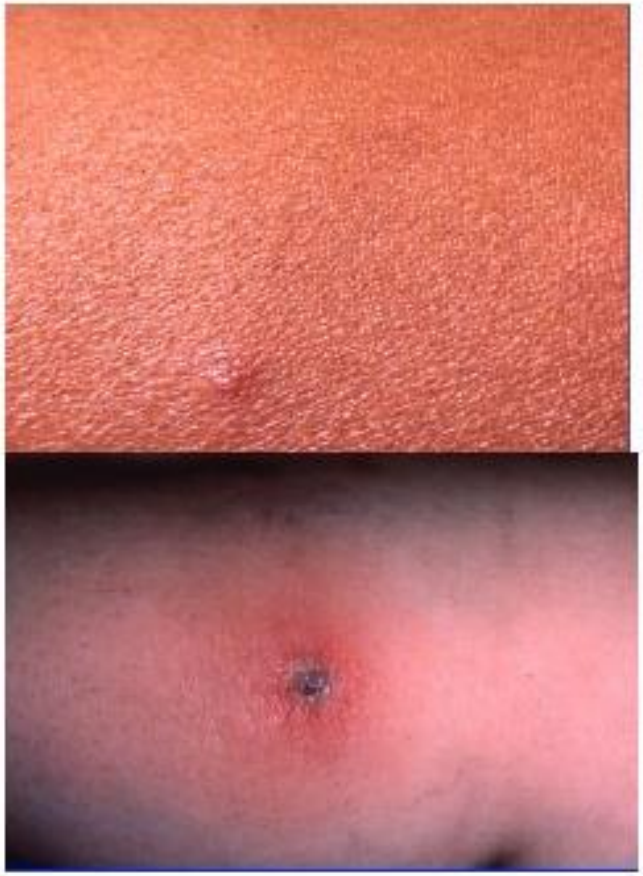
Early (macular) rash on sole of foot.



Late petechial rashes on palm and forearm.

Rickettsial pox

- Benign febrile illness with vesicular rash resembling chickenpox.
- Vector: Liponyssoides sanguineus
- Reservoir: Domestic mouse (Mus musculus)
- Self-limiting, non-fatal.



Complications of rickettsial diseases

- Bronchopneumonia
- Congestive heart failure
- Multi-organ failure
- Deafness
- Disseminated intravascular coagulopathy (DIC)
- Myocarditis (inflammation of heart muscle)
- Endocarditis (inflammation of heart lining)
- Glomerulonephritis (inflammation of kidney)

LABORATORY DIAGNOSIS

- **Isolation from experimental animals**
- **Serology**

Specimens:

Blood – collected in febrile illness

Note: Rickettsia is highly infectious so specimens should be handled very carefully.

ISOLATION

- Blood is inoculated in guinea pigs/mice.
- Observed on 3rd – 4th week.
- Animal responds to different rickettsial species can vary

Symptoms:

- Rise in temperature – all species.
- Scrotal inflammation,swelling,necrosis – R.typhi, R.conori, R.akari (except R.prowazekii)

Serology

- Reliable test to confirm rickettsial diseases
- Antibody detection by Weil-felix test
- Antigen detection by IFA

WEIL-FELIX TEST

- Heterophile agglutination test using

non motile proteus strains (OX 19, OX 2, OX K)
to find rickettsial antibodies in patient's serum.

Procedure:

- Serum is diluted in three separate series of tubes followed by the addition of equal amount of OX19,OX2,OXK in 3 separate series of tubes.
- Incubation at 37^0C for overnight.
- Observe for agglutination.

INTERPRETATION OF WEIL-FELIX TEST

- Strong Agglutination with OX 19 – means epidemic & endemic typhus.
- Strong agglutination with OX 19 & OX 2 – means Spotted fever
- Strong agglutination with OX K – Scrub typhus

(Scrub typhus by Orientia tsutsugamushi (one of the rickettsial disease)

Chlamydia

- Obligate intracellular coccoid parasites
- contain DNA and RNA, and ribosomes
- lack ATP, biosynthetic pathways
- cell wall but peptidoglycan absent -
  - use disulfide bonds
- non motile

Obligate Parasites

- Obligate intracellular parasites of mammals and birds
  - not transmitted by arthropods.
  - incorrectly called the PLT viruses or Bedsonia or basophilic viruses,
- Multiply in the cytoplasm of the host cell.
  - generally epithelial cells
  - Basophilic inclusions

Similar to Viral Infections

- The methods used to study Chlamydia are those of the virologist rather than the bacteriologist.
- The clinical features, pathogenesis, pathology and epidemiology of chlamydial infections are similar to those of viral infections.

Energy Parasites

- The cells can synthesize DNA, RNA and protein.
- No flavoproteins or cytochromes.
- lack of ATP-generating ability
- need to obtain ATP from the host cell.

Three species:

