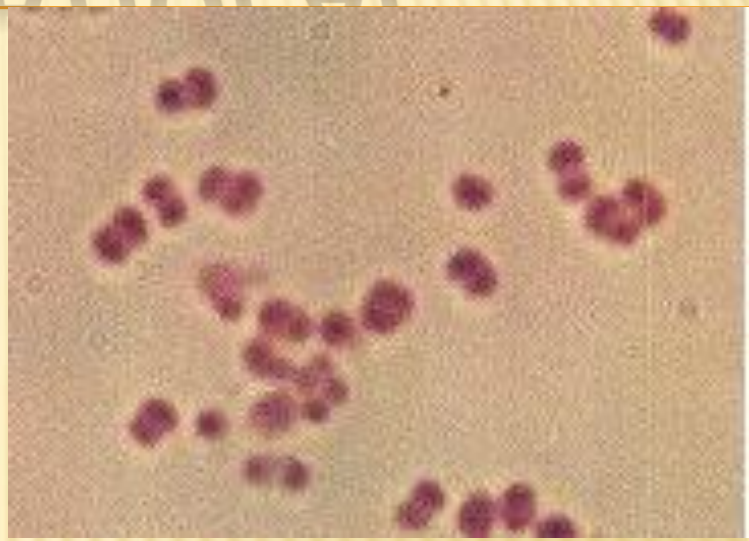
NEISSERIA GONORRHOEAE

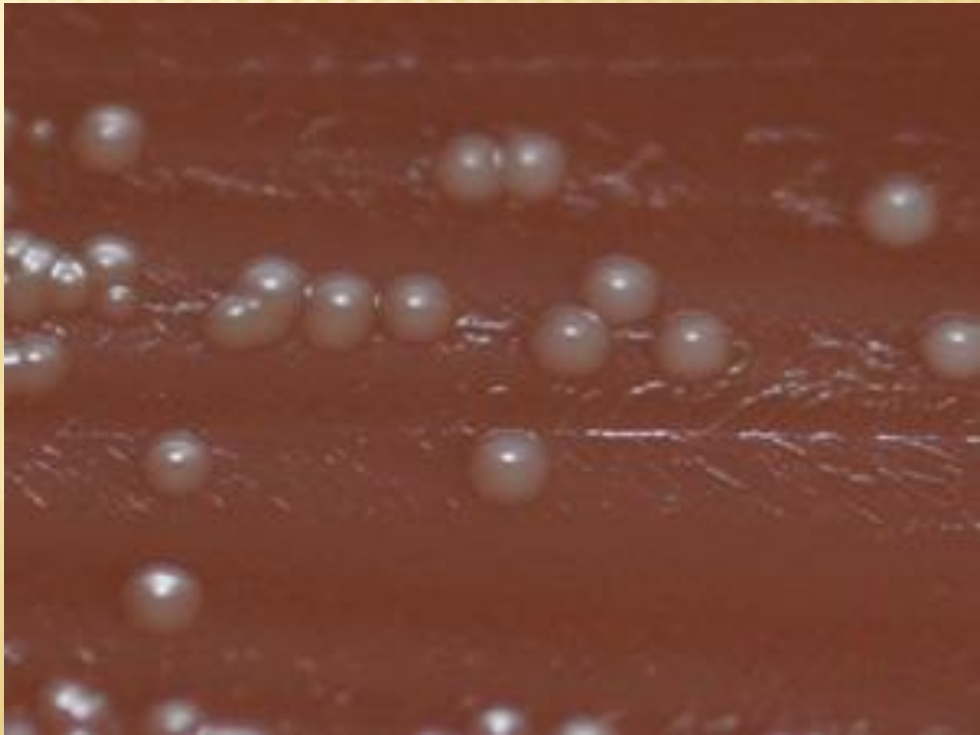


Family Neisseriaceae
Genera Neisseria
Species N. gonorrhoeae

Morphology and staining: a Gram-negative coccus, 0.6 to 1.0 μm in diameter, usually seen in pairs with adjacent flattened sides. The organism is frequently found intracellularly in polymorphonuclear leukocytes neutrophils) of the gonorrhea pustular exudate. Fimbriae, which play a major role in adherence, extend several micrometers from the cell surface.

CULTURAL CHARACTERISTIC

N. gonorrhoeae is aerobic. Strains of *N. gonorrhoeae* are variable in their cultural requirements so that media containing hemoglobin, NAD, yeast extract and other supplements are needed for isolation and growth of the organism**. Cultures are grown at 35-36 degrees in an moist aerobic atmosphere of 3-10% added CO_2 for 48 h.**



MTM Chocolate agar *is selective* **for *Neisseria***. The medium contains **enrichment factors** to promote the growth of *Neisseria*. In addition, it contains **antibiotics to inhibit normal body flora**: vancomycin to inhibit gram-positive bacteria; colistin to inhibit gram-negative bacteria; trimethoprim to suppress *Proteus*; and nystatin to inhibit yeast. The "chocolate" color is due to the hemoglobin enrichment added to the medium. Plates are then incubated under increased carbon dioxide tension as mentioned above. (Transgrow Medium is a convenient flask containing MTM Chocolate agar and CO_2.)

MTM CHOCOLATE AGAR IS SELECTIVE FOR PATHOGENIC *NEISSERIA*. *N. GONORRHOEAE* FORMS SMALL, CONVEX, GRAYISH-WHITE TO COLORLESS, MUCOID COLONIES IN 48 HOURS AT 35-37°C

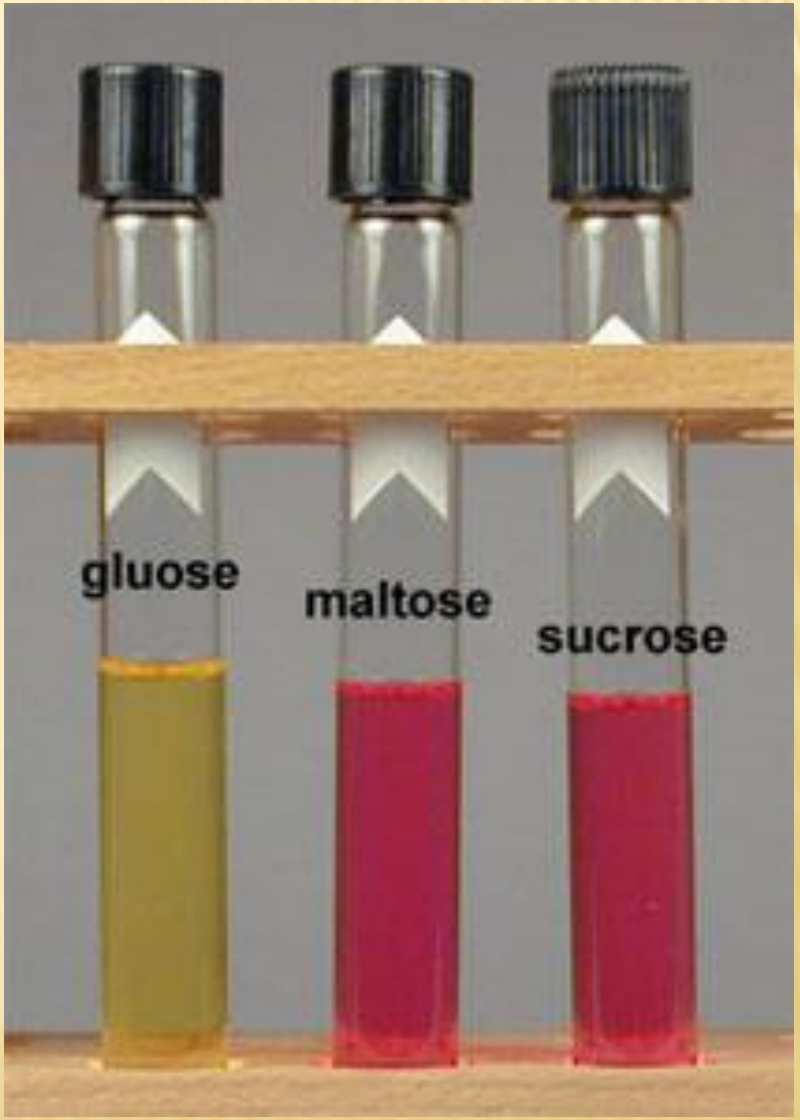
BIOCHEMICAL ACTIVITY

- *N. gonorrhoeae* are **oxidase positive**

Oxidase test suspect colonies of *N. gonorrhoeae* with tetramethyl-p-phenylenediamine (the “oxidase reagent”). Oxidase-positive colonies rapidly turn dark purple.

