Spirochetes

Dr Mehvash

- A medical illustration attributed to Albrecht Dürer (1496) depicting a person with syphilis.
- Here, the disease is believed to have astrological causes.

- The name "syphilis" was coined by the Italian physician and poet Girolamo Fracastoro in his pastoral noted poem, written in Latin, titled *Syphilis sive morbus gallicus*

- Syphilus is presented as the first man to contract the disease, sent by the god Apollo as punishment for the defiance that Syphilus and his followers had shown him

Fritz Richard Schaudinn
Zoologist

Paul Erich Hoffmann
Dermatologist

- Order Spirochaetales contains 2 families: Spirochaetaceae and Leptospiraceae (Leptospira and Leptonema)

- The family Spirochaetaceae contains the genera:
  - **Borrelia**
  - **Treponema**
  - **Spirochaeta**
  - **Christispira**

Subspecies

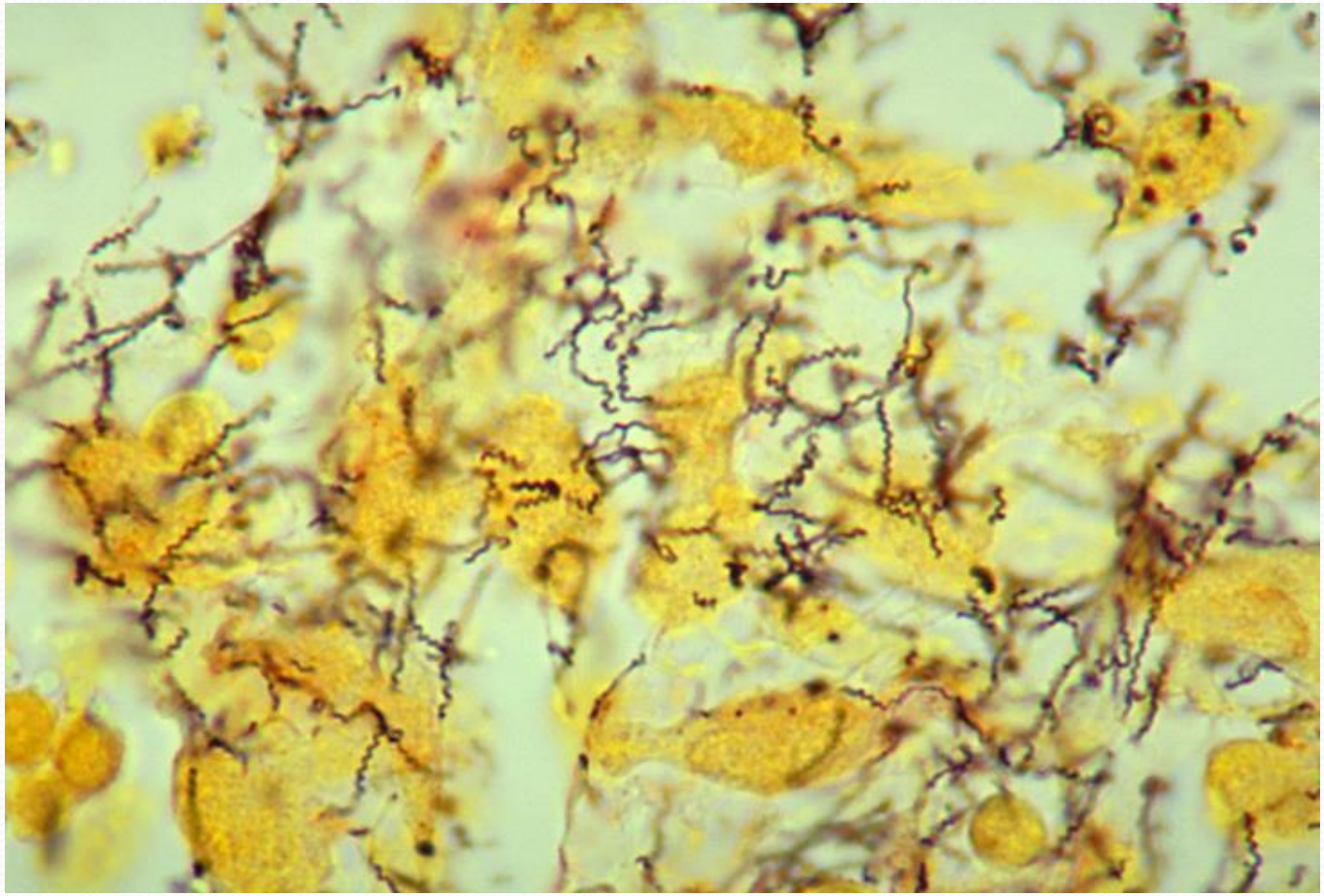
- There are at least four known subspecies of Treponema pallidum:
  - Treponema pallidum pallidum – Human Syphilis
  - T. pallidum endemicum - Bejel
  - T. pallidum pertenue – Yaws (elimination2006, free 2015)
  - T. pallidum carateum – Pinta
- Treponema cuniculi – Rabbit syphilis

Avirulent strains

- Avirulent strains of Treponema pallidum:
  - Nichols (pathogenic)
  - Reiter (non pathogenic- T phagedenis)
  - T. refringens
  - Noguchi
  - Kazan
  - Kroo