C. trachomatis

C. psittaci

C. pneumoniae

C trachomatis

Trachoma

conjunctivitis

proctitis

urethritis

salpingitis

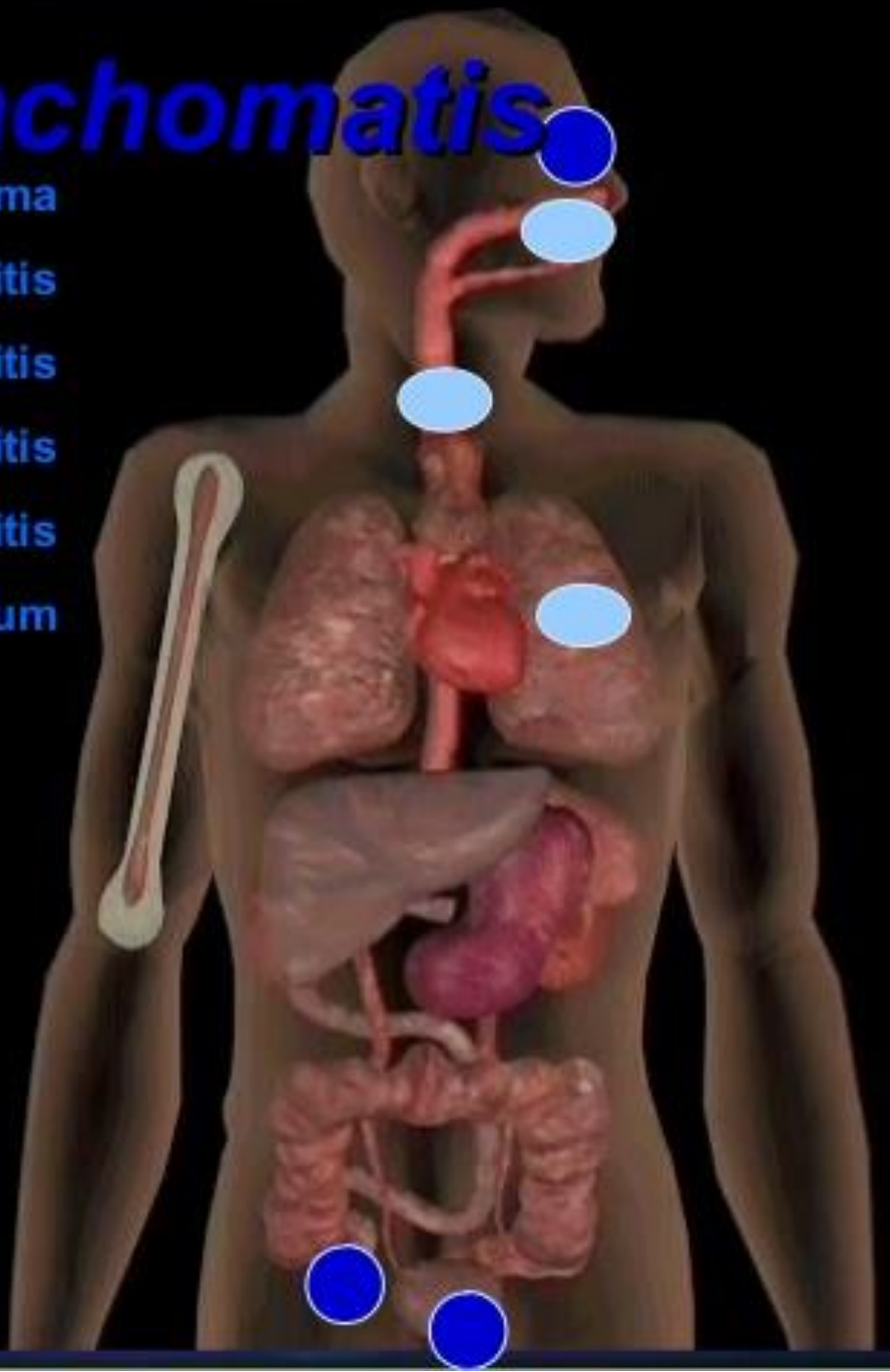
Lymphogranuloma venereum

C psittaci & C pneumoniae

Upper respiratory infection

Bronchitis

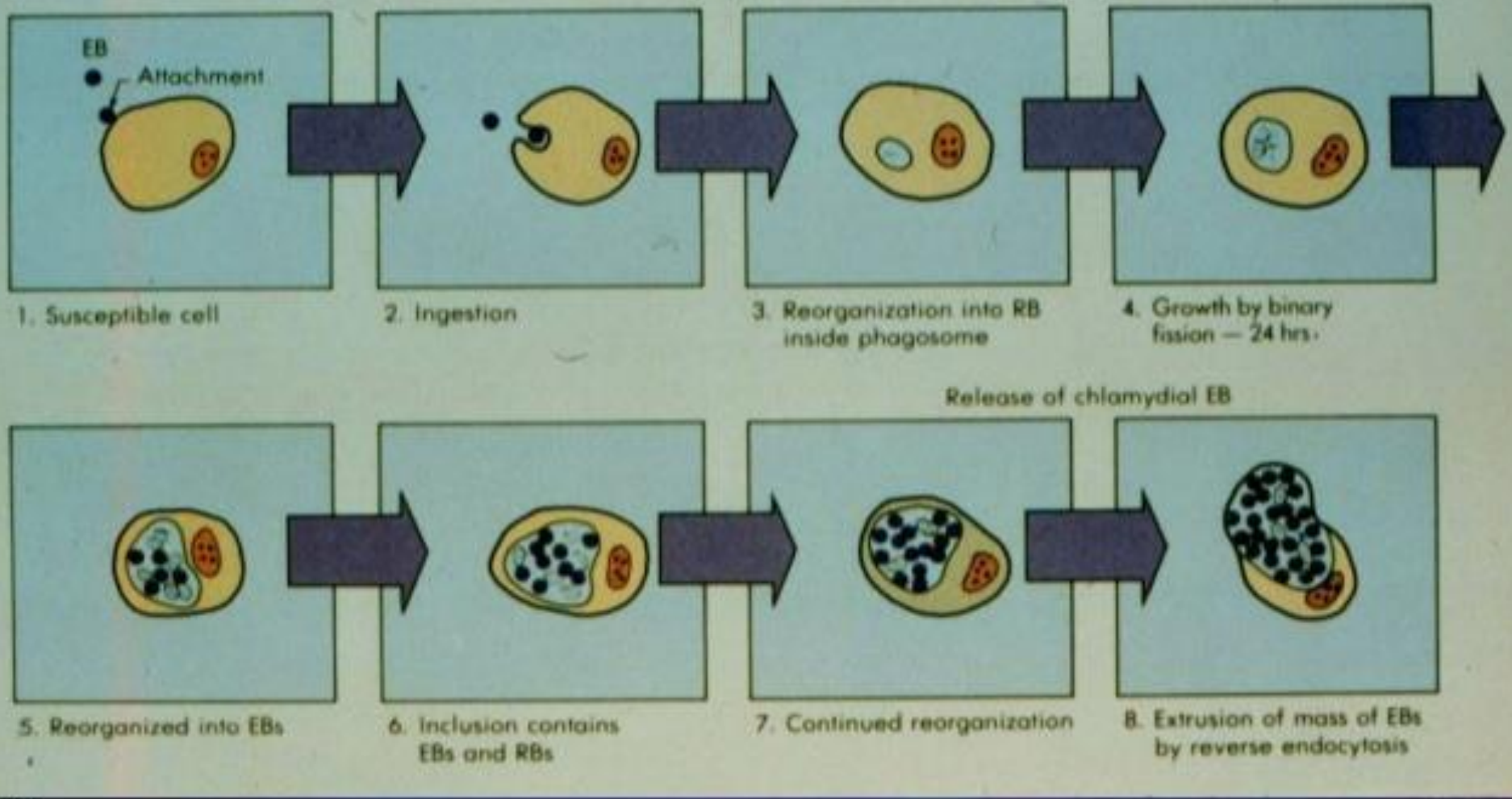
Pneumonia



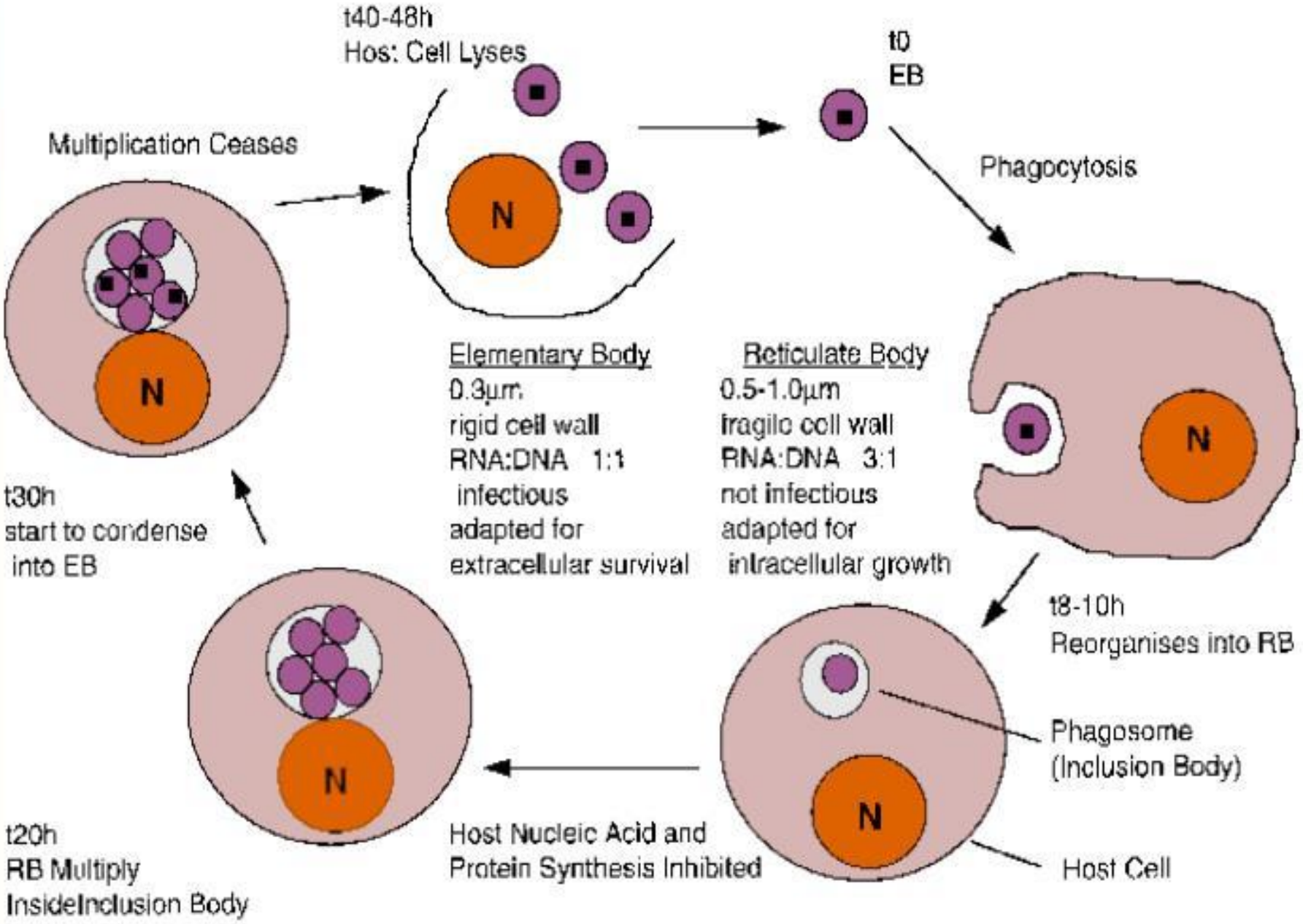
Chlamydial Morphologies

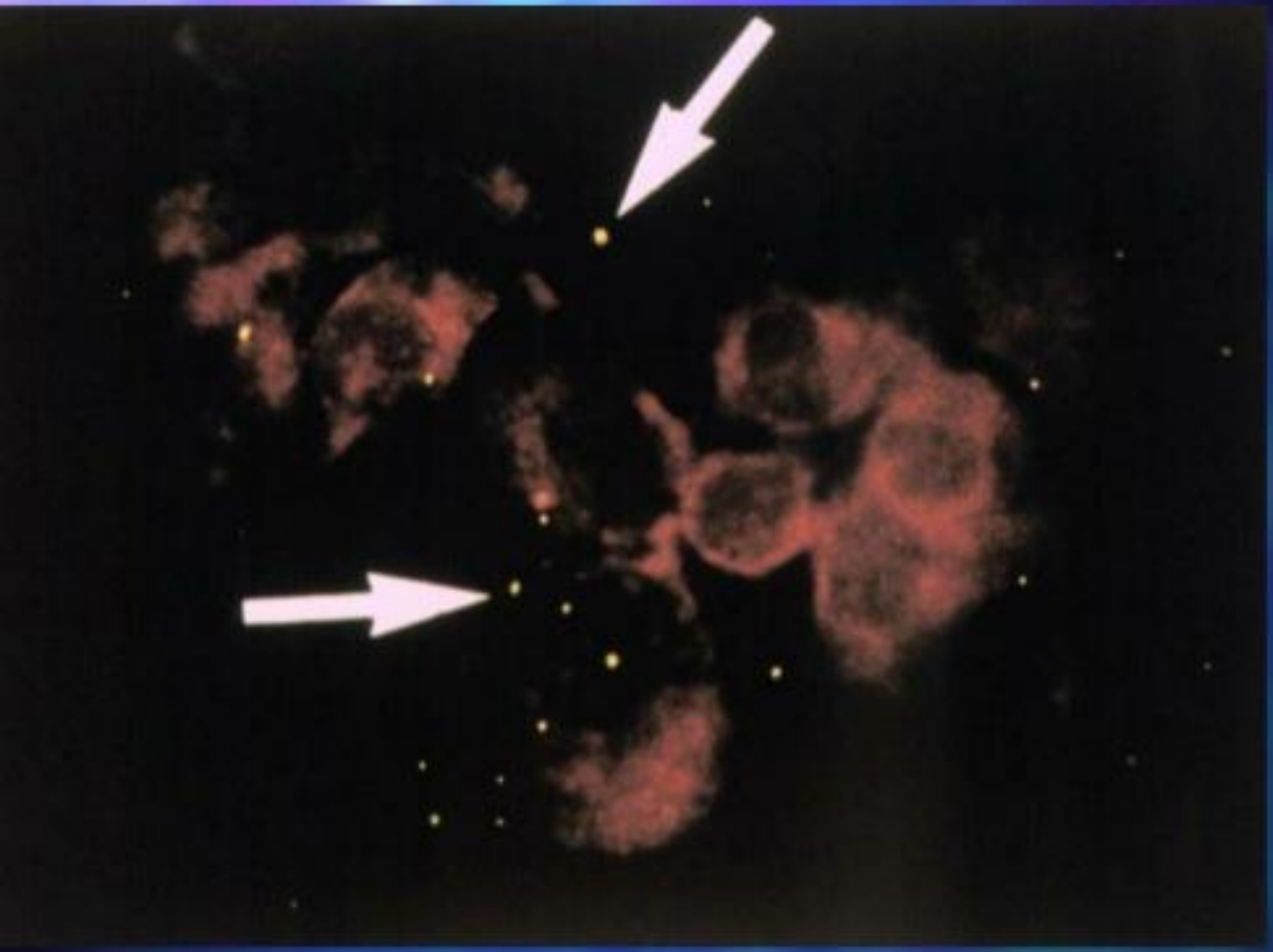
- Elementary body
  - 0.25 - 0.3 um diameter
  - electron-dense nucleoid
  - Released from ruptured infected cells. Human to human
  - & bird to human.
- Reticulate Body
  - Intracytoplasmic form 0.5 - 1.0 um
  - Replication and growth. (Inclusion body)
  - without a dense center.