- **Carbohydrate utilization**

Neisseria gonorrhoeae produces acid from oxidation of glucose but not from maltose, sucrose, or lactose. *Neisseria* species produce acid end products from an oxidative pathway rather than from fermentation. The acid turns the pH indicator phenol red from red to yellow. Lactose, not shown here, is not utilized by *N. gonorrhoeae*.



- *N. gonorrhoeae* are **catalase positive**

RESISTANCE

N. gonorrhoeae is a relatively fragile organism, susceptible to temperature changes, drying, UV light, and other environmental conditions.

Typing

N gonorrhoeae strains have been typed on the basis of their growth requirements (auxotyping) or by antigenic differences in the porin protein (serotyping).

VIRULENCE FACTORS

- In the outer membrane of the gram-negative cell wall, the lipooligosaccharide (LOS) functions as an **endotoxin** .
- Various types of **pili** enable the bacterium to initially adhere to epithelial cells of the mucous membranes.
- A variety of cell wall **adhesins** enable the bacterium to make a more intimate contact with the mucous membranes.
- Transferrin-binding **proteins**, lactoferrin-binding proteins, and hemoglobin-binding proteins allow the gonococcus to obtain iron bound to human iron-binding proteins.
- IgA1 **protease** degrades the human antibody IgA1.

Habitat

Humans are the only natural host.

Source

Transmitted sexually by contact with an infected individual; may be transmitted from mother to baby during birth.

Pathogenesis

Gonococci adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is facilitated through pili and adhesins. Gonococcal lipopolysaccharide stimulates the production of tumor necrosis factor, which causes cell damage. Gonococci may disseminate via the bloodstream. Strains that cause disseminated infections are usually resistant to serum and complement.

CLINICAL DISEASE

- The incubation period averaging 2-7 days.

- In **males**, the gonococcus typically invades the anterior **urethra**, usually producing a **purulent discharge, pain upon urination, and a frequency of urination.** Approximately **5-10% of infected males are asymptomatic** but will still be infectious. The infection may spread up the reproductive tract, infecting the **prostate, vas deferens, epididymis, and testes**, causing painful inflammation and scar tissue formation that can result in **sterility.**

- In **females, 30-50 percent** of those initially infected are **asymptomatic** or show mild symptoms. They are, however, still infectious. Initially, the organism invades the **cervix**, the **urethra**, and frequently the **rectum**. In about 15 percent of the cases, the organism spreads up the reproductive tract and infects the **fallopian tubes** causing **pelvic inflammatory disease (PID).** The resulting inflammation and scar tissue formation may result in **sterility or abnormal (ectopic or tubal) pregnancies**.

- The gonococcus may also cause **extragenital infections** such as **pharyngitis** (from oral-genital sex), **ophthalmia** (from inoculation of the eyes with contaminated fingers), and **proctitis** (from anal sex). In 1 - 3% of infected women and a lower percentage of infected males, the organism invades the blood and disseminates, causing a **rash, septic arthritis, endocarditis, and/or meningitis**. Dissemination occurs more frequently in females.

- **Congenital gonorrhea** is known as **ophthalmia neonatorum** and occurs as a result of the eyes of newborns becoming infected as the baby passes through the birth canal.

DIAGNOSIS OF GONORRHEA

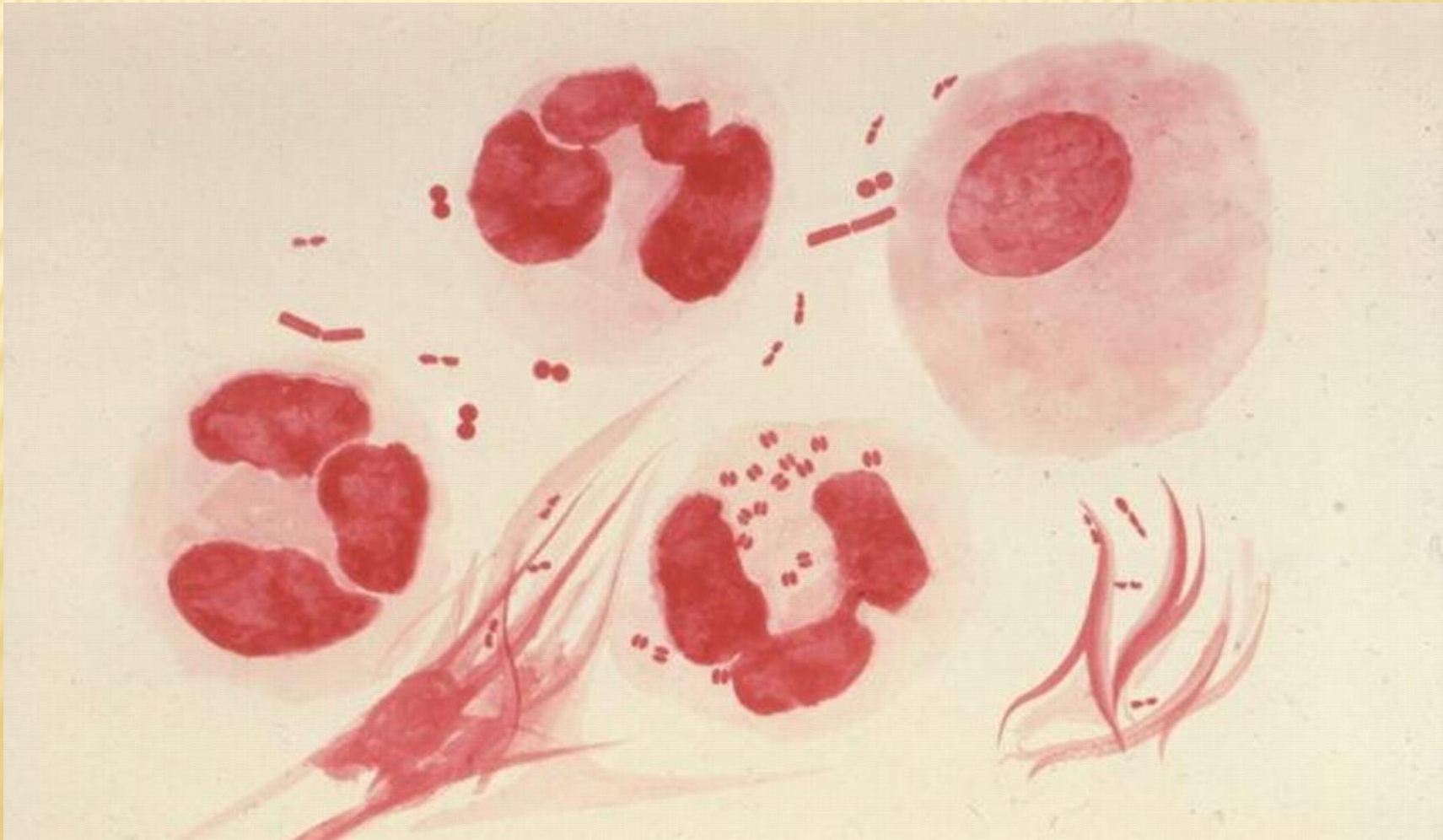
A definitive diagnosis of gonorrhea is made on the basis of:

1. Isolation of *N. gonorrhoeae* from the site of exposure by culture, usually on a selective medium, demonstration of typical colony morphology, positive oxidase reaction, and gram-negative coccal morphology in a gram stain
2. Conformation of isolates by biochemical, enzymatic, serologic, or nucleic acid testing, e.g., carbohydrate utilization, rapid enzyme substrate tests, serologic methods such as fluorescent antibody tests, or a DNA probe culture confirmation technique.