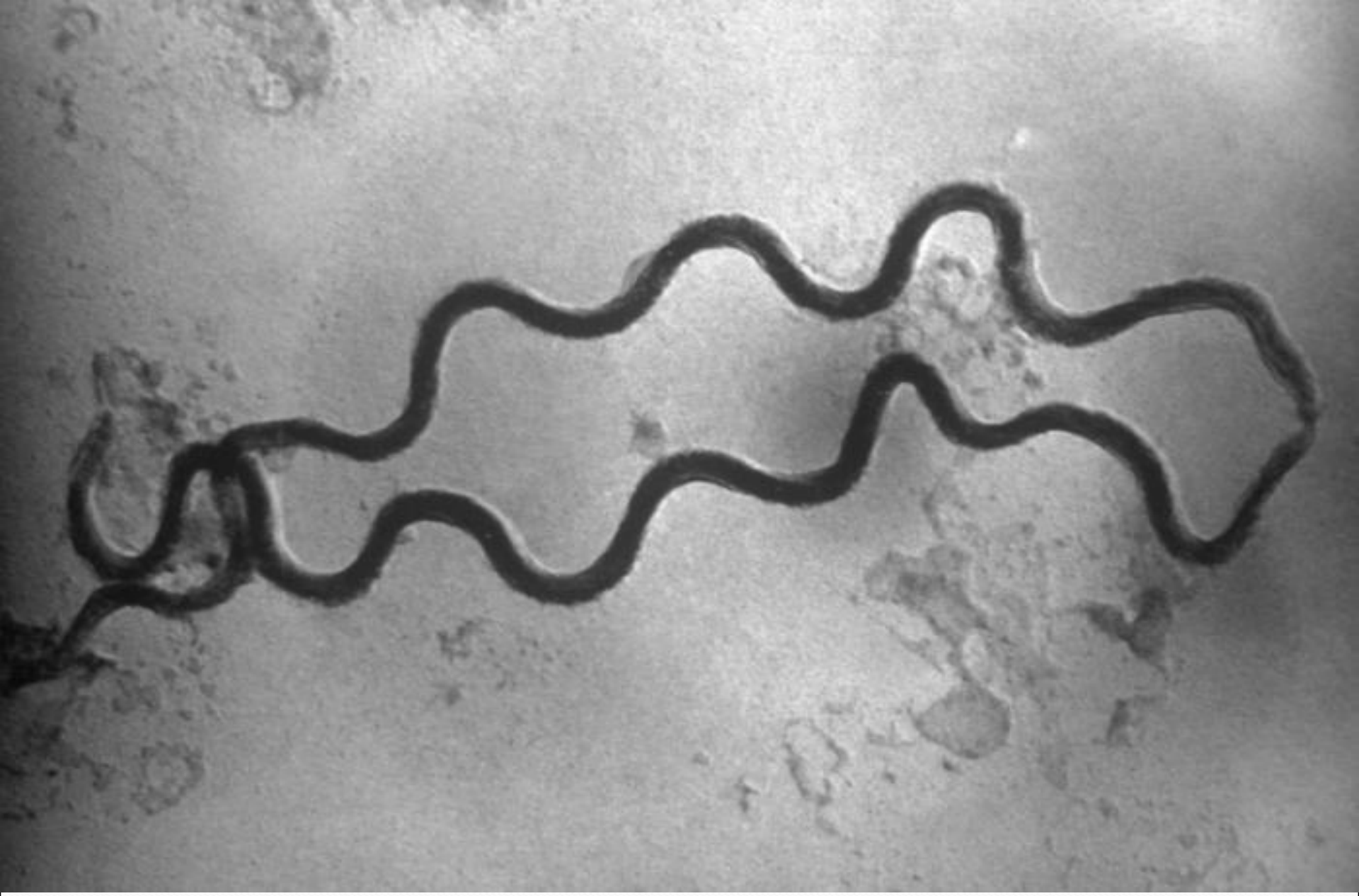
Microscopy

- Live treponemes are too slender to be seen by conventional light microscopy.
- Can be seen with :
  - Negative staining with Indian Ink.
  - Dieterle stain.
  - Steiner stain.
  - Warthin Starry stain. (Fontana method for films)
- Can also be visualized by using dark-field microscopy.

