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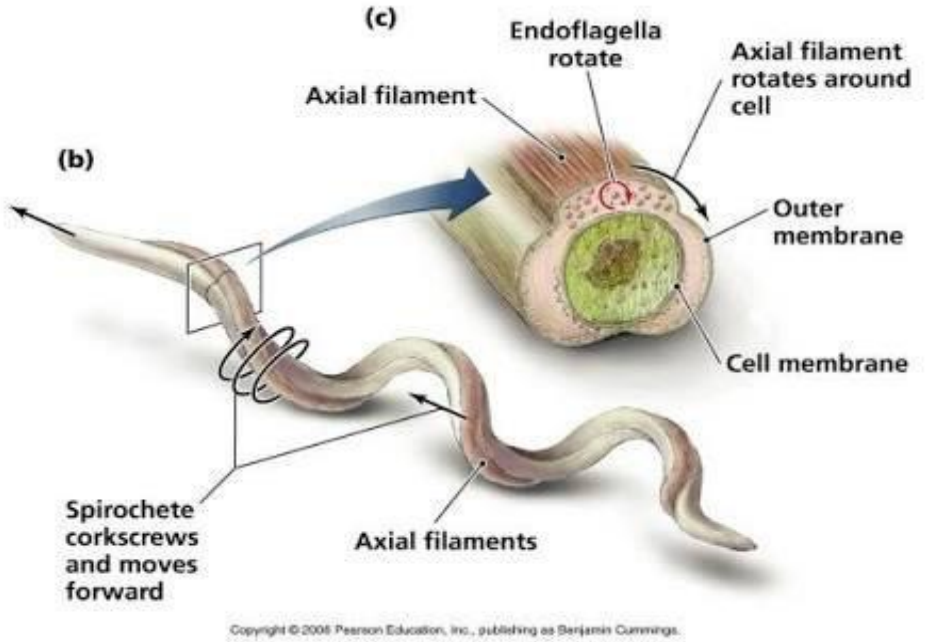
Lecture. 13
Second Stage

College of Health & Medical Techniques
Department of Medical Laboratories Techniques

SPIROCHETES

Spirochetes

The spirochetes are **long**, **slender**, **helically coiled**, **spiral**, or **corkscrew** shaped bacilli. *T. pallidum* has an **outer sheath**. Inside the sheath is the outer membrane, which contains peptidoglycan and maintains the structural integrity of the organisms. **Endoflagella** (**axial filaments**) are the **flagella-like organelles** in the **periplasmic** space coated by the outer membrane. The endoflagella begin at each end of the organism and wind around it, extending to and **overlapping** at the **midpoint**. Inside the endoflagella is the inner membrane (cytoplasmic membrane) that provides osmotic stability and covers the protoplasmic cylinder. A series of cytoplasmic tubules (body fibrils) are inside the cell near the inner membrane. Treponemes reproduce by **transverse fission**.



TREPONEMA PALLIDUM AND SYPHILIS

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Morphology and Identification

A. Typical Organisms: *T. pallidum* are **slender** spirals measuring about 0.2 μm in width and 5–15 μm in length. The spiral **coils** are **regularly spaced** at a distance of 1 μm from one another. The organisms are **actively motile**, rotating steadily بثبات تدور around their endoflagella even after attaching to cells by their tapered ends نهايات مستدقة. The **long axis** of the spiral is ordinarily straight but may sometimes bend ينحني so that the organism forms a complete circle for moments at a time, returning then to its normal straight position. The spirals are so **thin** that they are not readily seen unless **immunofluorescent** stain or **dark-field** illumination is used. They do not stain well with **aniline dyes**, but they can be seen in tissues when stained by a **silver impregnation** method.

B. Culture Pathogenic *T. pallidum* has never been cultured continuously on artificial media, in fertile eggs, or in tissue culture.

In proper suspending fluids and in the presence of reducing substances, *T. pallidum* may remain motile for 3–6 days at 25°C. In whole blood or plasma stored at 4°C, organisms remain viable for at least 24 hours, which is of potential importance in blood transfusions.

Antigenic Structure:

- The outer membrane
- The peptidoglycan–cytoplasmic membrane complex.
- Membrane proteins are present that contain covalently bound lipids at their amino terminals.
- The lipids appear to anchor the proteins to the cytoplasmic or outer membranes.
- The endoflagella are in the periplasmic space.
- *T. pallidum* has hyaluronidase.

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- The endoflagella are composed of three core proteins that are homologous to other bacterial flagellin proteins plus an unrelated sheath protein.
- Cardiolipin is an important component of the treponemal antigens.

Pathogenesis, Pathology, and Clinical Findings

Acquired Syphilis:

- Natural infection with *T. pallidum* is limited to the human host.
- Human infection is usually transmitted by sexual contact, and the infectious lesion is on the skin or mucous membranes of genitalia.
- *T. pallidum* can probably penetrate intact mucous membranes, or the organisms may enter through a break in the epidermis.
- Spirochetes multiply locally at the site of entry, and some spread to nearby lymph nodes and then reach the bloodstream. Within 2–10 weeks after infection, a papule develops at the site of infection and breaks down to form an ulcer with a clean, hard base (“hard chancre”). e. t. c.

B. Congenital Syphilis A pregnant woman with syphilis can transmit *T. pallidum* to the fetus through the placenta beginning in the 10th–15th weeks of gestation.

Diagnostic Laboratory Tests

A. Specimens: Specimens include tissue fluid expressed from early surface lesions for demonstration of spirochetes by either dark-field microscopy or immunofluorescence; such specimens can also be tested by nucleic acid amplification. Blood can be obtained for serologic tests; cerebrospinal fluid (CSF) is useful for Venereal Disease Research Laboratory.

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B. Dark-Field Examination A drop of tissue fluid or exudate is placed on a slide, and a coverslip is pressed over it to make a thin layer.

C. Immunofluorescence Tissue fluid or exudate is spread on a glass slide, air-dried, and sent to the laboratory.

D. Serologic Tests for Syphilis These tests use either nontreponemal or treponemal antigens.

Table-1: Diagnosis and tests of syphilis

A. Demonstration of treponemes in the exudate	1. Dark-ground microscopy 2. Direct fluorescent-antibody staining for *Treponema pallidum* (DFA- Tp) 3. Silver impregnation method (Levaditi's stain) 4. Enzyme immunoassay, Polymerase chain reaction (PCR).
B. Serological tests	**a. Nontreponemal tests** **Nonspecific *(reagin antibody)* tests using the cardiolipin antigen *(standard tests for syphilis or STS)*.** 1. Wassermann complement fixation test 2. Kahn flocculation test 3. Venereal Disease Research Laboratory (VDRL) test 4. Rapid Plasma Reagin (RPR) test 5. Toluidine red unheated serum test (TRUST) **b. Treponemal tests** **a. Group specific test using cultivable treponemal (Reiter strain) antigen** I. Reiter Protein CF (RPCF) test (1953) **b. Specific tests using pathogenic treponemes *(T. palidum)*** I. Test using live *T. pallidum* *Treponema pallidum* Immobilization (TPI) test **II. Tests using killed *T. pallidum*** a. Treponema pallidum agglutination (TPA) test b. Treponema pallidum immune adherence (TPIA) test c. Fluorescent Treponemal Antibody Absorption (FTA-ABS) test **III. Tests using *T. pallidum* extract** a. *Treponema pallidum* Hemagglutination Assay (TPHA) Microhemagglutination test for *Treponema pallidum* (MHA-TP) b. *Treponema pallidum* Enzyme Immunoassays (TP-EIA):