BACTERIOSCOPIC METHOD

The GC smear (gonococcus smear) is a gram stain of urethral exudates in men and endocervical secretions in women. One looks for **gram-negative diplococci with flattened adjacent walls that are seen both inside and outside of polymorphonuclear leukocytes**.
This test is quite sensitive in symptomatic males but only 40-60% sensitive in symptomatic females. In asymptomatic males and females the gram stain has a lower predictive value.

This illustration depicts a Gram-stain of a urethral exudate showing typical intracellular gram-negative diplococci, and pleomorphic extracellular gram-negative *N. gonorrhoea* organisms.



BACTERIOLOGICAL METHOD

Isolation and Identification of *Neisseria gonorrhoeae*

To diagnose genital gonorrhea in males, the sample to be **cultured** is taken from the **urethra.** In females, cultures are taken from the **cervix and the rectum**. In non-genital gonorrhea, the infected site is cultured.

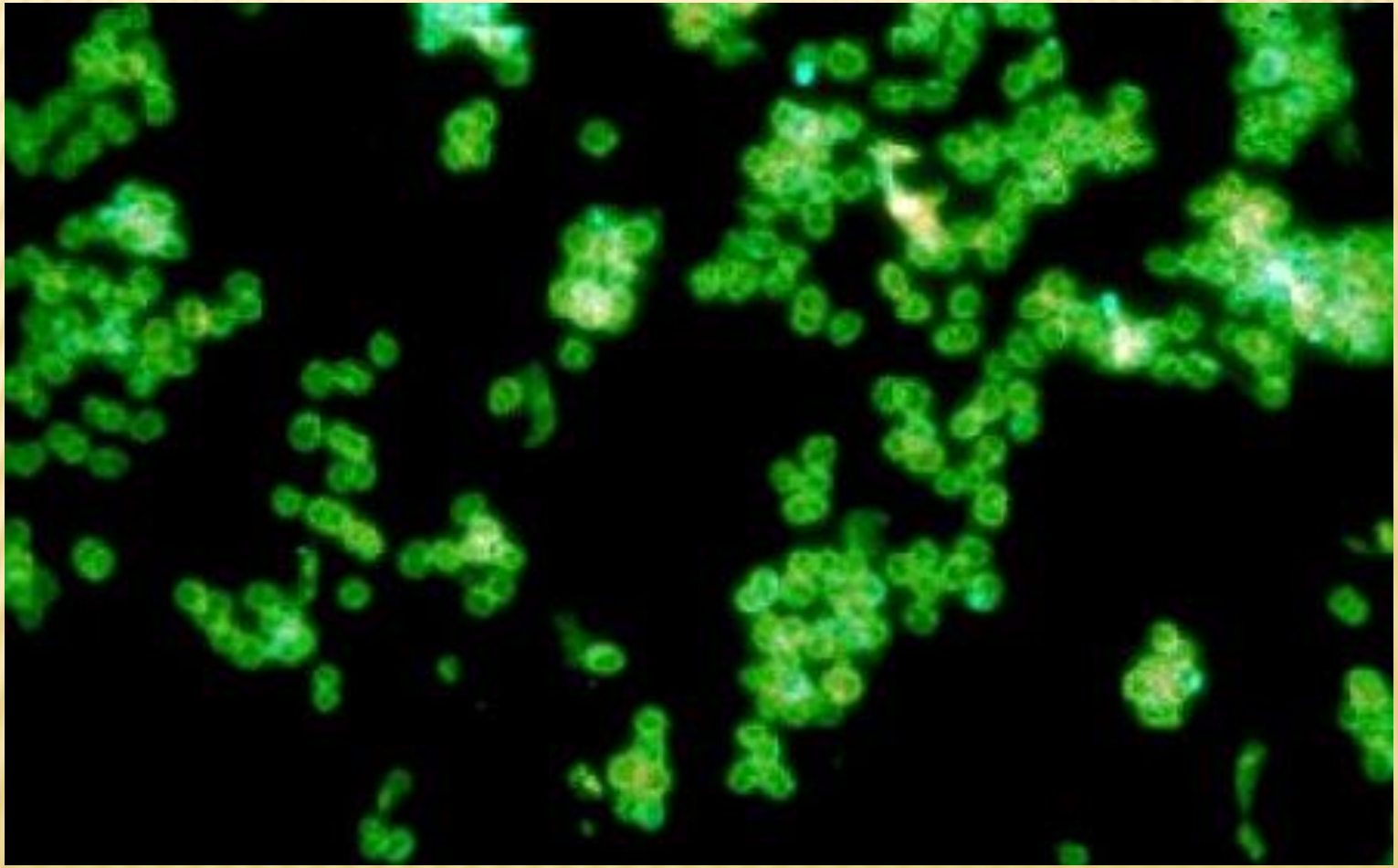
Once isolated, *N. gonorrhoeae* can be **identified** by the oxidase test, gram-staining, carbohydrate utilization reactions, rapid enzyme substrate tests, serologic methods such as fluorescent antibody tests, or a DNA probe culture confirmation technique.

SEROLOGIC AND NUCLEIC ACID TESTS

Serologic tests are also available for rapidly identifying *N. gonorrhoeae*. These include an ELISA test to detect gonococci in urethral pus or on a cervical swab, as well as a direct serologic test using fluorescent monoclonal antibodies to detect *N. gonorrhoeae*.

Nucleic acid probes and nucleic acid amplification tests are also available for identifying *N. gonorrhoeae*.

A POSITIVE DIRECT FLUORESCENT ANTIBODY TEST FOR *N. GONORRHOEAE*
NOTE GREEN-FLUORESCENT DIPLOCOCCI.

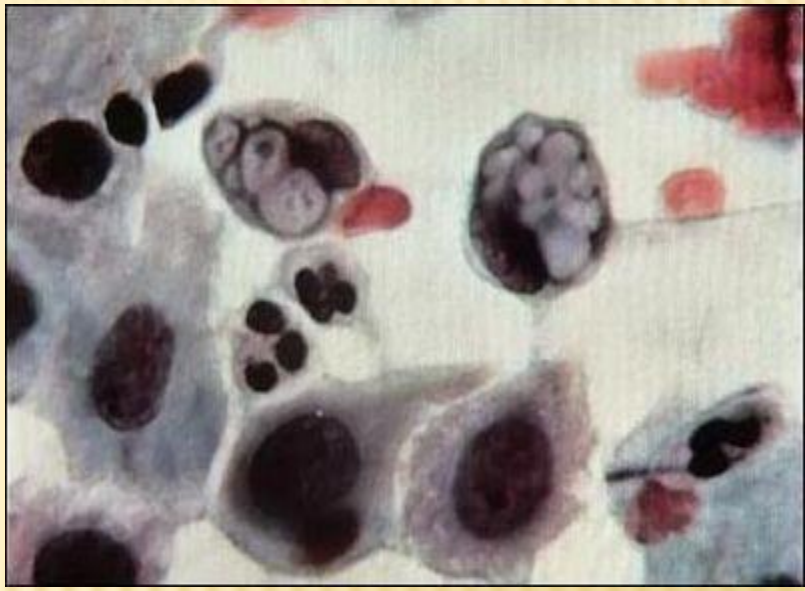


TREATMENT AND PREVENTION

- The recommended treatment for uncomplicated infections is a third-generation cephalosporin or a fluoroquinolone plus an antibiotic (e.g., doxycycline or erythromycin) effective against possible coinfection with *Chlamydia trachomatis.* Sex partners should be referred and treated. The recommended antimicrobial agents are ceftriaxone, cefixime, ciprofloxacin, or oflaxacin, doxycycline or azrithromycin.