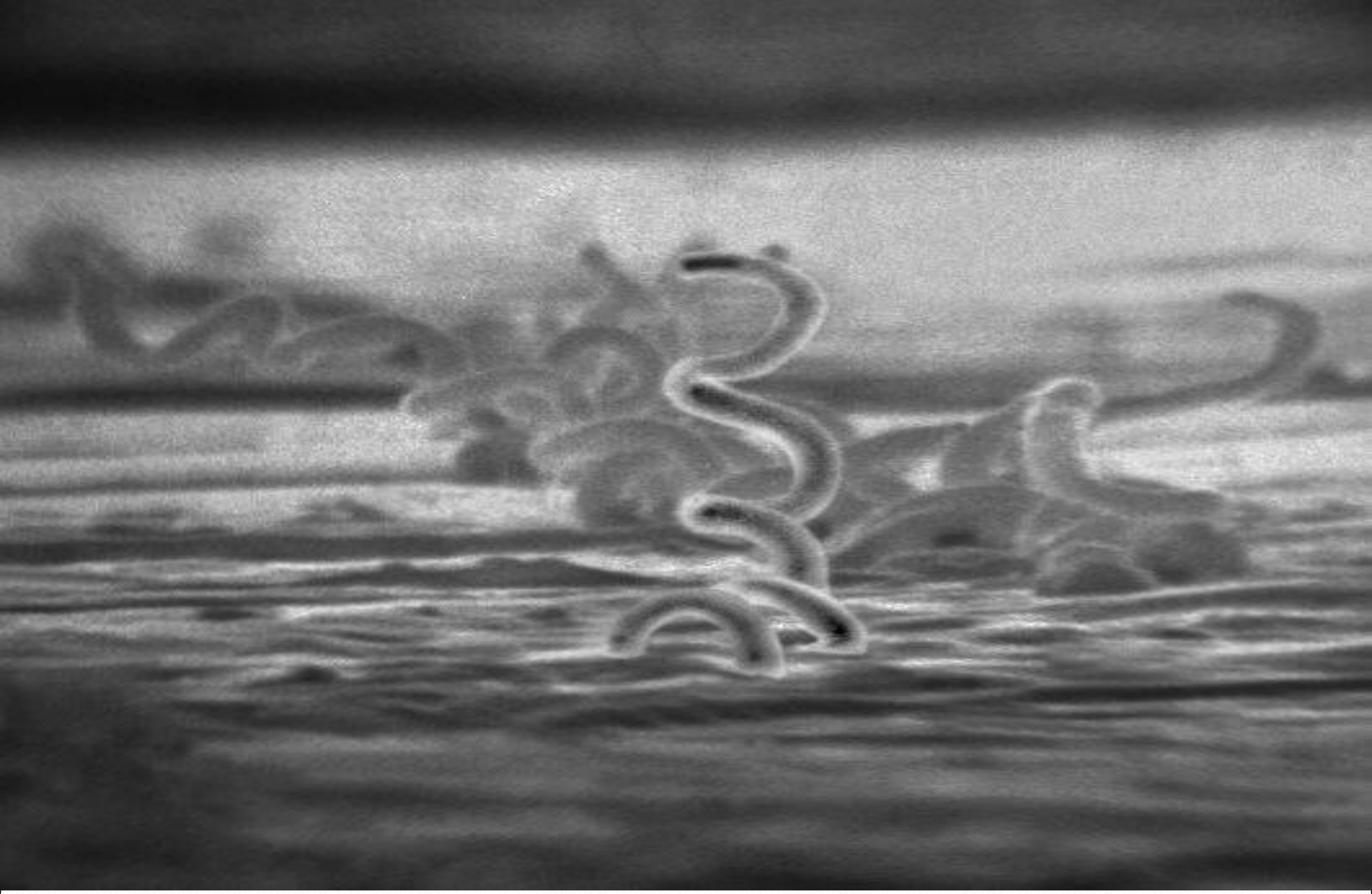


Structure

- Helically coiled, corkscrew-shaped organisms.
- 6 to 15 μ long and 0.1 to 0.2 μ wide.
- Present 6-14 spirals.
- Ends are pointed with finely spiral terminal filaments.
- Exhibits characteristic motility.
- Consists of rapid rotation about its longitudinal axis and bending, flexing, and snapping about its full length.