Developmental Cycle of Chlamydia



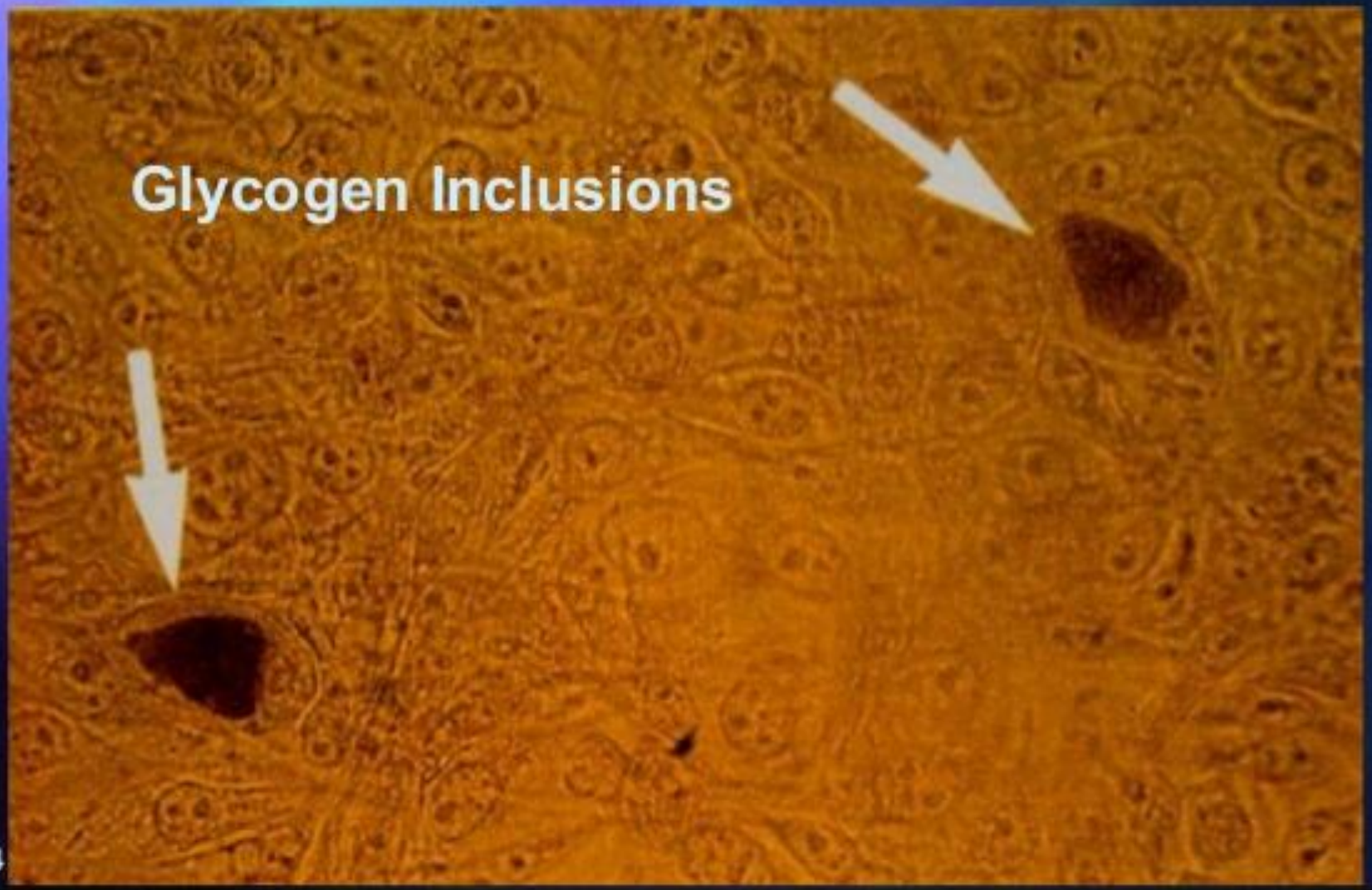
Chlamydial Life Cycle



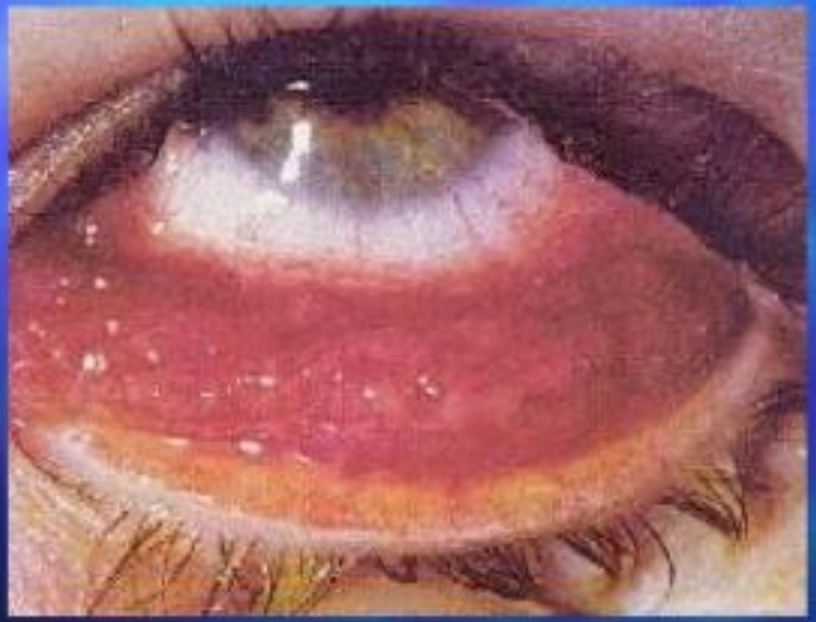


C trachomatis elementary bodies

C trachomatis inclusions



Chlamydial Infection of Ocular Conjunctiva



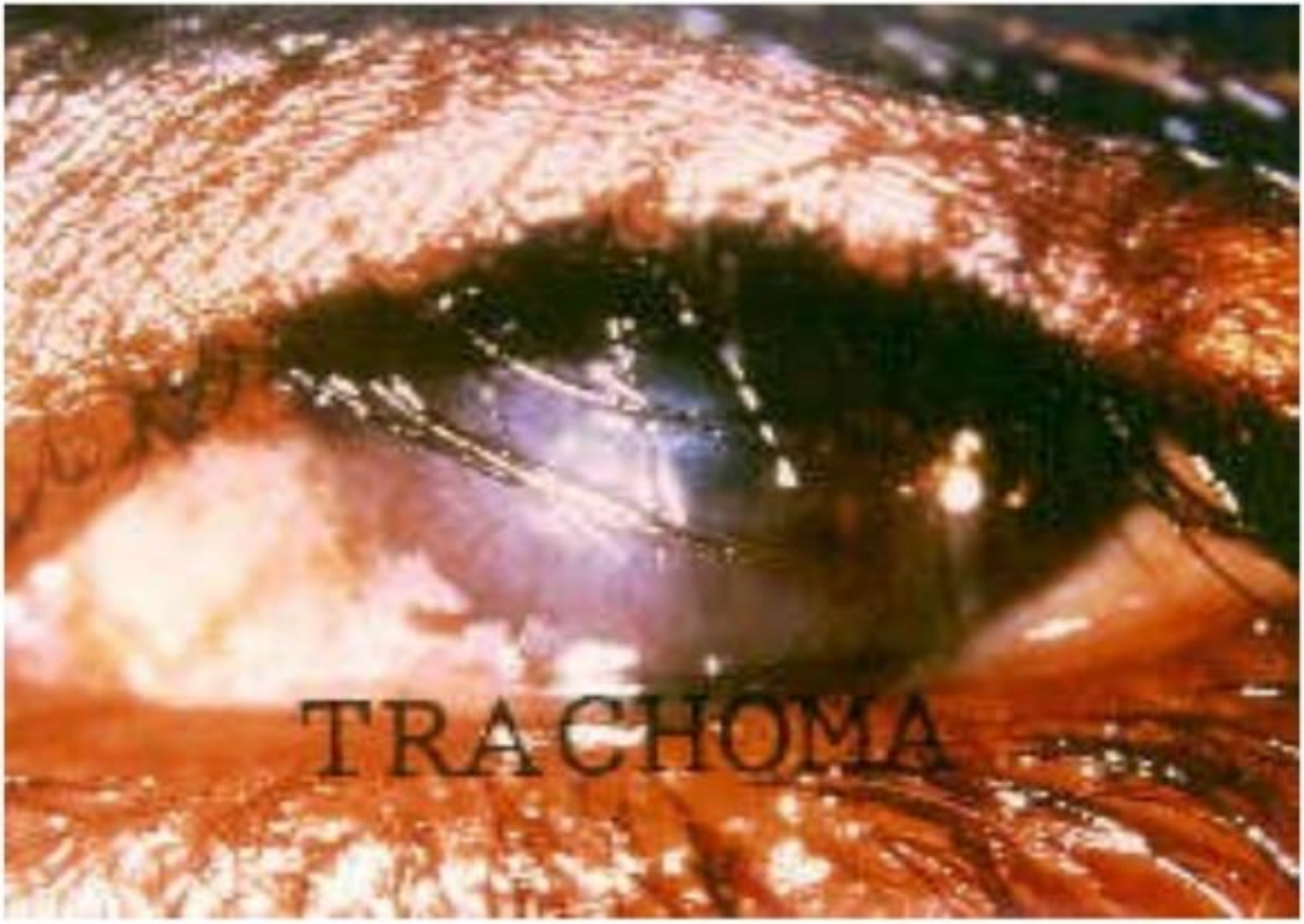
Trachoma

- infection of conjunctival epithelial cells.
  - subepithelial infiltration of lymphocytes.
  - Infected epithelial cells contain cytoplasmic inclusion bodies
  - Cell infiltrations to cornea cause clouding
- Trichiasis: abnormal inward growth of eyelashes.

Trachoma

- Almost six million people have become blind and another 540 million almost 10% of the world's population are at risk

Trachoma

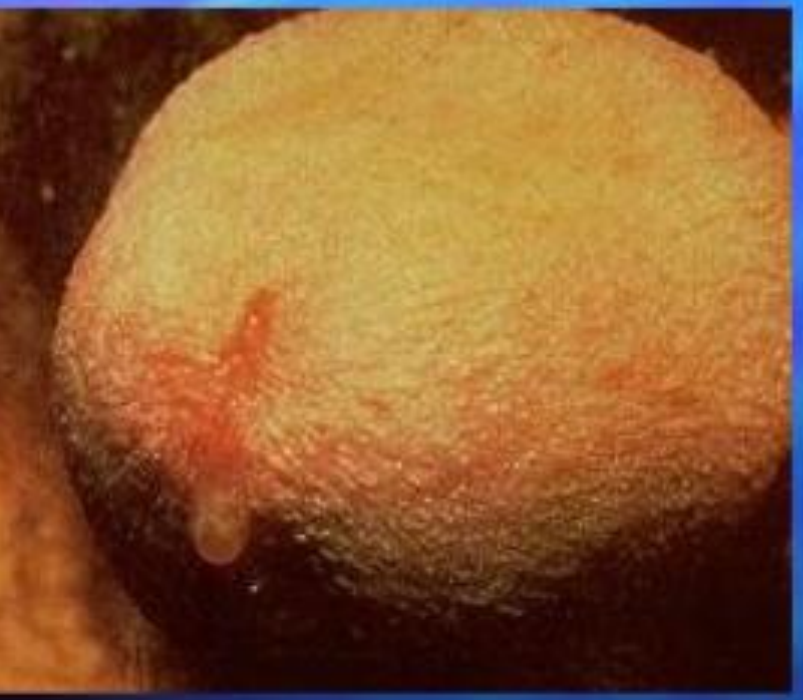


Chlamydial infection in the eye causing trachoma. From the Dana Center Trachoma Study.

Chlamydia trachomatis
Clinical disease

- lymphogranuloma venereum
- nongonoccal urethritis (NGU)
- epididymitis
- salpingitis
- mucopurulent cervicitis
- pelvic inflammatory disease (PID)
- Reiter's syndrome
- neonatal chlamydia

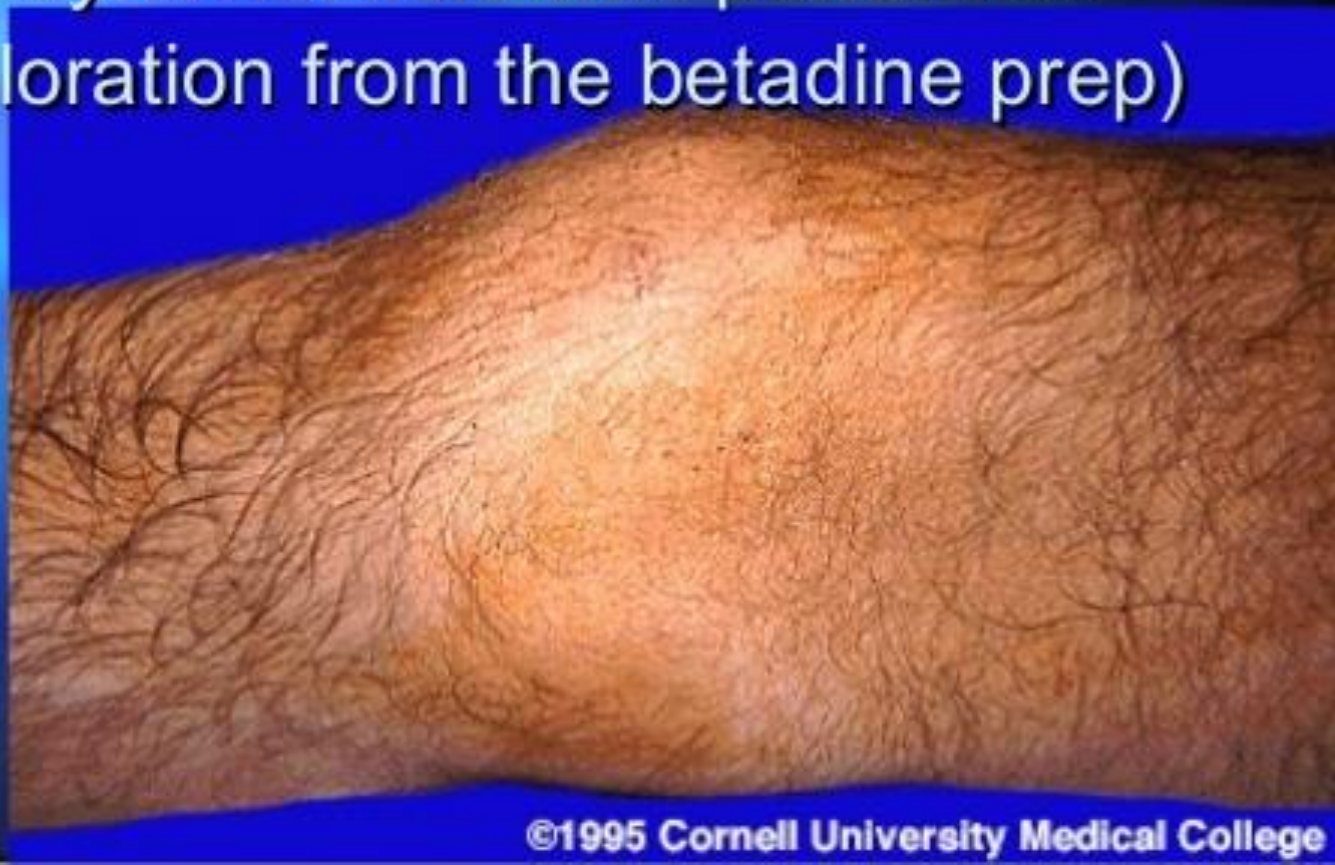
Chlamydia Symptoms In Men



- Symptoms usually appear between 7 and 28 days after infection, usually with mild burning when urinating, a more frequent need to urinate, and a white discharge from the penis. Occasionally, blood may appear in the urine. The symptoms occur most frequently in the morning.

Nongonococcal urethritis (NGU) - Reiter's syndrome

- Swollen, painful right knee in which needle aspiration for synovial fluid was performed (yellow discoloration from the betadine prep)

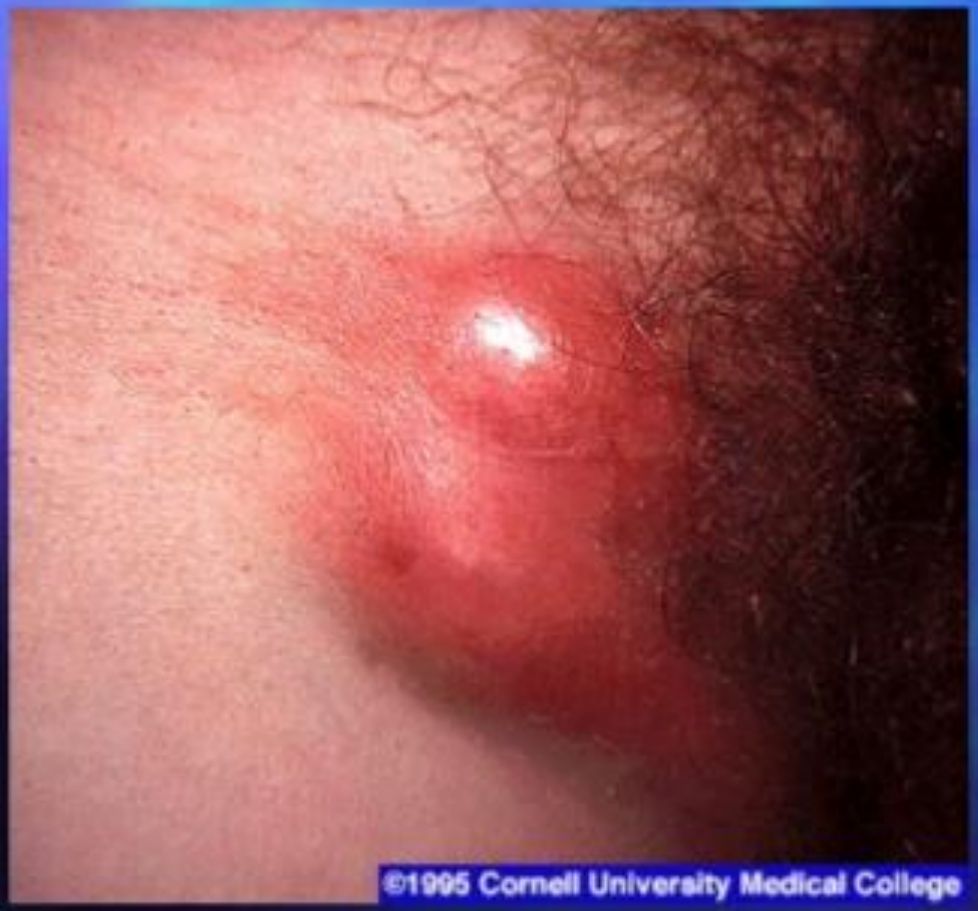


Hyperlink to original site

Lymphogranuloma venereum LGV

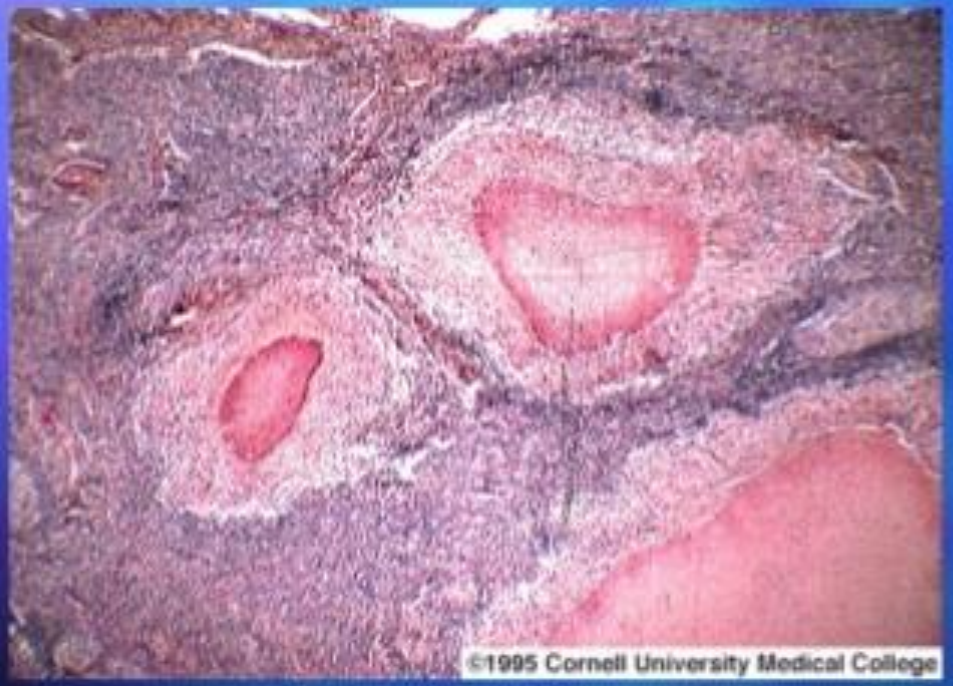
- 200 reported cases per year.
- Incubation period is 5 to 20 days.
- Lesion: Transient vesicles on penis or vagina that are often unnoticed and patients do not usually seek medical advice.