- There is no effective vaccine to prevent gonorrhea.

CHLAMYDIA TRACHOMATIS



Family Chlamydiaceae
Genera *Chlamydia*
Species *C. trachomatis*

Morphology

Chlamydiae are obligate intracellular parasites that differ from other prokaryotes in their growth cycle, in which there are two morphologically and functionally distinct cell types: the infectious, elementary body (EB) and the reproductive, reticulate body (RB). Gram-negative cocci

CHLAMYDIA

- Obligate intracellular coccoid parasites
- contain DNA and RNA, and ribosomes
- lack ATP, biosynthetic pathways
- cell wall absent peptidoglycan -
  - use disulfide bonds
- non motile
- Multiply in the cytoplasm of the host cell.

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.

ECOLOGY

- Chlamydia form two main ecological groups.
- Infect only humans - Subgroup A
  - trachoma, inclusion conjunctivitis, and lymphogranuloma venereum
- Zoonotic Infections - Subgroup B
  - Respiratory tract infections

C trachomatis

Trachoma

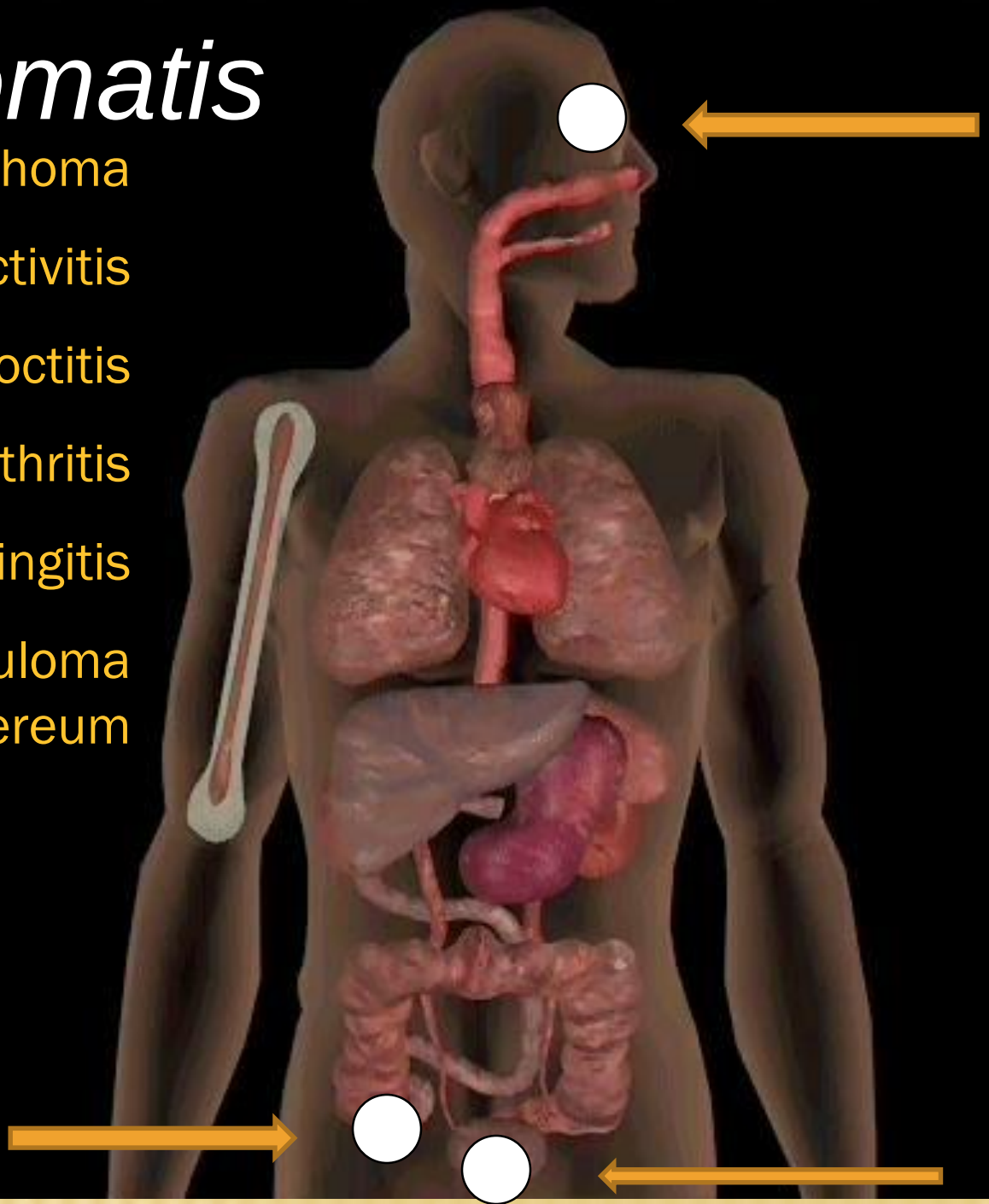
conjunctivitis

proctitis

urethritis

salpingitis

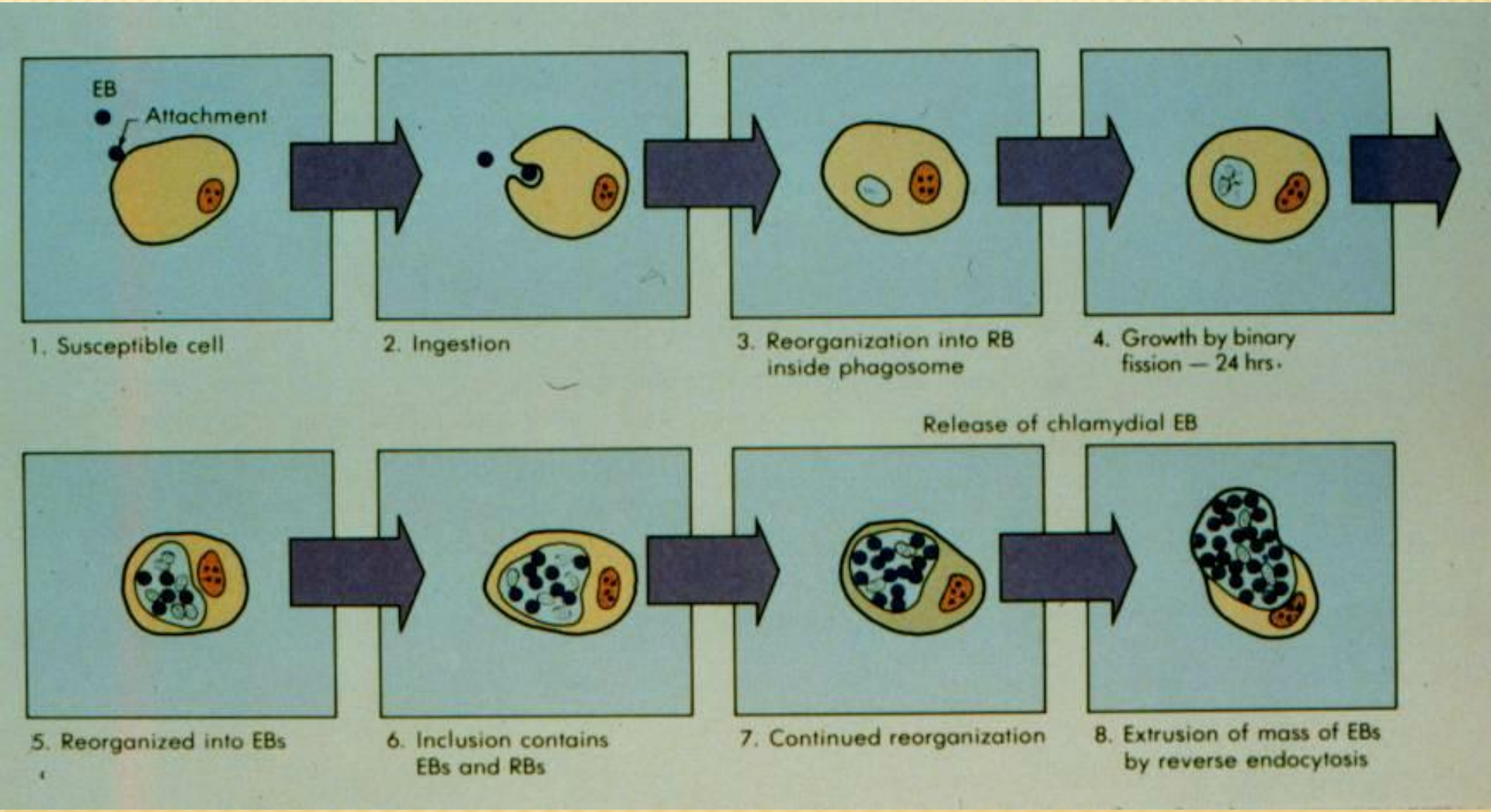
Lymphogranuloma venereum



CHLAMYDIAL MORPHOLOGIES

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

DEVELOPMENTAL CYCLE OF CHLAMYDIA



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 MAN

- **Most Common**
- Silent or no symptoms (in 50% of men with chlamydia)
- **Less Common**
- Abnormal penile discharge (thick, yellow-white, milky or watery)
- Pain during urination
- Rectal pain, discharge or bleeding
- Inflamed eye
- **Least Common**
- Itching and burning around the opening of the penis
- Testicular pain and swelling
- Sore throat

CHLAMYDIA SYMPTOMS IN WOMEN

Most Common

Silent or no symptoms (in 75% of women with chlamydia)

Less Common

Abnormal vaginal discharge (may have an odor)

Pain during urination

Rectal pain, discharge or bleeding

Inflamed eye

Least Common

Bleeding between menstrual cycles

Lower bellyache

Lower back pain

Nausea

Fever

Pain during sex

Sore throat

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)