Dark Field Microscopy



Electron Microscopy

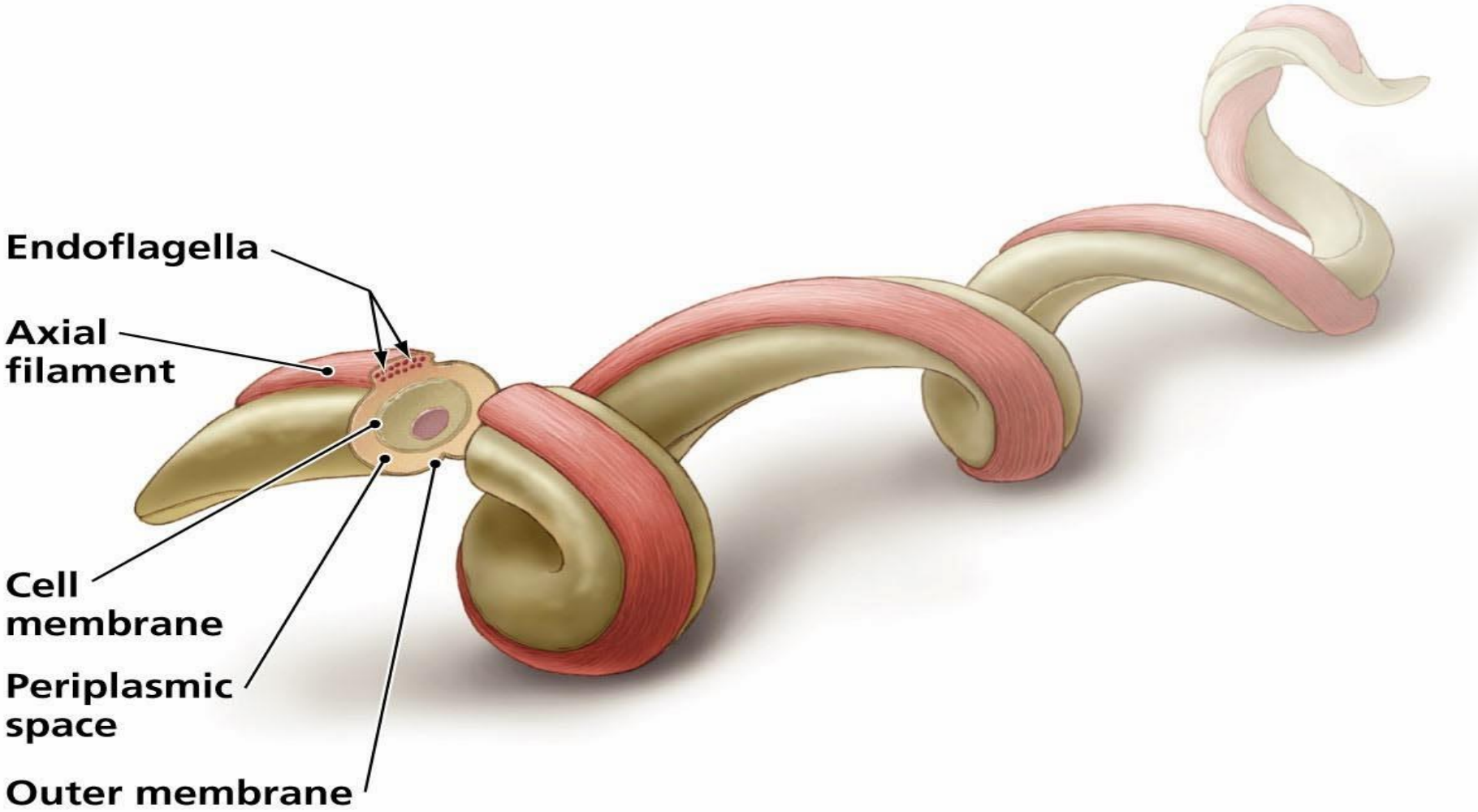
Characteristics

- Fastidious organism that exhibits narrow optimal ranges of :
  - pH (7.2 to 7.4)
  - Temperature (30 to 37°C).
- It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants.
- microaerophilic.
- Treponemes multiply by binary transverse fission.

- In Vivo generation time is about 30 hours.
- Not been successfully cultured In Vitro.
- Viable organisms can be maintained for 18 to 21 days in complex media.
- Limited replication has been obtained by co-cultivation with tissue culture cells.

Ultra Structure

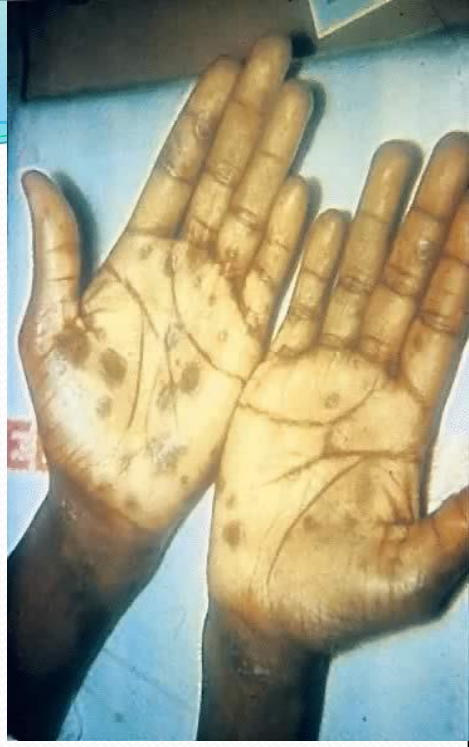
- Cytoplasmic membrane
- Cell wall (a thin peptidoglycan layer)
- Outer membrane layer
- 3-4 Periplasmic flagella (also called endoflagella)
- Cytoplasmic filaments, also known as Cytoplasmic fibrils