LGY: "Groove sign"



- Pompart's ligament is preserved despite the involvement of multiple inguinal nodes

LGV: Microscopy



- lymph node shows both necrosis and granulomatous reaction (dimorphic necrotizing granulomatous reaction)

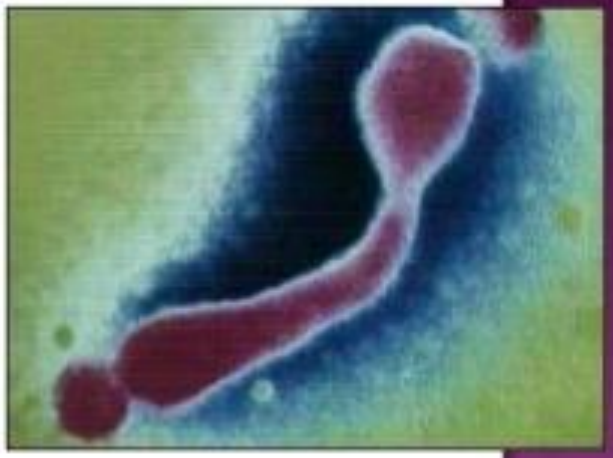
Mycoplasma

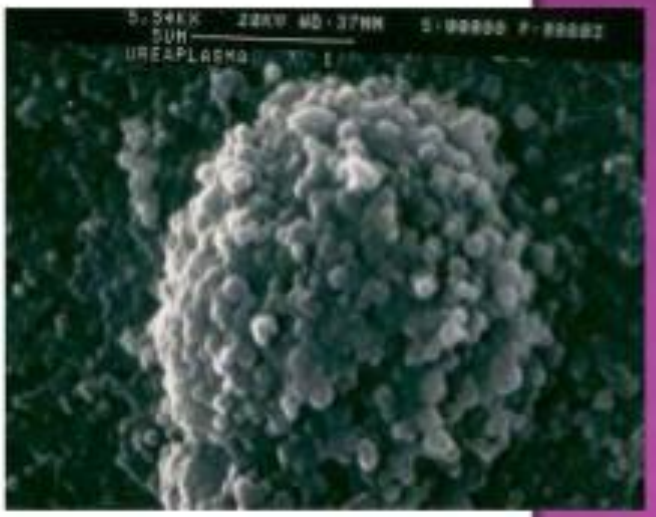
- ***Mycoplasma*** species are the smallest free-living organisms. These organisms are unique among prokaryotes in that they lack a cell wall.

- Mollicutes

- Familia *Mycoplasmataceae*

- *Mycoplasma pneumoneae.*
- *Mycoplasma hominis*
- *Mycoplasma genitalium*
- *Ureaplasma urealyticum*





Urogenital mycoplasmas

Table 3.1: Disease associations of urogenital mycoplasmas

Species	Disease associations[a]						
	Urethritis	Cervicitis	Bacterial vaginosis	Endometritis and/or PID	Preterm birth	Infertility (Women)	HIV transmission
M. genitalium	++++	+++	–	+++	+/–	+	+
M. hominis	–	–	++++	+/–	+/–	–	ND
Ureaplasmas (undifferentiated)	+/–	–	+++	ND	+	+/–	ND
U. urealyticum	+	ND	ND	ND	ND	ND	ND
U. parvum	–	ND	ND	ND	ND	ND	ND

ND, not determined; PID, pelvic inflammatory disease.

[a] ++++ strong association, +++ association in most studies, + association only from a few studies, +/– conflicting results.

Jensen and Unemo. WHO Manual. 2013

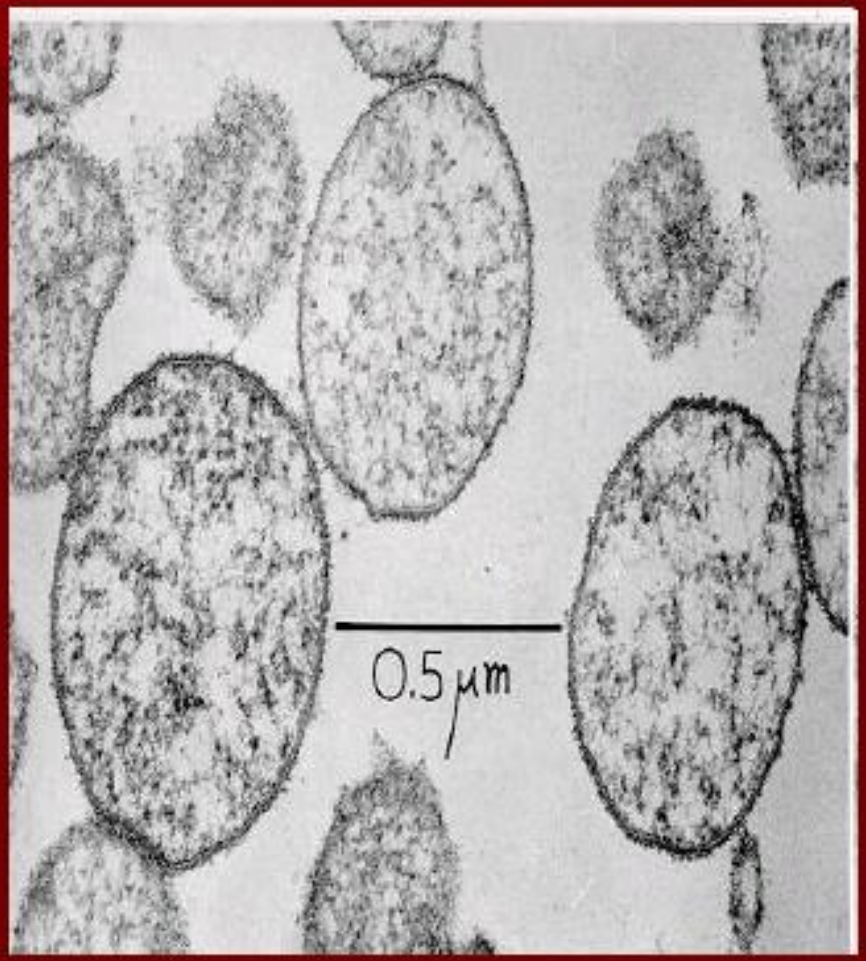
Urogenital mycoplasmas

- *M.hominis, U.urealyticum, U.parvum*- commonly detected in healthy individuals
- Their association with urogenital infection in either men or women remains to be conclusively proven

Basic Characters of Mycoplasma

- Prokaryotic microbes
- Size of 150-250 nm
- Lack of a cell wall
- Sterol-containing cell membrane
- Fastidious growth requirements
- Fried-egg or mulberry colonies on agar

Mycoplasma are cell wall deficient microorganisms



- Cross-section of **Mycoplasma** bacteria, a common cause of atypical pneumonia. This bacteria is unusual in that it lacks a cell wall.

Culturing Mycoplasma

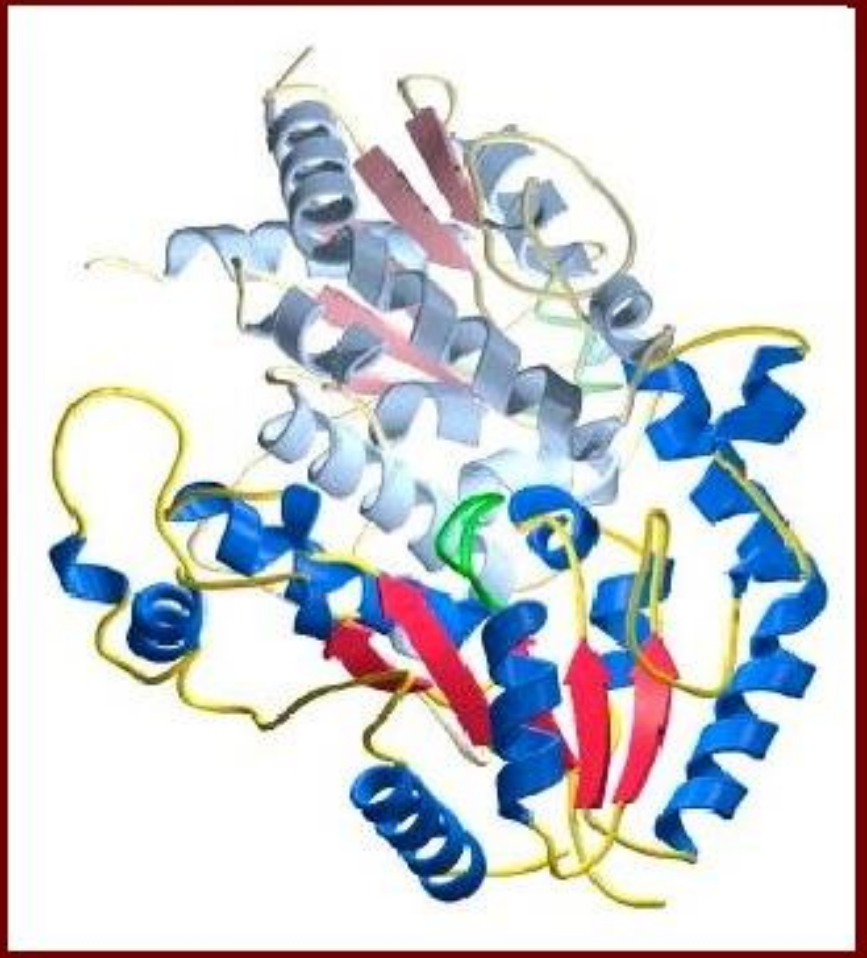
- Mycoplasma can be cultured on liquid or solid medium
- Growths optimally at 35 to 37^{0}c
- Medium of growth should be enriched with 20% horse or human serum.
- The colonies appears as fried egg appearance

Characters of Mycoplasma

- They are prokaryotes but lack a cell wall. However, they have a unique cell membrane that contains **sterols,** which are not present in either bacteria or viruses. *Mycoplasma* organisms are small (150-250 nm) and have deformable membranes. The name *Mycoplasma* refers to the plasticity of the bacterial forms resembling fungal elements.

Antigenic properties

- The surface antigens are glycolipids and protiens
- Glycolipids are identified by complement fixation.
- Proteins antigens detected by ELISA method.



Resistance

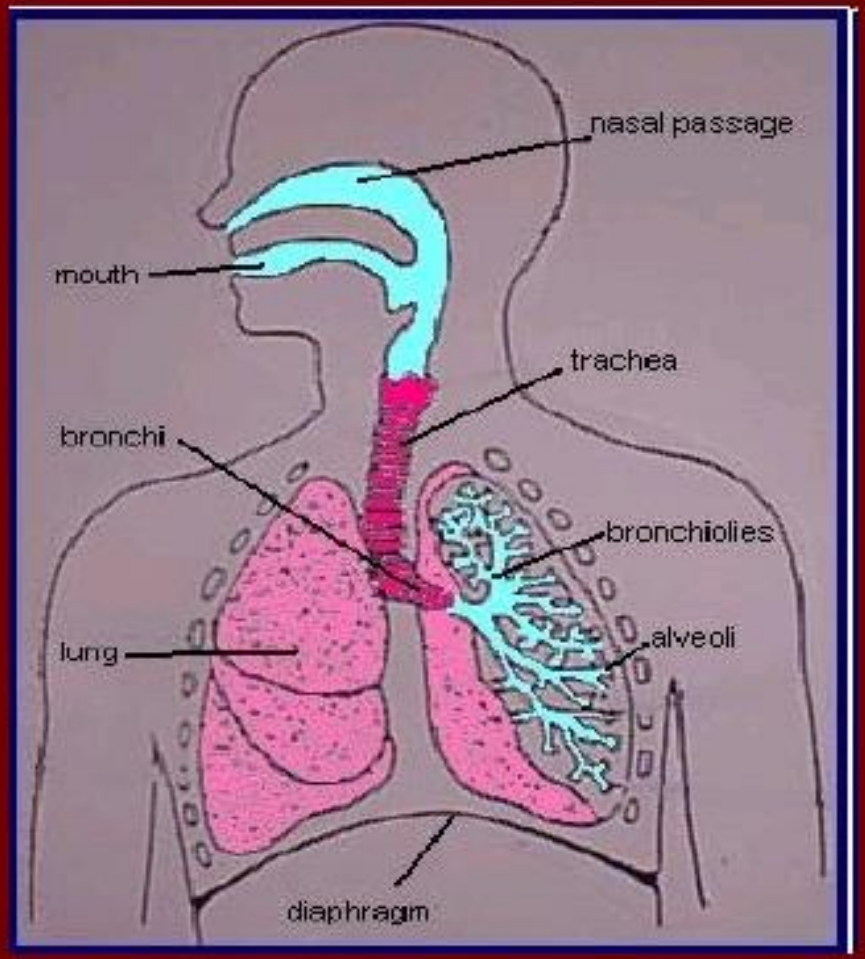
- They are normally destroyed by heat at 45^{0}c in 15 minutes.
- They are relatively resistant to pencillins, and Cephalosporins
- Sensitive to Tetracyclnes, and several other antibiotics

Clinical Manifestations

- Generalized aches and pains
- Fever (usually 102°F)
- Cough - Usually non-productive
- Sore throat (nonexudative Pharyngitis)
- Headache/ myalgias
- Chills but not rigors
- Nasal congestion with coryza
- Earache
- General malaise