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.

TREATMENT CHLAMYDIA

- Adults Conjunctival, urethral, cervical, rectal:
  - Azithromycin 1 gm x 1 dose
  - Doxycycline 100 mg bis in die for 7 days
  - Ofloxacin 300 mg bis in die for 7 days
  - Erythromycin 500 mg Quater In Die for 7 days
  - Amoxacillin 500 mg three times daily 7-10 days
- Children < 45 kg, urogenital & rectal:
  - erythromycin 50 mg/kg/day in 4 doses for 10-14 days
- Neonates (ophthalmia, infants born to infected mothers):
  - erthromycin 50 mg/kg/day in 4 doses for 10-14 days

CHLAMYDIA ANTIGENS

Antigens: group specific & species specific

- Major outer membrane protein (cysteine-rich)
- Eucaryotic cell binding protein

Host response: damage to specific tissues

LABORATORY DIAGNOSIS

- Isolate the organism from infected tissue.
  - Inoculate the yolk sac of seven-day chick embryos
  - Inoculate McCoy human cells.
- Characteristic cytoplasmic inclusion bodies in infected cells.

IMMUNOFLUORECENT TESTS

- Microimmunofluorescent tests
  - patients with eye infections
  - Check tears for the presence of anti-chlamydia antibody.
- Direct immunofluorescence
  - of conjunctive cells with fluorescein - conjugated monoclonal antibody is sensitive and specific.
  - In neonatal conjunctivitis and early trachoma

SEROLOGICAL DIAGNOSIS:

- **Enzyme immunoassay** (EIA), which uses polyclonal antibodies to detect chlamydial lipopolysaccharide (LPS). This is the first method for testing a large number of people, such as pregnant women, sexual contacts and young adolescents.

- **Direct cytological examination of smears using fluorescein** (FITC)-conjugated monoclonal antibodies directed against OMP_1. This method can be used for large groups of patients with 88% sensitivity and 98-99% specificity.

- **Nucleic acid amplification methods (NAATs)** are the most sensitive tests today and can be used for large groups of symptomatic and asymptomatic patients.

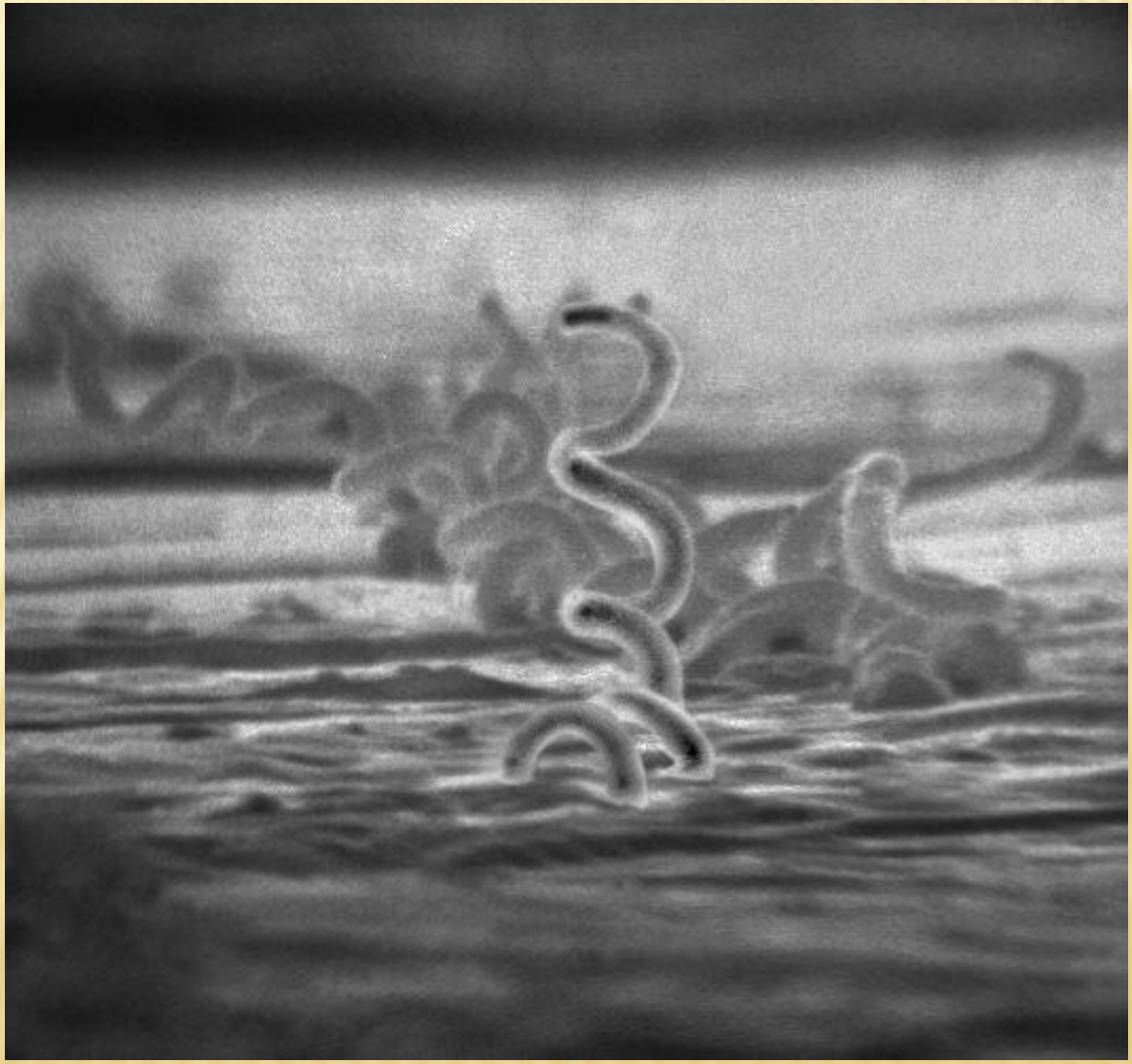
These methods have a sensitivity of between 90.3% and 97% and a specificity between 98% and 99.1%.