Endoflagella
Axial filament
Cell membrane
Periplasmic space
Outer membrane

Biochemical Structure

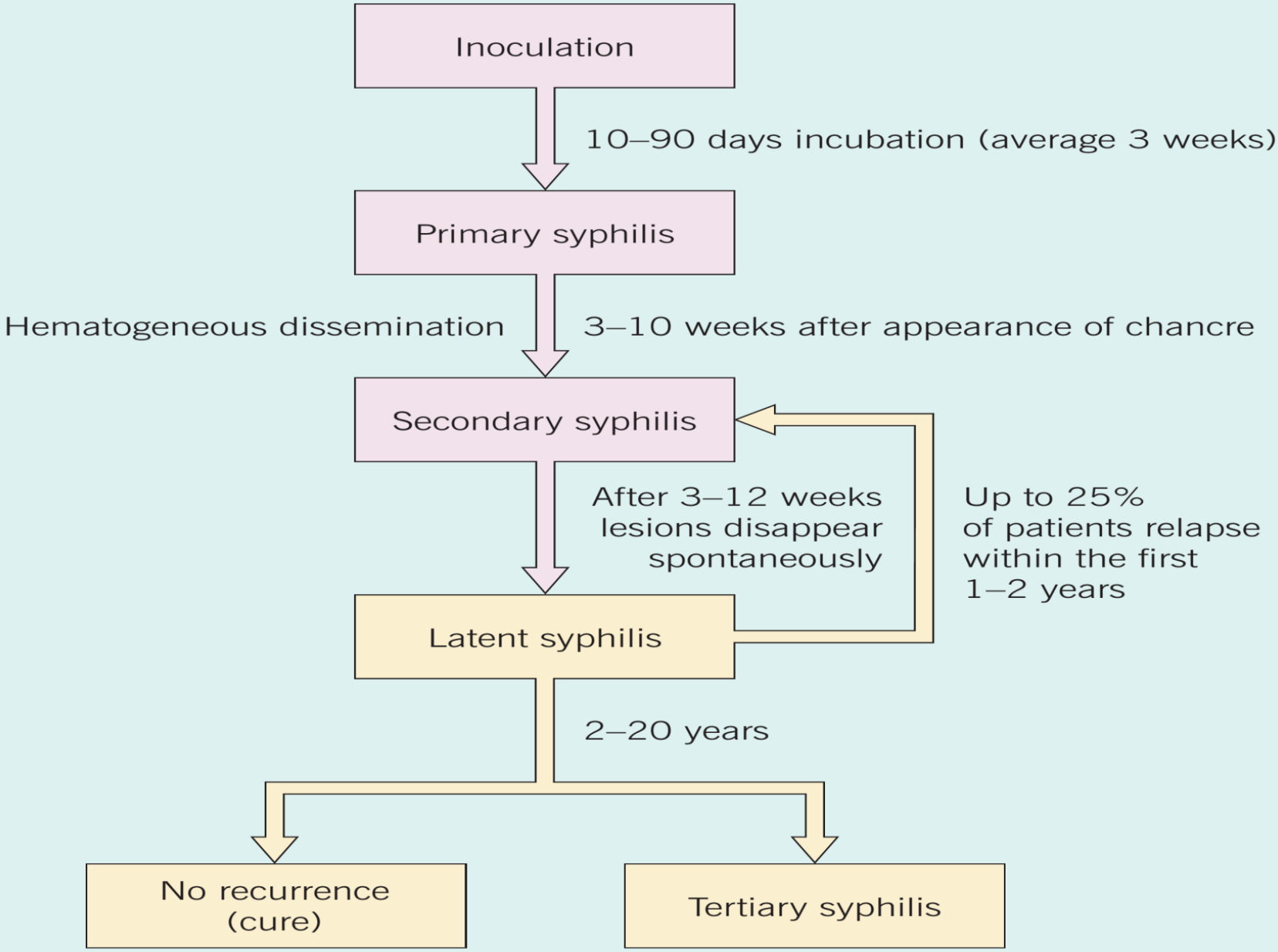
- Treponema is composed of approximately 70 % proteins, 20 % lipids, and 5 % carbohydrates.
- This lipid content is relatively high for bacteria.
- The lipid composition of *T pallidum* is complex, consisting of several phospholipids, including cardiolipin, and a poorly characterized glycolipid.

Syphilis – The "Great Imitator"



- Infectious Dose: ~57 organisms[1]
- Incubation Period – 21 days (median)
- 3 clinical stages of syphilis
  - Primary:
    - Painless sore (chancre) at inoculation site
  - Secondary:
    - Rash, Fever, Lymphadenopathy, Malaise
  - Tertiary/Latent:
    - CNS invasion, organ damage
- "The physician that knows syphilis knows medicine."
  – Sir William Osler

NATURAL HISTORY OF UNTREATED SYPHILIS



LAB DIAGNOSIS OF SYPHILIS

Sample collection

- For direct examination, exudates from lesions of primary, secondary and early congenital syphilis are the most useful.
- Clear, serous fluid free of erythrocytes, tissue debris and other organisms is collected.
- Serum is the specimen of choice for both nontreponemal and treponemal serological tests.
- Cerebrospinal fluid (CSF) testing is indicated in congenital and tertiary syphilis and when neurological symptoms are present.

Laboratory Diagnosis

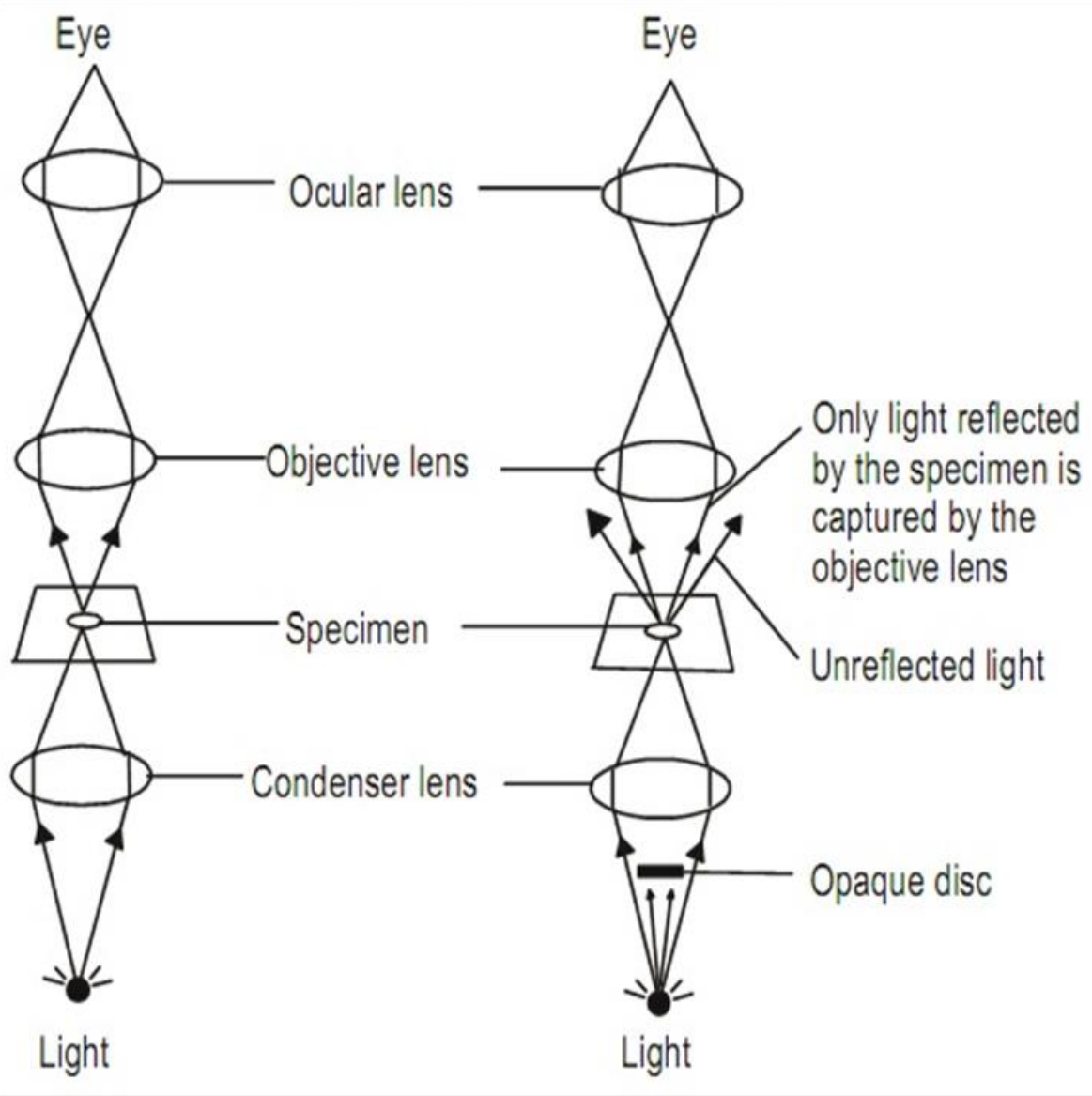
- Identification of *Treponema pallidum* in lesions