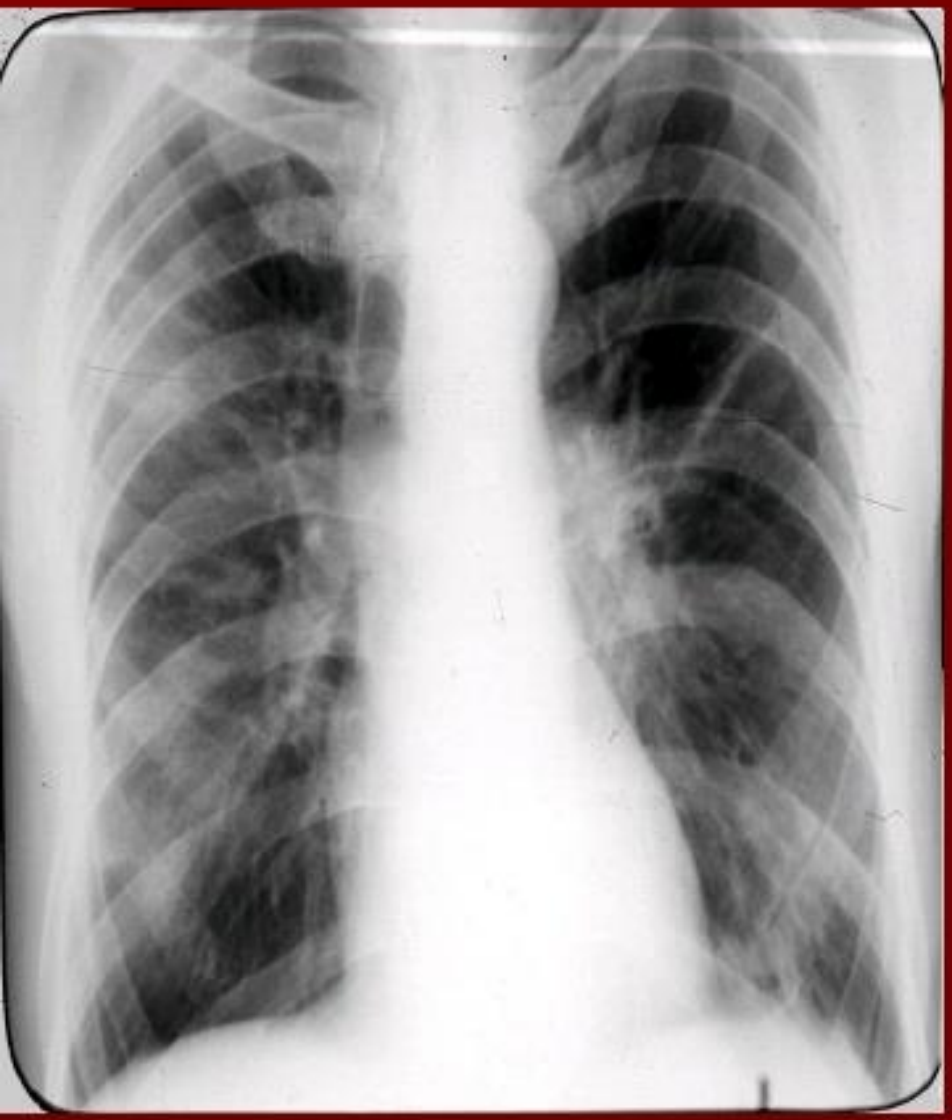
Respiratory spread

- Infection moves easily among people in close contact because it is spread primarily when infected droplets from the respiratory system circulate in the air due to coughing, spitting, or sneezing



Pneumonia leading Manifestation in Mycoplasma infections

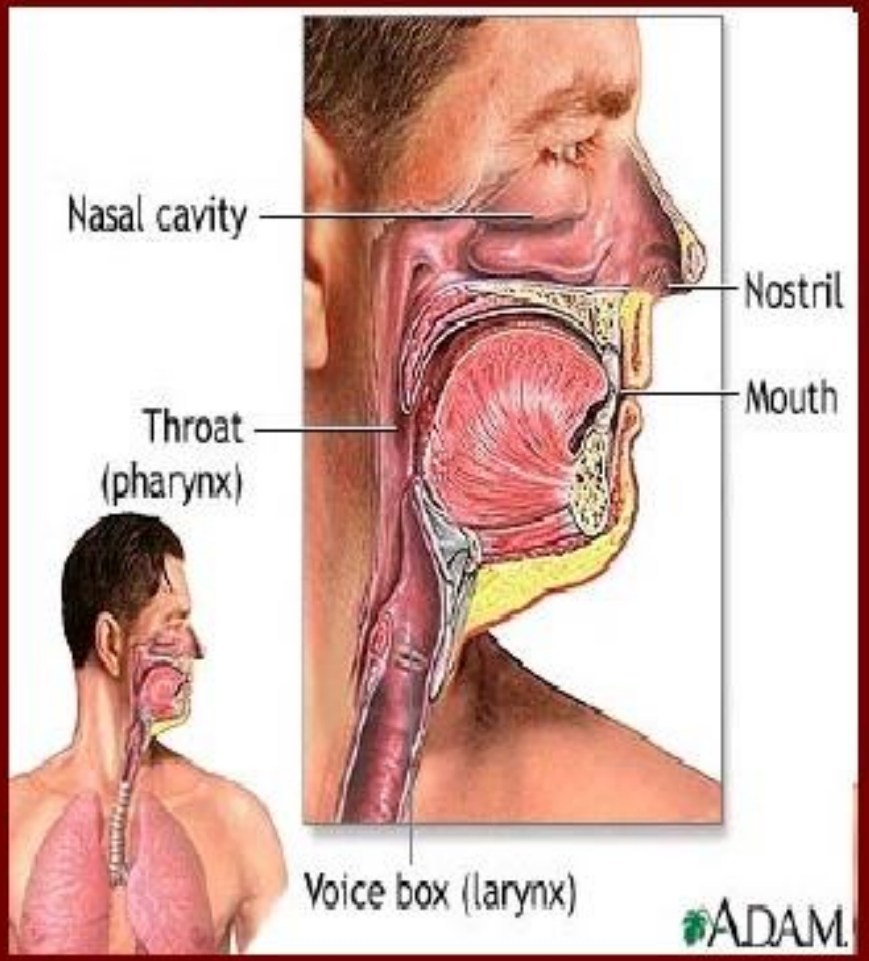
Pneumonia



- Pneumonia caused by Mycoplasma is also called atypical pneumonia, walking pneumonia, or community-acquired pneumonia.

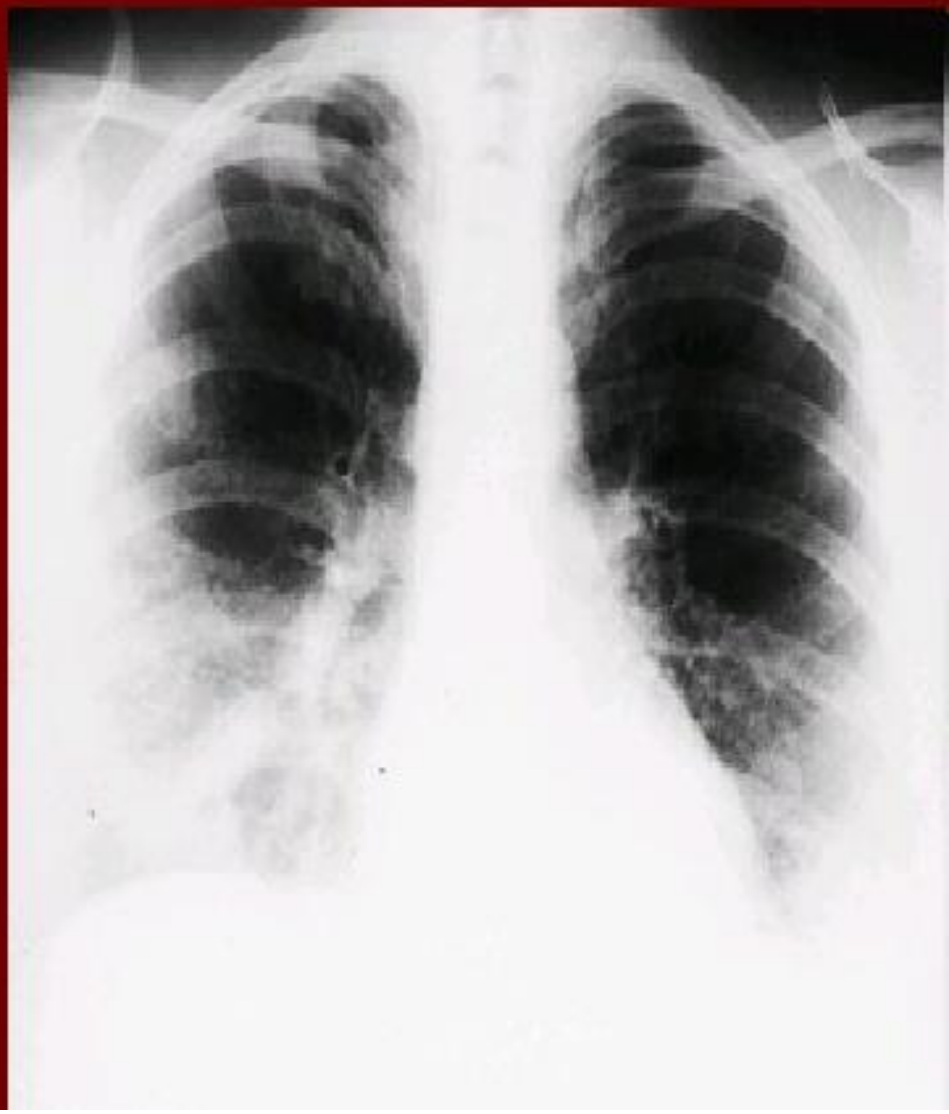
Mycoplasma presents as non specific Respiratory infections

- Infections commonly involve the oropharynx, trachea, bronchi, and lungs, usually causing unilateral pneumonia of the lower lobe.
  The radiographic appearance cannot be distinguished from that of other nonbacterial pneumonias.



Radiological presentation

- The radiological picture is extremely variable, but one or both lower lobes are usually involved. The opacities usually start as partly mottled, partly node-like peribronchial opacities, which may gradually develop to involve whole segments or lobes



Mycoplasma in New born

- Colonization of infants by genital *Mycoplasma* species usually occurs during passage through an infected birth canal, and genital mycoplasmal organisms have been isolated from the upper respiratory tract in 15% of infants. Colonization usually does not persist beyond 2 years.

Cardiac Manifestations

- Arrhythmia and/or ECG abnormalities (conduction defects)
- Congestive failure
- Pericarditis
- Myocarditis
- Endocarditis

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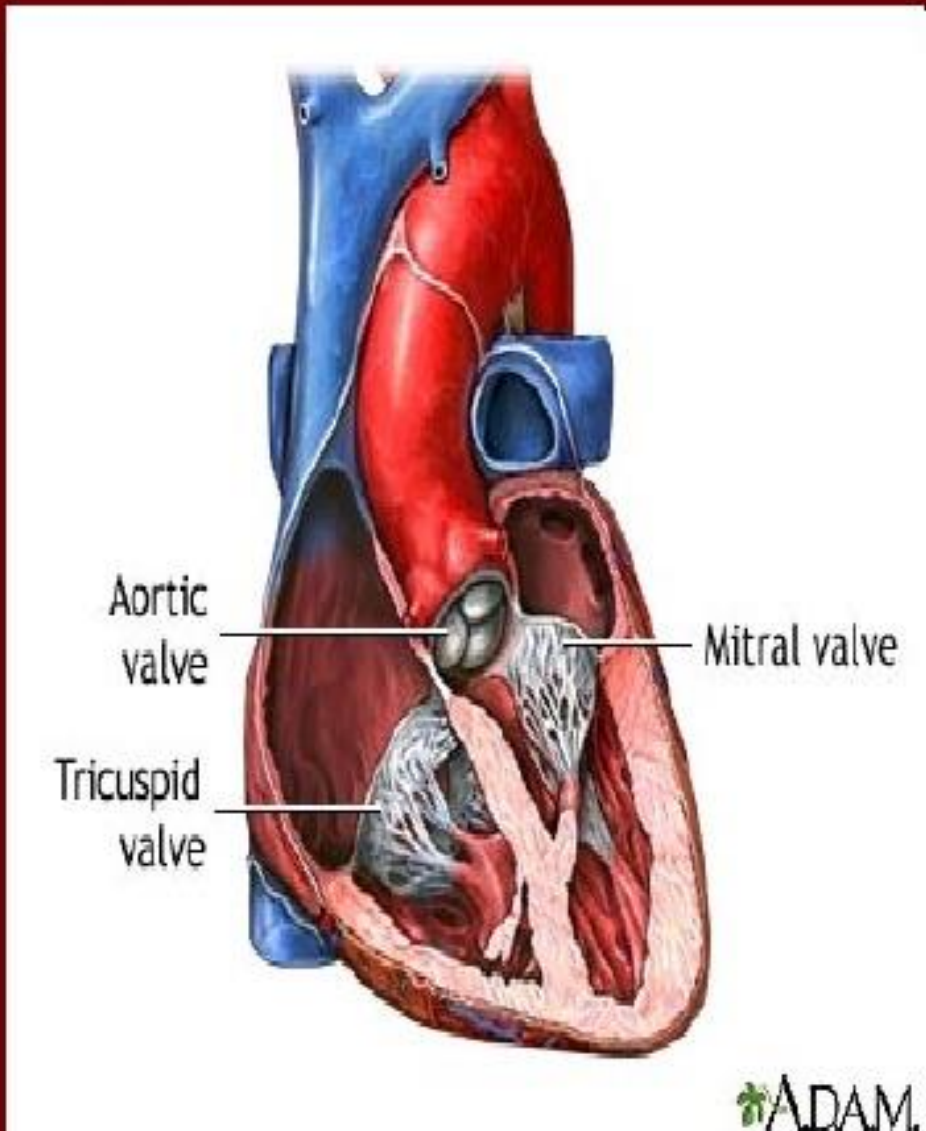
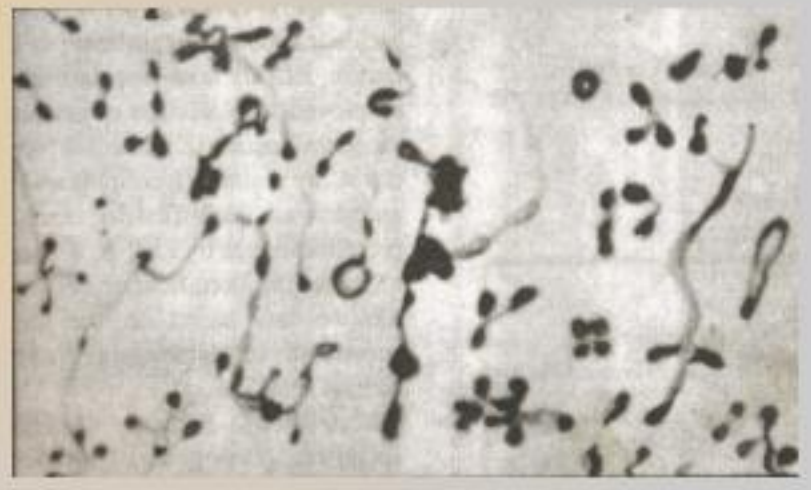


Laboratory Diagnosis

- **Specimens** – throat swabs, respiratory secretions.