TREPONEMA PALLIDUM *SYPHILIS*

Family Spirochaetaceae
Genera Treponema
Species Treponema pallidum

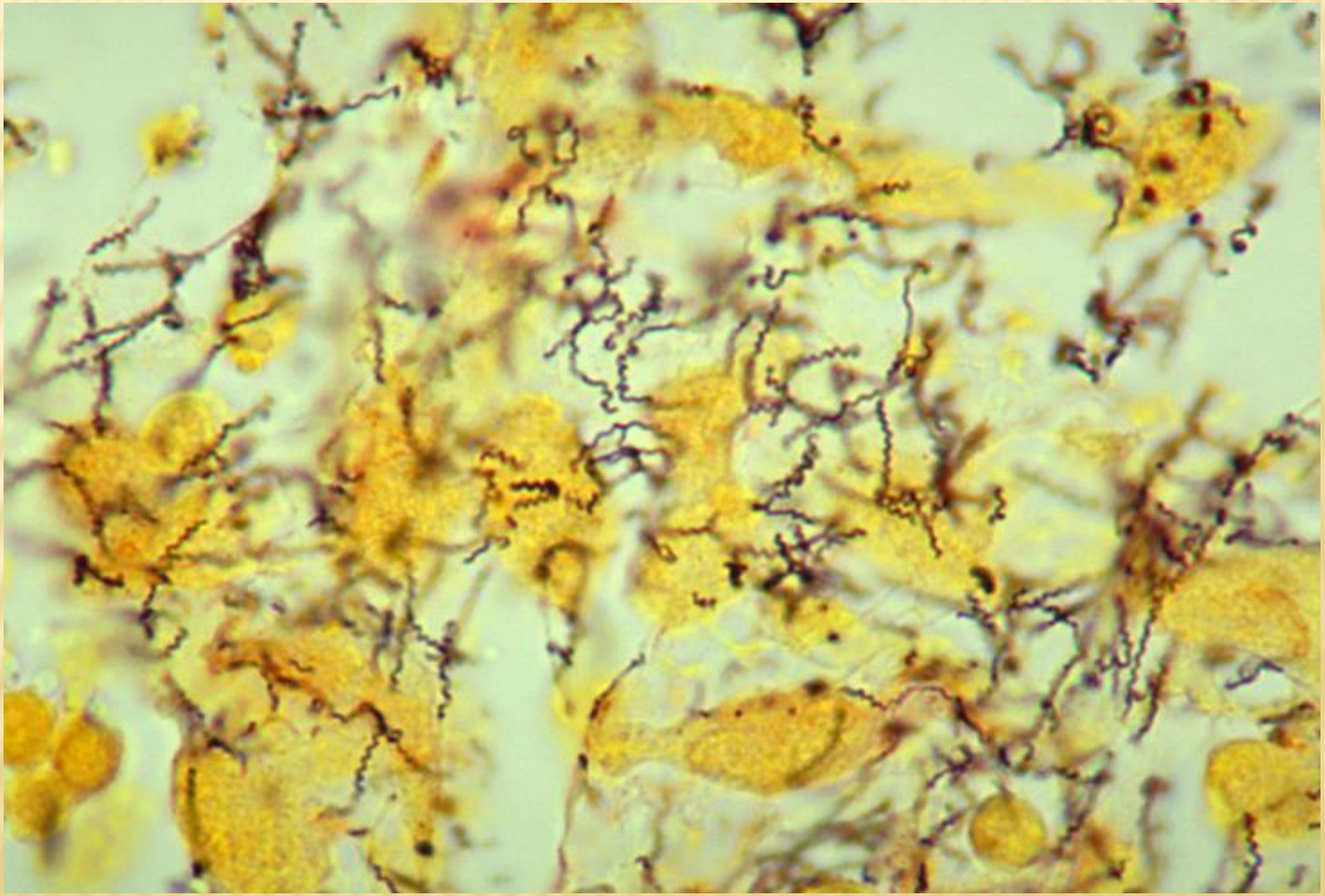
Morphology and staining: Treponemes are helically coiled, corkscrew-shaped organisms 6 to 15 µm long and 0.1 to 0.2 µm wide. The organisms stain poorly with aniline dyes. Treponemes in tissues can be visualized by silver impregnation methods. Live treponemes, which are too slender to be seen by conventional light microscopy, can be visualized by using dark-field microscopy. *Treponema pallidum* exhibits characteristic motility that consists of rapid rotation about its longitudinal axis and bending, flexing, and snapping about its full length.

ELECTRON MICROGRAPH OF TREPONEMA



TREPONEMA PALLIDUM SPIROCHETES SEEN WITH DARKFIELD MICROSCOPE

TREPONEMA PALLIDUM SPIROCHETES USING A MODIFIED STEINER SILVER STAIN.



PHYSIOLOGY

Treponema pallidum is a fastidious organism that exhibits narrow optimal ranges of pH (7.2 to 7.4) and temperature (30 to 37 °C). It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants. Traditionally this organism has been considered a *obligate anaerobes*.

Treponema is *chemoheterotroph*; it ferments sugars and amino acids.The fermentation products are CO2, small organic acids and alcohols.

Treponemes multiply by binary transverse fission. The in vivo generation time is relatively long (30 hours). Despite intense efforts over the past 75 years, *T pallidum* subsp *pallidum* has not been successfully cultured in vitro. Viable organisms can be maintained for 18 to 21 days in complex media, while limited replication has been obtained by co-cultivation with tissue culture cells.

VIRULENCE FACTORS

The outer **membrane proteins** are associated with adherence to the surface of host cells, and virulent spirochetes produce **hyaluronidase**, which may facilitate perivascular infiltration. Virulent spirochetes are also coated with host cell **fibronectin**, which can protect against phagocytosis.

SYPHILIS: EPIDEMIOLOGY, PATHOGENESIS, CLINICAL FEATURES.

Venereal syphilis is a contagious sexually transmitted disease caused by the spirochete *Treponema pallidum*.

Congenital syphilis is the disease acquired in utero from the mother.

T. pallidum enters the body through mucous membranes or minor breaks or abrasions of the skin. It migrates to the regional lymph nodes and rapidly spreads throughout the body.

The disease is not highly contagious, and there is only about a 1 in 10 chance of acquiring it from a single exposure to an infected sex partner.