- Serologic tests
  - Nontreponemal tests
  - Treponemal tests

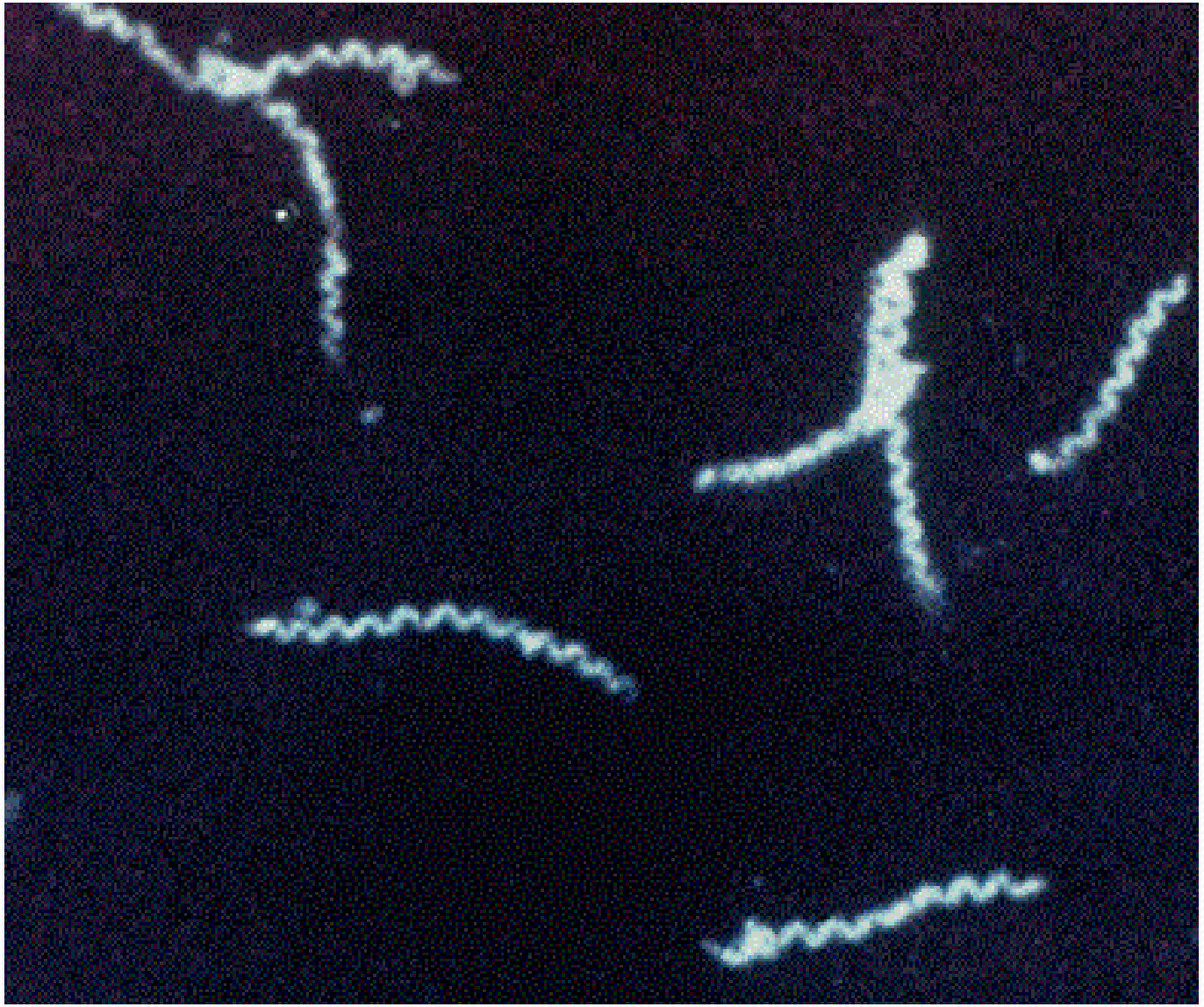
Dark-field microscopy

- Simplest and most reliable method.
- Exudates and fluids from lesions are examined as a wet mount.
- Identification of *T pallidum* is based on the characteristic morphology and motility.
- This method is suitable when the lesions are moist.
- Examination should be done immediately after specimen collection.