- **Microscopy** –

1. Highly pleomorphic, varying from small spherical shapes to longer branching filaments.
2. Gram negative, but better stained with Giemsa.

Laboratory Diagnosis

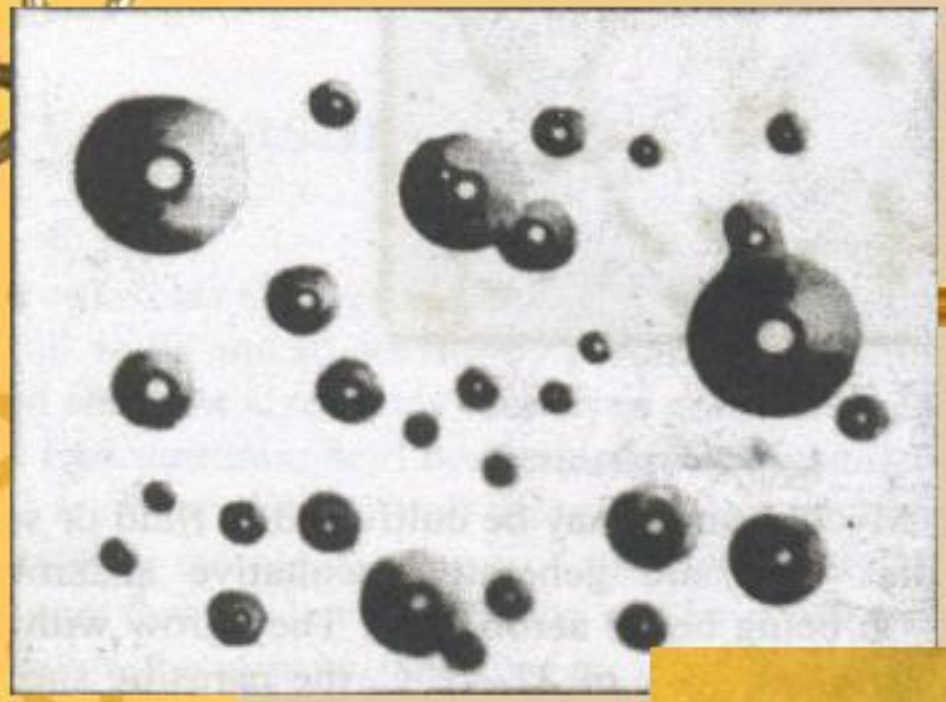
- Isolation of Mycoplasma (Culture) -

1. Semi solid enriched medium containing 20% horse or human serum, yeast extract & DNA. Penicillium & Thallium acetate are selective agents.
(serum – source of cholesterol & other lipids)

2. Incubate aerobically for 7 -12 days with 5-10% CO_2 at 35-37°C. (temp range 22- 41°C, parasites 35- 37°C, saprophytes – lower temp)

Laboratory Diagnosis

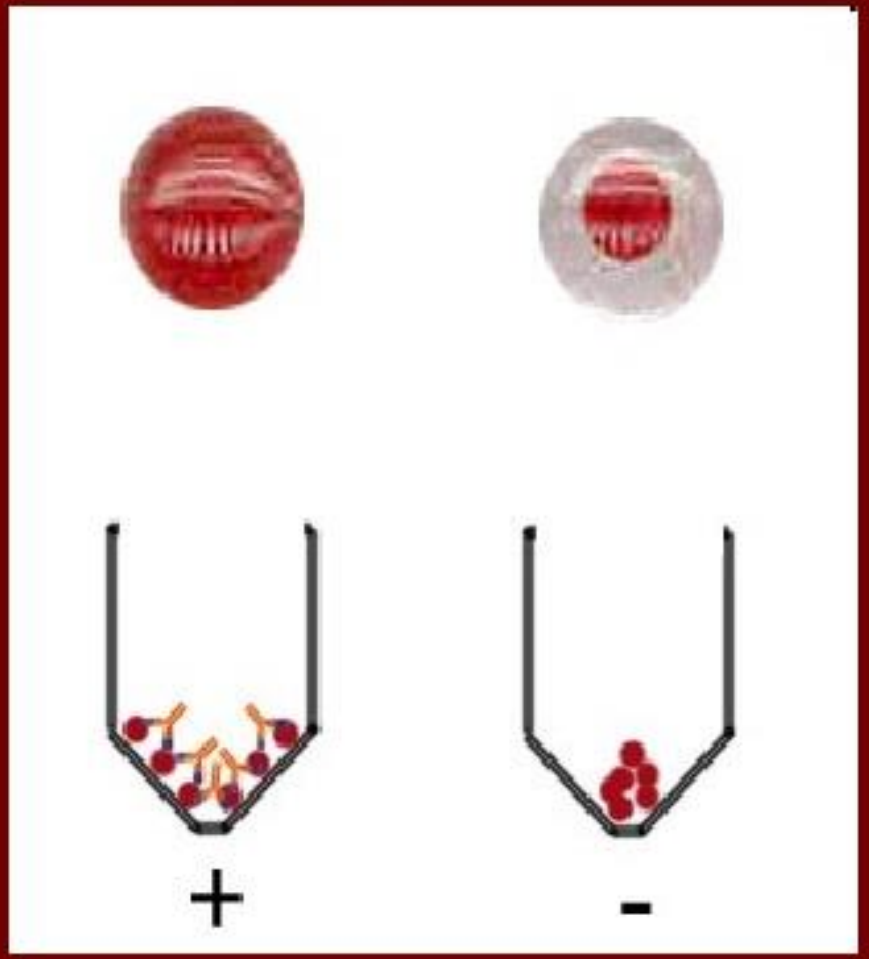
3. Typical "fried egg" appearance of colonies - Central opaque granular area of growth extending into the depth of the medium, surrounded by a flat, translucent peripheral zone.
4. Colonies best seen with a hand lens after staining with Diene's method.
5. Produce beta hemolytic colonies, can agglutinate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



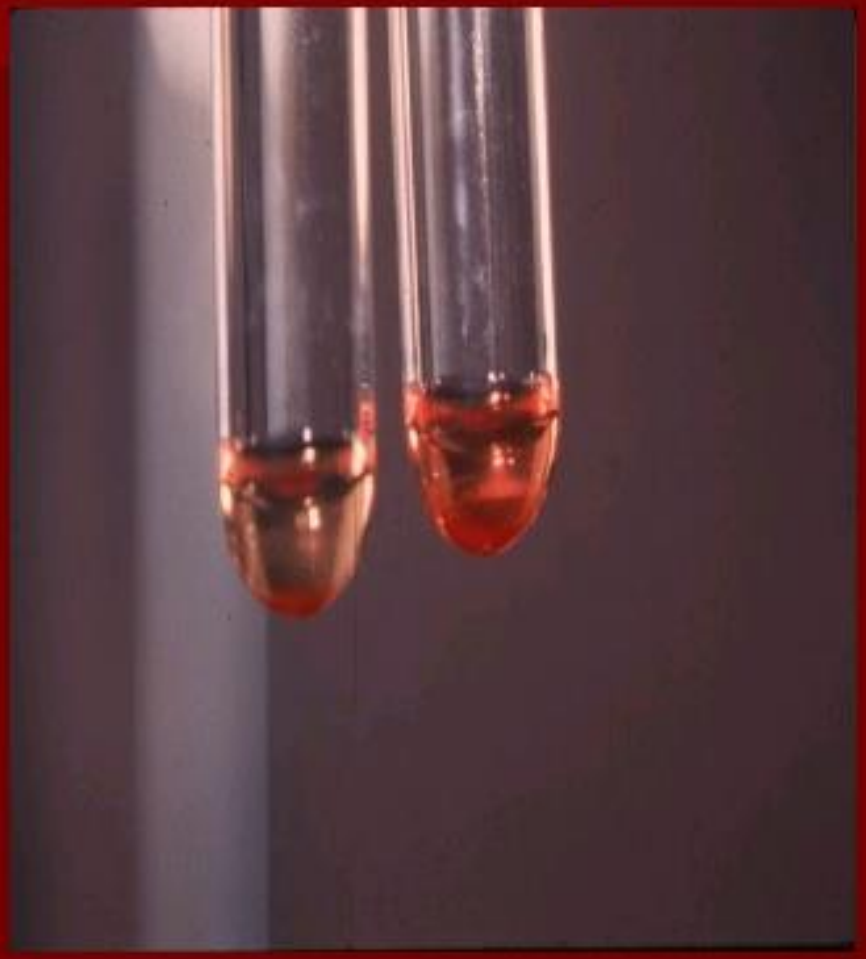
Fried egg colonies

Cold Agglutination Test

- Cold Agglutination test is associated with macroglobulin antibodies that agglutinate human o RBC at low temperature