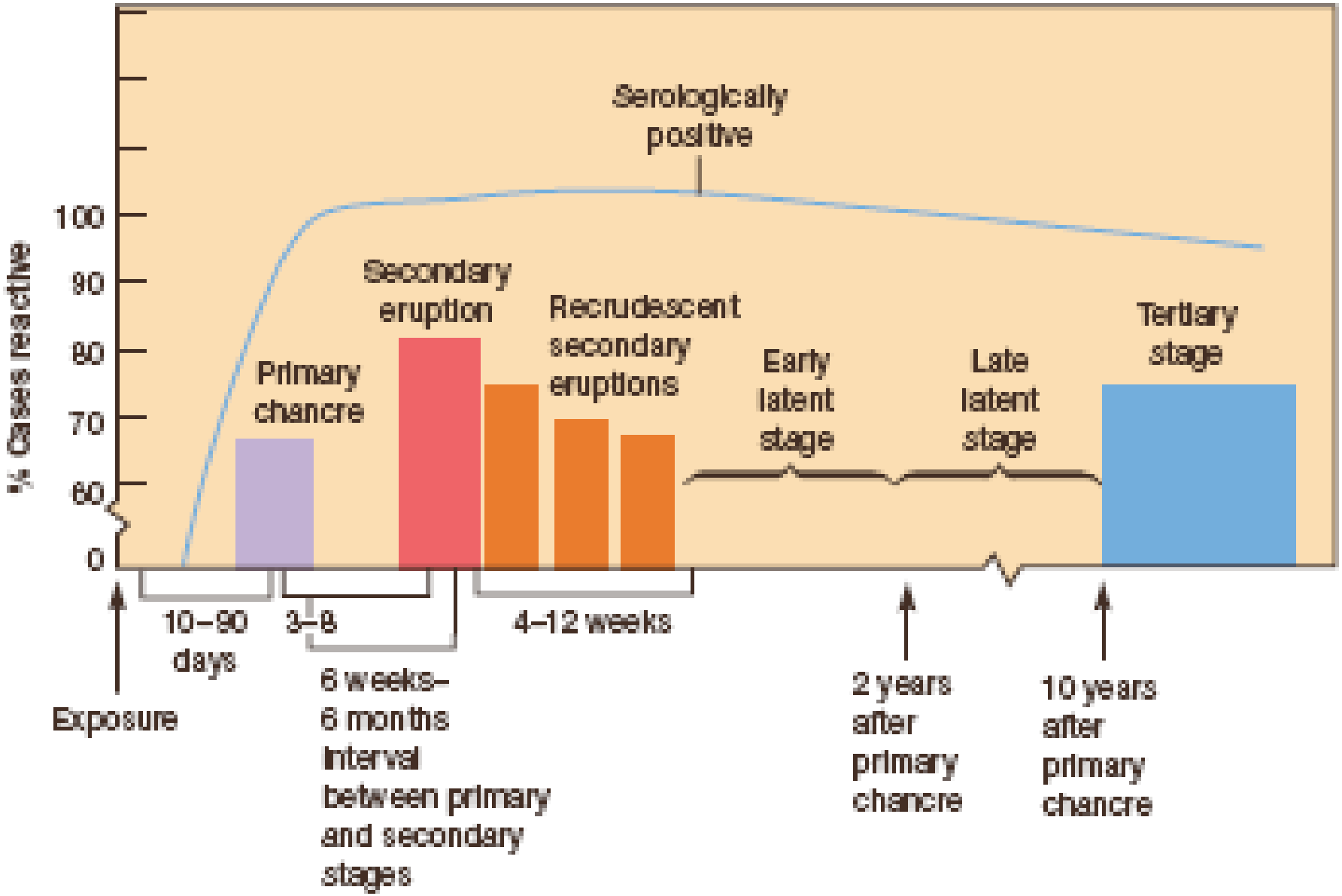
Three recognizable stages of syphilis occur in untreated adults.

In the primary stage, after an incubation period of about 10 days to 3 weeks or more, the initial symptom is a small, painless, reddened ulcer, or **chancre** [French *canker,* a destructive sore] with a hard ridge that appears at the infection site and contains spirochetes. Contact with the chancre during sexual intercourse may result in disease transmission. In about 1/3 of the cases, the disease does not progress further and the chancre disappears. Serological tests are positive in about 80% of the individuals during this stage.

In the remaining cases the spirochetes enter the bloodstream and are distributed throughout the body. Within 2 to 10 weeks after the primary lesion, the disease may enter **the secondary stage**, which is characterized by a skin rash. By this time 100% of the individuals are serologically positive. Other symptoms during this stage Include the loss of patches of hair, malaise, and fever. Both the chancre and the rash lesions are infectious. After several weeks the disease becomes latent. During the latent period the disease is not normally infectious, except for possible transmission from mother to fetus (congenital syphilis).

After many years a **tertiary stage** develops in about 40% of untreated individuals with secondary syphilis. During this stage degenerative lesions called **gummas** form in the skin, bone, and nervous system as the result of hypersensitivity reactions. This stage also is characterized by a great reduction in the number of spirochetes in the body. Involvement of the central nervous system may result in tissue loss that can lead to mental retardation, blindness, a “shuffle” walk (tabes), or insanity. Many of these symptoms have been associated with such well-known people as Al Capone, Francisco Goya, Henry VIII, Adolf Hitler, Scott Joplin, Friedrich Nietzsche, Franz Schubert, Oscar Wilde, and Kaiser Wilhelm.

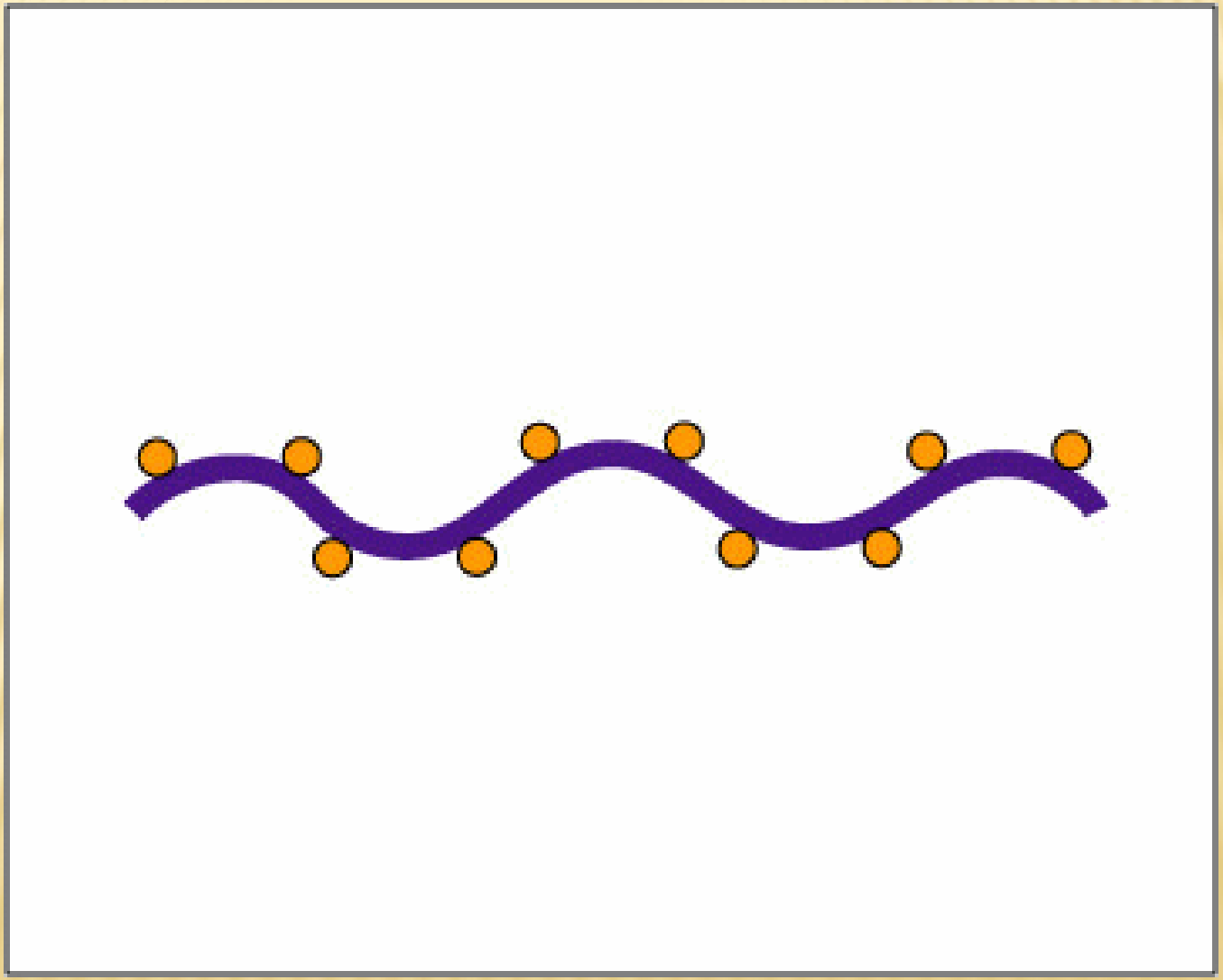
THE COURSE OF UNTREATED SYPHILIS.