- This technique requires a trained, experienced microscopist.
- Treatment with antibiotics may result in a false-negative finding.
- Dark-field microscopy has limited sensitivity.

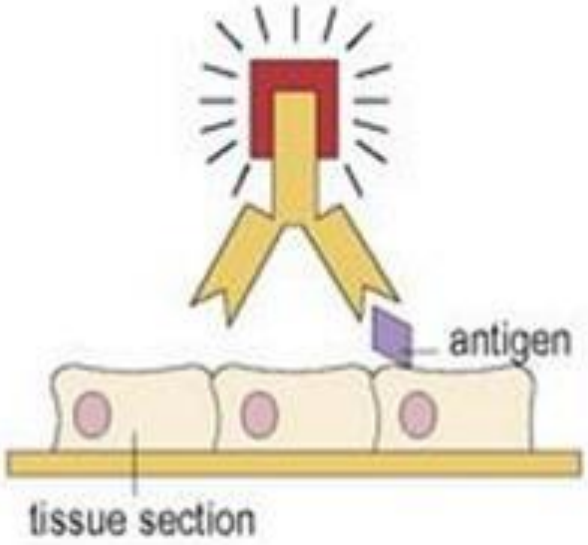
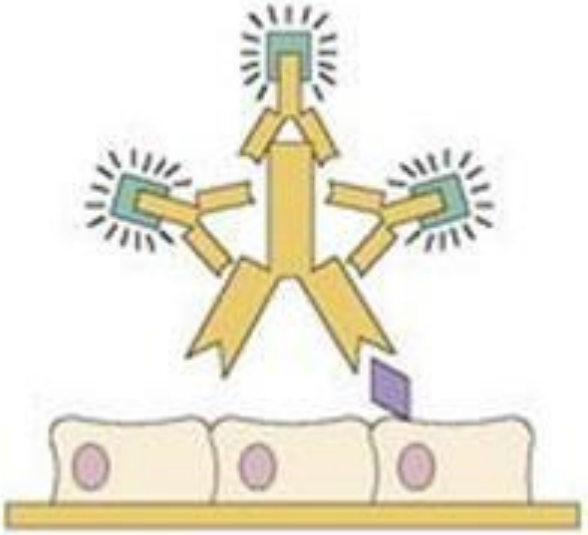

Eye
Eye
Ocular lens
Objective lens
Specimen
Condenser lens
Only light reflected by the specimen is captured by the objective lens
Unreflected light
Opaque disc
Light
Light

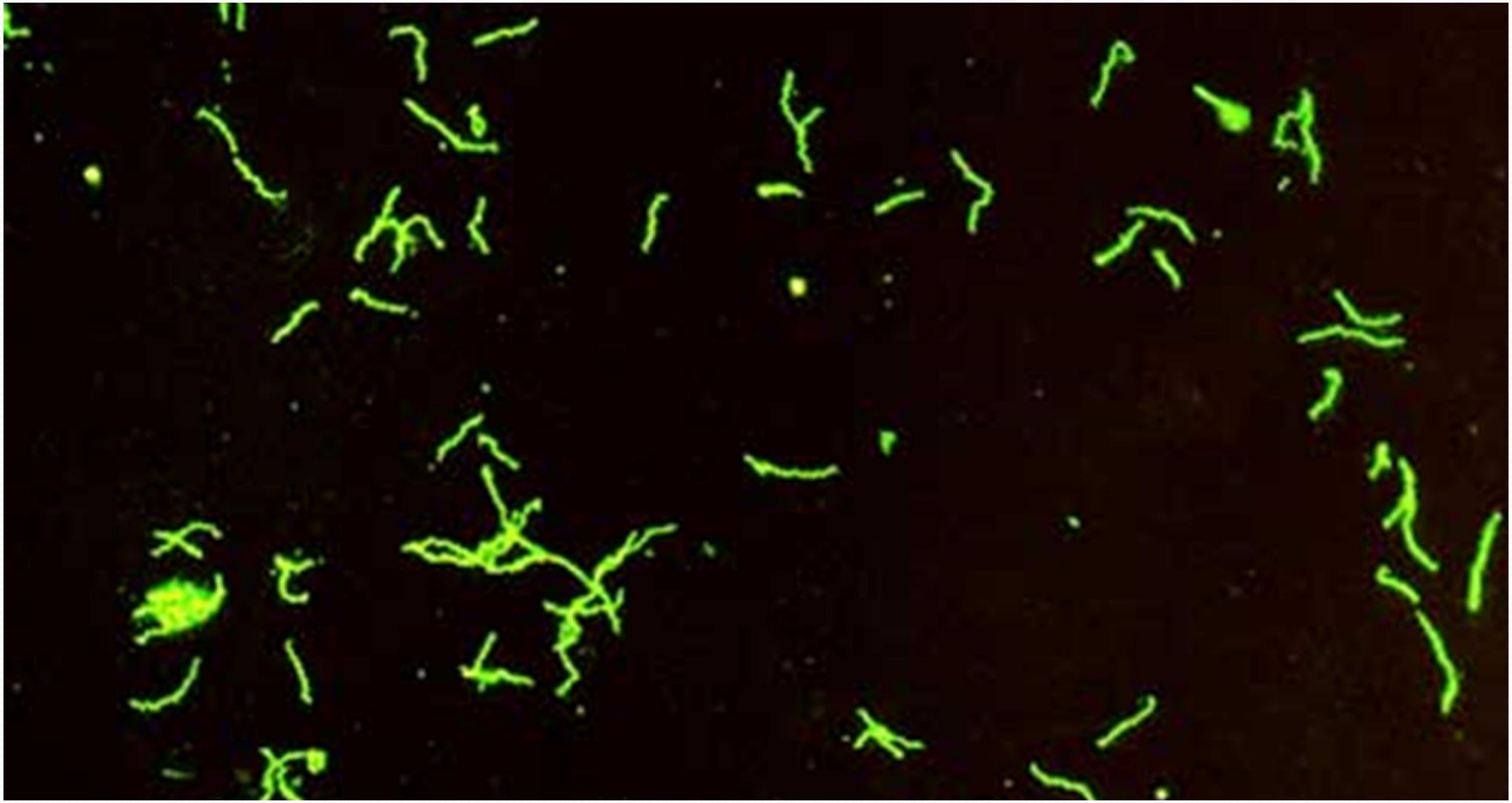
Dark Field Microscopy showed Treponema pallidum