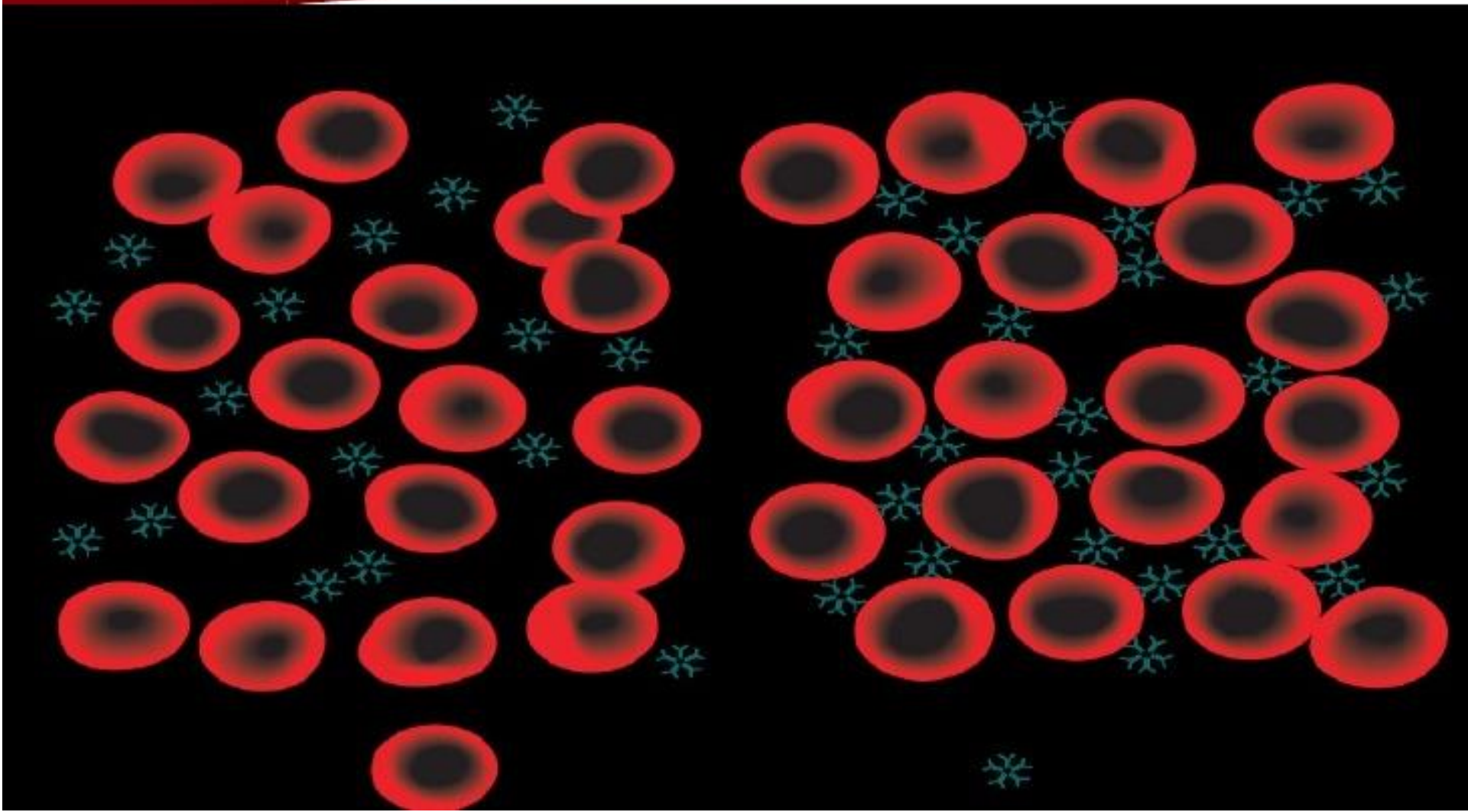


Cold Agglutination test procedure



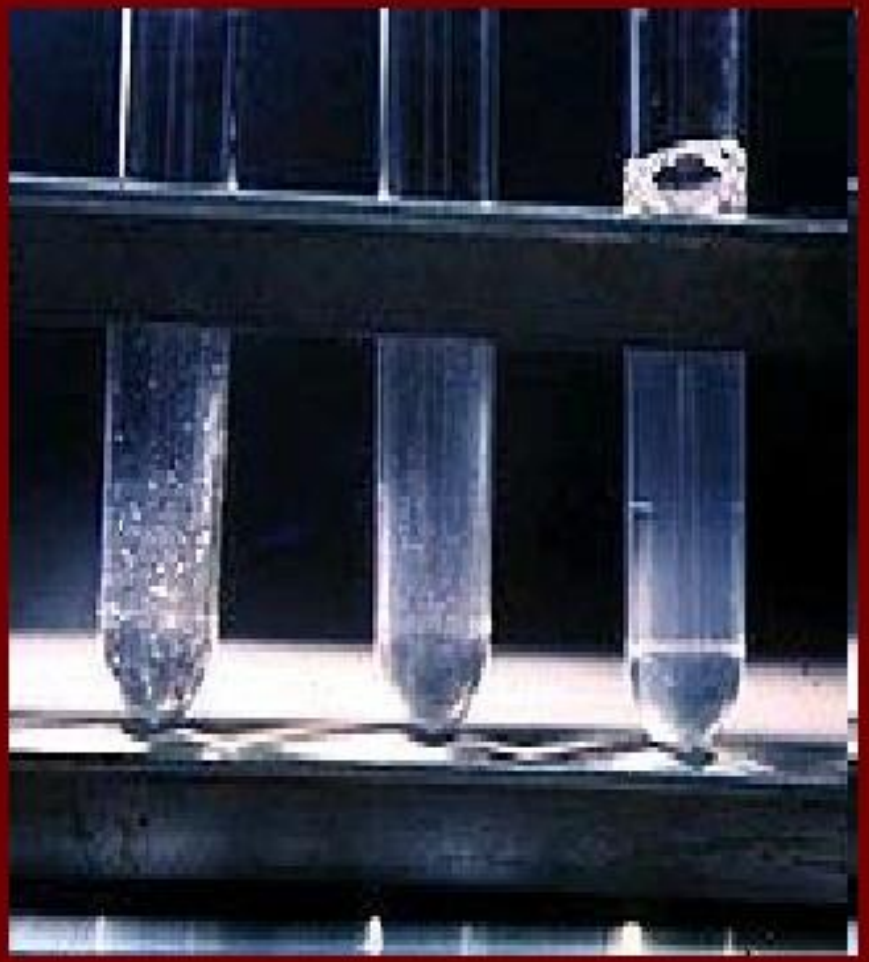
- The serial dilutions of patients serum are mixed with an equal volume of 0.2% washed human O group erythrocytes at low temperature
- The clumping is observed at 4^0c overnight.
- However the clumping is dissociated at 37^0c
- A titer of 1:32 or > is suggestive.
- A raised titer in paired serum sample is more suggestive of infection.

RBC showing non agglutinating and agglutinating RBC



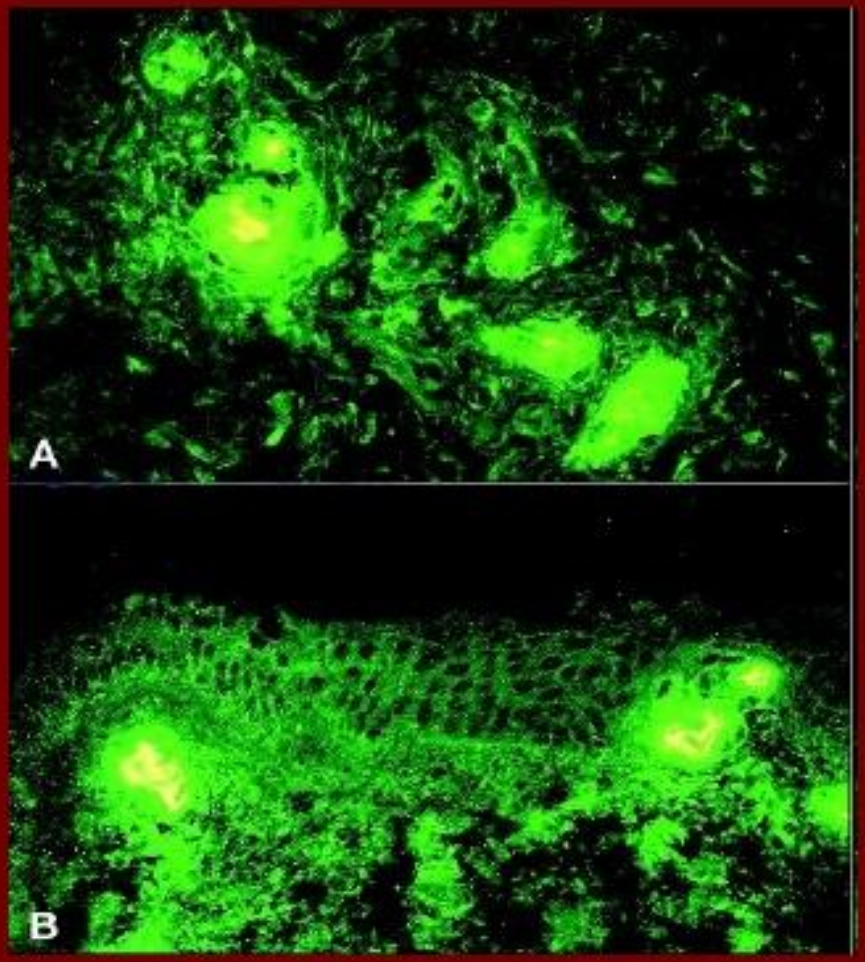
Streptococcal MG test

- The test is performed by mixing serial dilutions of patients serum with heat killed suspension of Streptococcus MG.
- The sample is incubated at 37^{0}c
- The agglutination titer of 1:20 or > is suggestive.

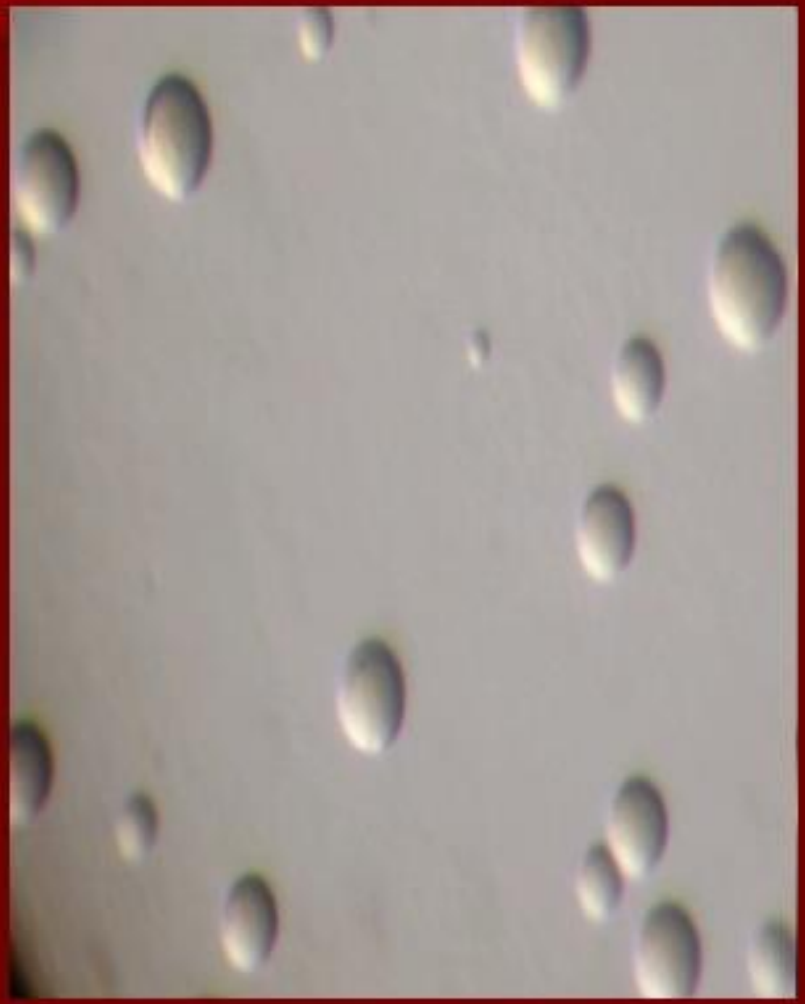


Other Serological Tests

- Immunofluorescence
- Hemagglutination inhibition test
- Complement fixation test *less sensitivie*.



Growth of Bacteriological Medium

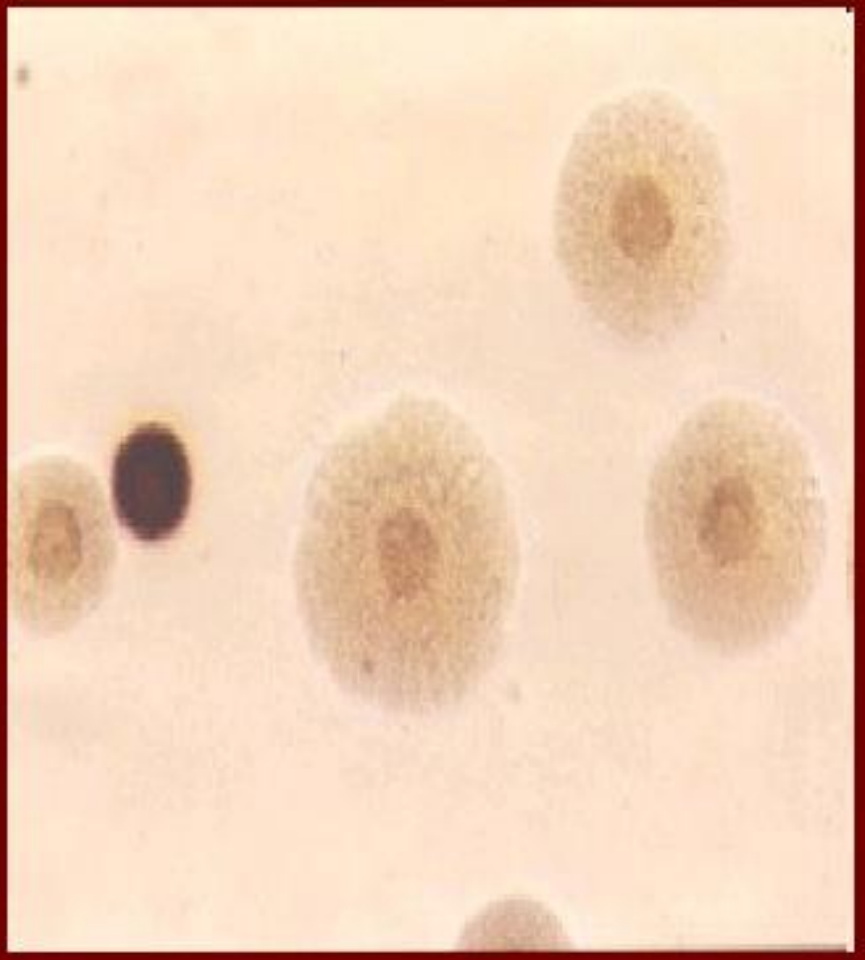


- For isolation swabs from throat or respiratory secretions inoculated not Mycoplasma medium
- The growth is slow and takes 1 – 3 weeks
- The colonies appear as fried egg, with central opaque granular area surrounded by flat translucent peripheral zone

Mycoplasma on PPLO agar

Typical Mycoplasma colonies on enriched medium

- The colonies showing typical fried egg appearance.
- The colonies appear 2-6 days of incubation.
- The size of the colonies can be from 10 – 600 microns in size.