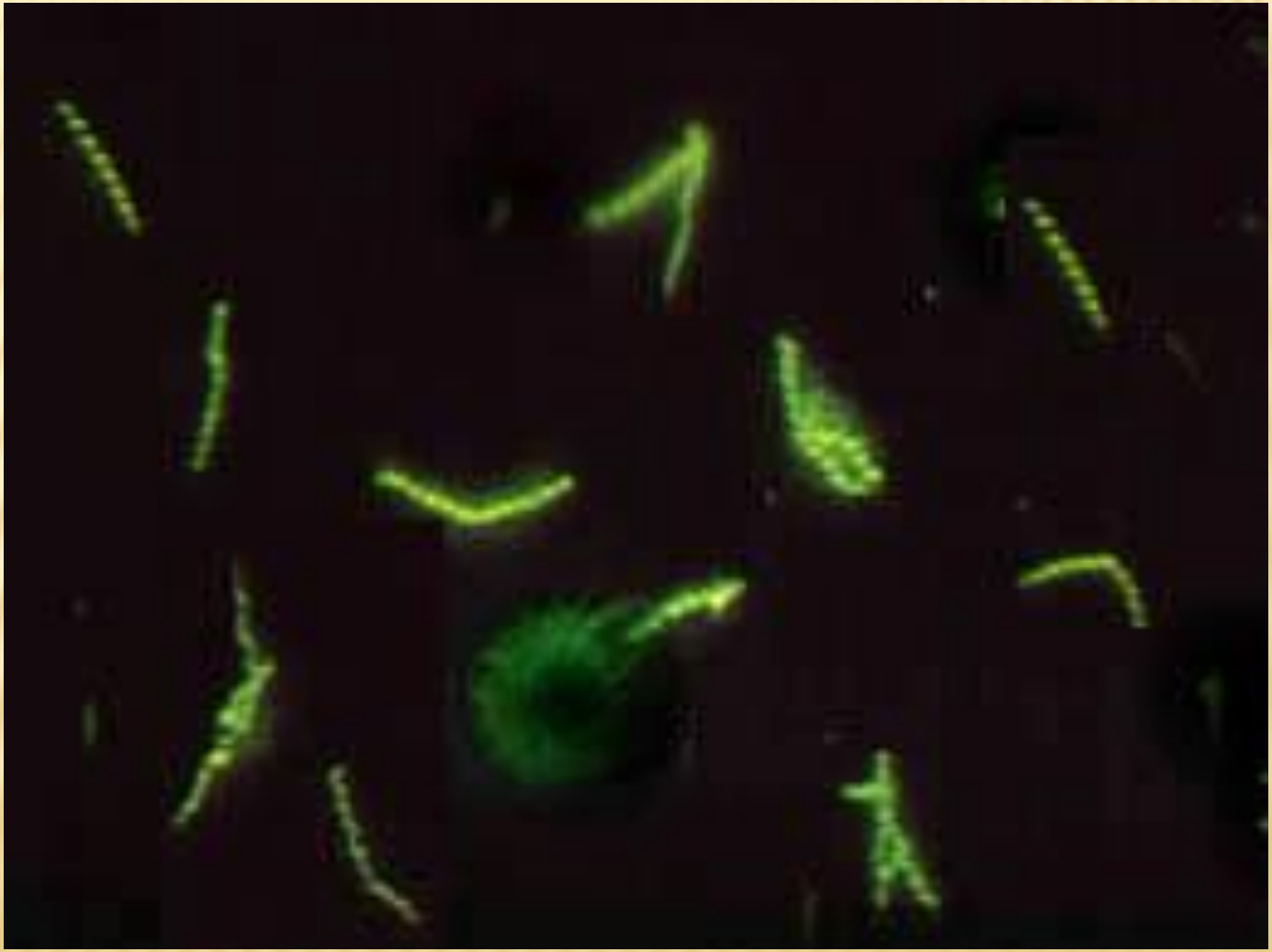
Diagnosis of syphilis is through a clinical history, a thorough physical examination, and dark-field and immunofluorescence examination of fluids from the lesions (except oral lesions) for typical motile or fluorescent spirochetes. Because humans respond to *T.pallidum* with the formation of antitreponemal antibody and a complement-fixing reagin, serological tests are very informative.

Examples include tests for nontreponemal antigens (VDRL, **V**enereal**D**isease**R**esearch**L**aboratories test; RPR,**R**apid **P**lasma**R**eagin test; **complement fixation or the Wassermann test) and treponemal antibodies** (FTA-ABS, **f**luorescent **t**reponemal **a**ntibody-**abs**orption test; TPI, *T. **p**allidum* **i**mmobilization; *T. pallidum* complement fixation; TPHA, *T. **p**allidum* **h**em**a**gglutination).

FTA TEST FOR ANTIBODIES AGAINST TREPONEMA PALLIDUM



TREPONEMA ORGANISMS STAINED BY FLUORESCENT-TAGGED ANTIBODIES.



FTA TEST FOR ANTIBODIES AGAINST *TREPONEMA PALLIDUM*

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. Fluorescent anti-human gamma globulin (anti-HGG) is added to the well. (Anti-HGG is an antibody made by another animal against human IgG antibodies. A fluorescent dye is then attached to the antibody.) The anti-HGG will with any human IgG antibodies bound to the *Treponema pallidum* on the slide. All unbound anti-HGG is then washed from the slide. When viewed with a flourescent microscope, the spirochetes will fluoresce.

Treatment in the early stages of the disease is easily accomplished with long-acting **benzathine penicillin G** or aqueous procaine penicillin. Later stages of syphilis are more difficult to treat with drugs and require much larger doses over a longer period. For example, in neurosyphilis cases, treponemes occasionally survive such drug treatment.

Immunity to syphilis is not complete, and subsequent infections can occur once the first infection has spontaneously disappeared or has been eliminated with antibiotics.

PREVENTION AND CONTROL OF SYPHILIS

Prevention and control of syphilis depends on

(1) public education

(2) prompt and adequate treatment of all new cases,

(3) follow-up on sources of infection and contact so they can be treated,

(4) sexual hygiene, and

(5) prophylaxis (condoms) to prevent exposure.