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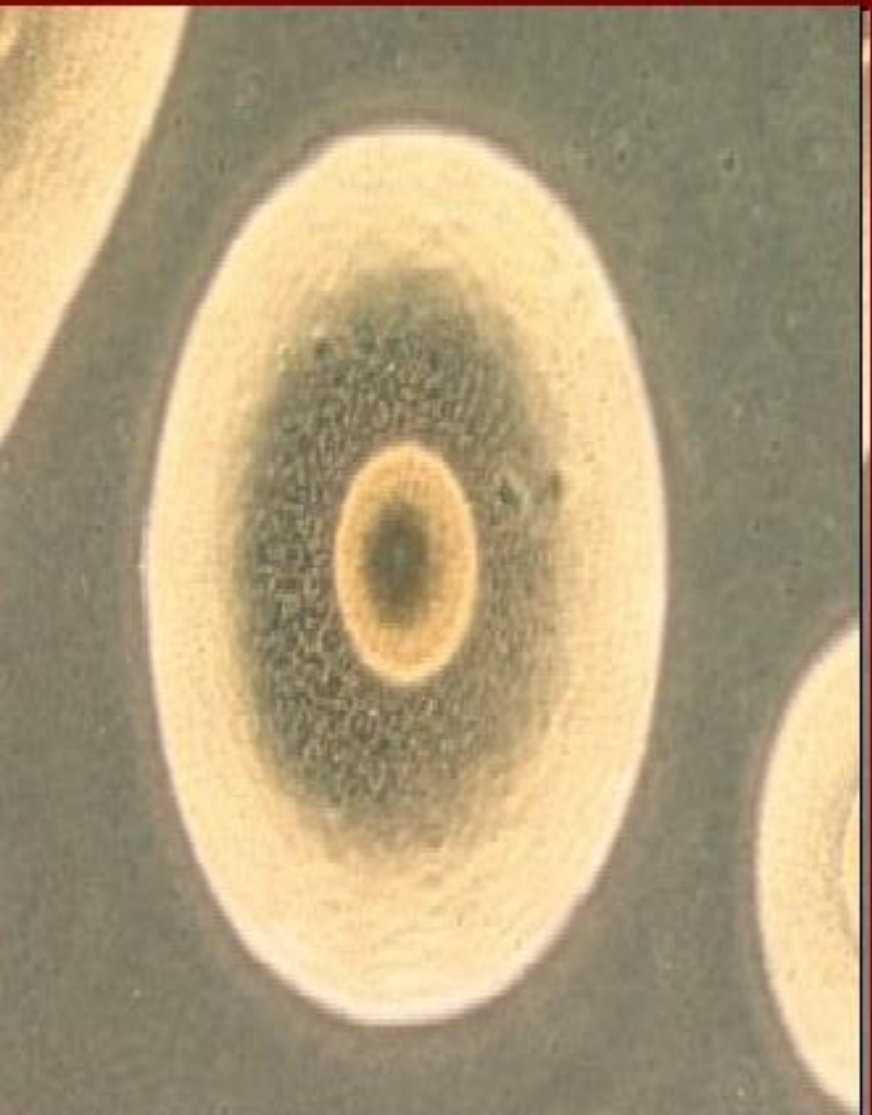
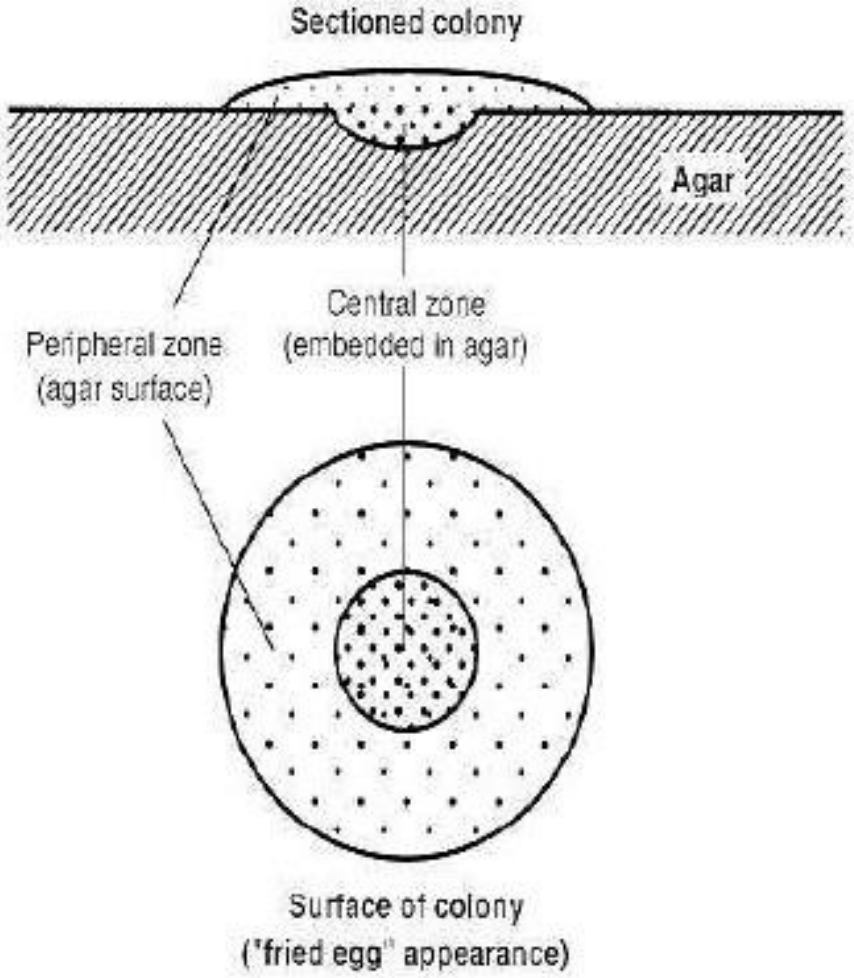
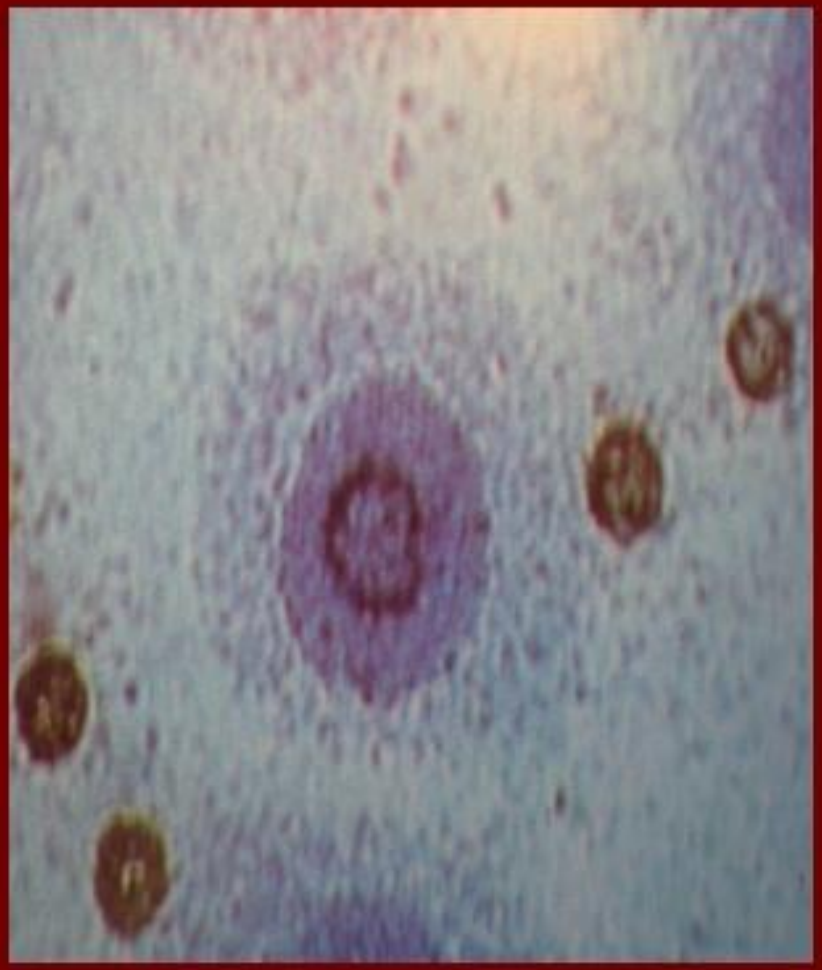
Colony characters of Mycoplasma isolates



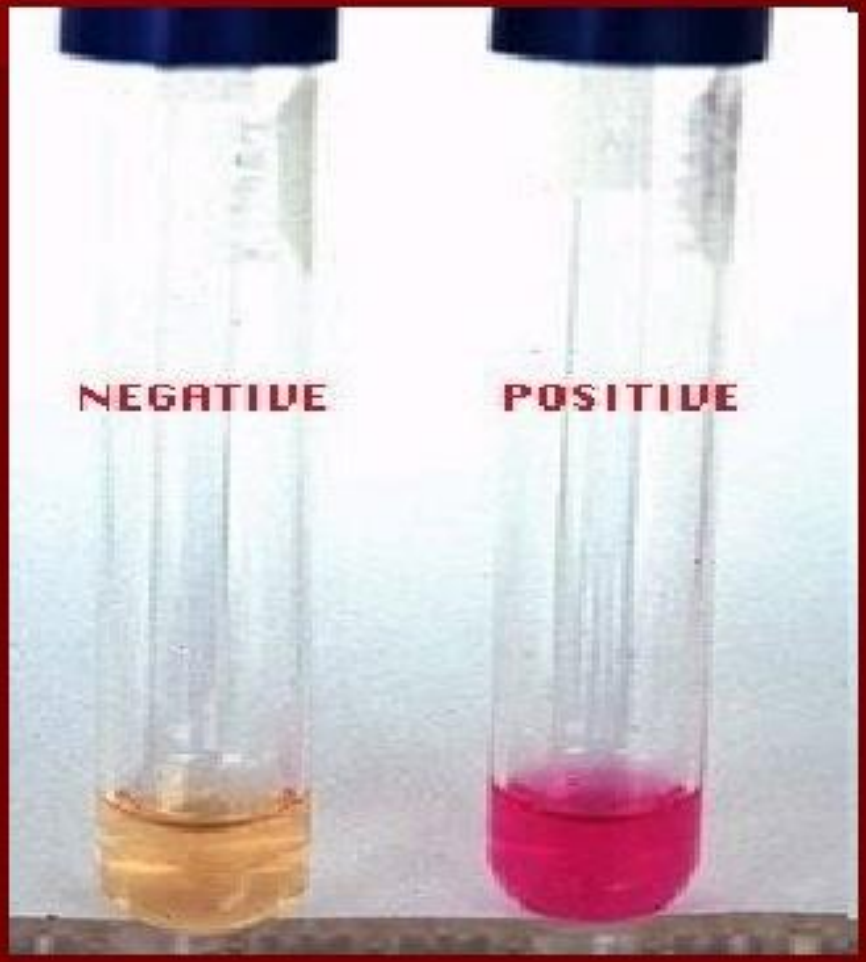


M. pneumoniae colonies demonostrated in Dienes method

- The colonies can be demonostrated by Dienes method.
- In which a block of agar containing the colony is cut and placed on a slide, covered with a cover slip on which has been dried in alcoholic solution of methylene blue and azure.



Biochemical Characters of Mycoplasma



- The metabolism of Mycoplasma are fermentative
- Most species utilize glucose or arginine
- Urea is hydrolyzed by ***Ureaplasma only***

Diagnosis of Urogenital Infections

- Material from urethra, cervical, or vaginal or centrifuged deposit of urine is added to separate vials with liquid mycoplasmal medium containing phenol red and 0.1% glucose, arginine or urea
- The Ureaplasmal urease also breaks down urea to ammonia

Newer methods in Diagnosis

- Phylogeny based rapid identification of urogenital Mycoplasmas and ureaplasmas based on amplification of part of 165rRNA gene by PCR is available

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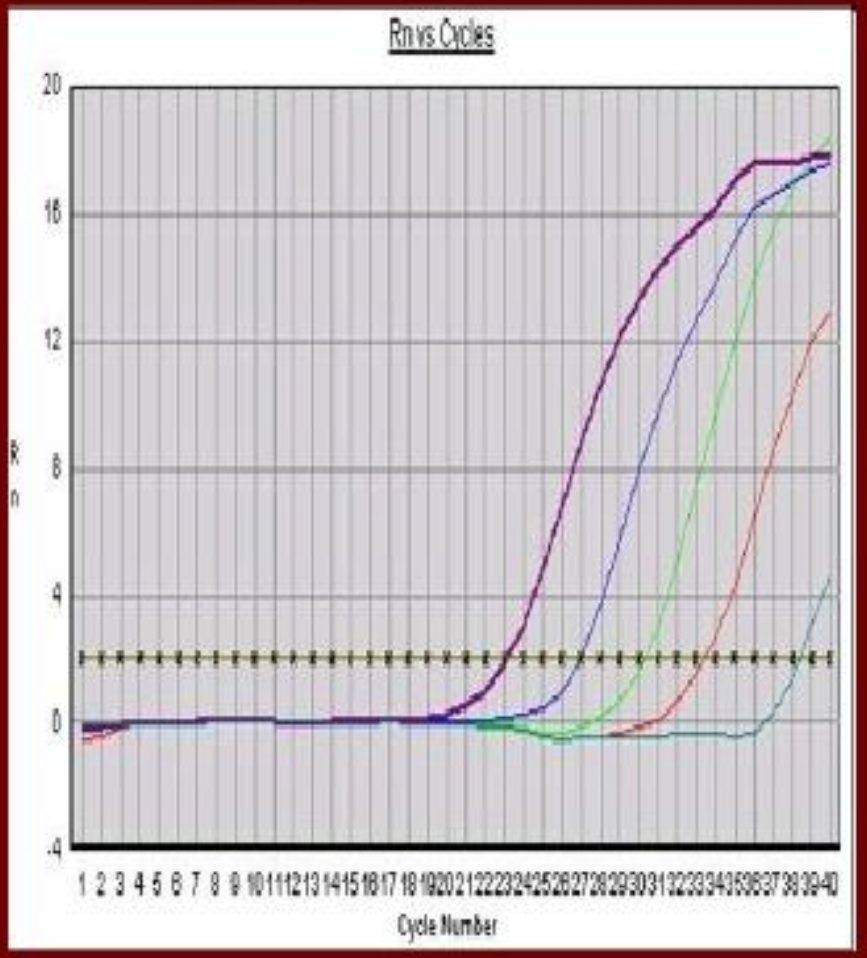
Advantages of PCR methods

- PCR methods are proving to be rapid, sensitive, and specific



Ureaplasma species

Important species in Ureaplasma

- The *Ureaplasma* genus now is subdivided into 2 species: *U urealyticum* and *U parvum.* For clinical purposes, separating infections caused by the different 2 species is not possible or necessary. In both the clinical setting and in the diagnostic laboratory, they are considered *Ureaplasma* species.

Ureaplasma differs from Mycoplasma

- The Ureaplasma are the only non fermentative mollicutes i.e., they do not ferment the growth substrates such as carbohydrates and amino acids like other mollicutes but they depend on the hydrolysis of urea for their energy

Urease test differentiates Mycoplasma from Ureaplasma species