Direct fluorescent antibody test for *T. pallidum*

- It detects antigen and, thus, does not require the presence of motile treponemes.
- Uses fluorescein isothiocyanate-labelled antibody specific to pathogenic treponemes
- Suitable for the examination of specimens from oral and rectal lesions.
- Does not differentiate between *T pallidum* and other pathogenic treponemes.

direct test	indirect test
	



Demonstration in tissue

- Immunofluorescence
- Silver impregnation (Levaditi method)

Animal Inoculation

- Rabbits were inoculated intratesticularly with T. Pallidum .

Nucleic acid amplification methods

- Highly sensitive
- Able to detect as low as one to 10 organisms per specimen with high specificity.
- Used to monitor treatment .
- Used to differentiate new infections from old infections.
- May be available only through select laboratories.

NON TREPONEMAL TESTS

- Nontreponemal tests are rapid, simple and inexpensive.
- They are the only tests recommended to monitor the course of disease during and after treatment.
- Nontreponemal tests can also serve to detect reinfection.
- They are also used as screening tests.
- Limitations – low specificity, low sensitivity in primary and late latent syphilis, false-positive results

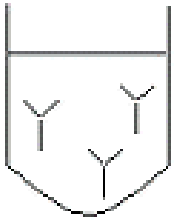
These include :

- Complement Fixation Tests
- Kahn Flocculation test
- VDRL
- Unheated Serum Reagin Test (USR)
- Rapid Plasma Reagin Test (RPR)
- Toluidine Red Unheated Serum Test (TRUST)

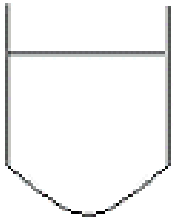
COMPLEMENT FIXATION TEST

- First developed by Wassermann in 1906.
- In this method, the patient's serum containing antibodies is made to react with a standardised antigen.
- Wassermann antigen – extract of liver from newborns who had died of congenital syphilis.
- Cholesterol and Lecithin were added to increase sensitivity of antigens.
- Complicated to perform, required many reagents and 24 h to complete

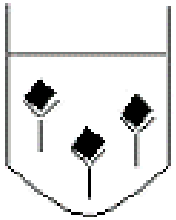
Complement Fixation Test



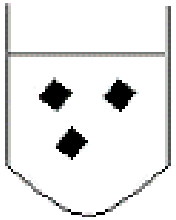
Serum with antibodies



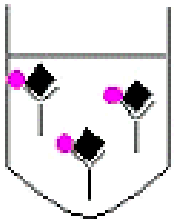
Serum without antibodies



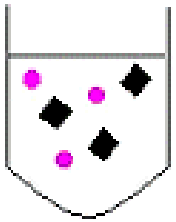
Antigen binds with antibodies



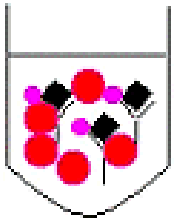
Unbound Antigen



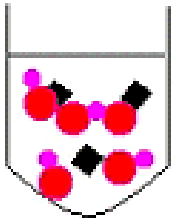
Complement binds with Ag/Ab complex



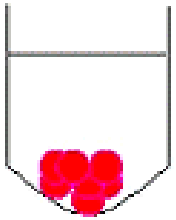
Unbound complement



Hemolysin Sensitized red blood cells serve as an indicator

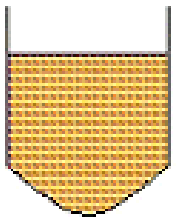


Hemolysin Sensitized RBCs serve as an indicator



RBCs settle into a pellet

no lysis



RBCs lysed by unbound complement

lysis

Reactive

Nonreactive

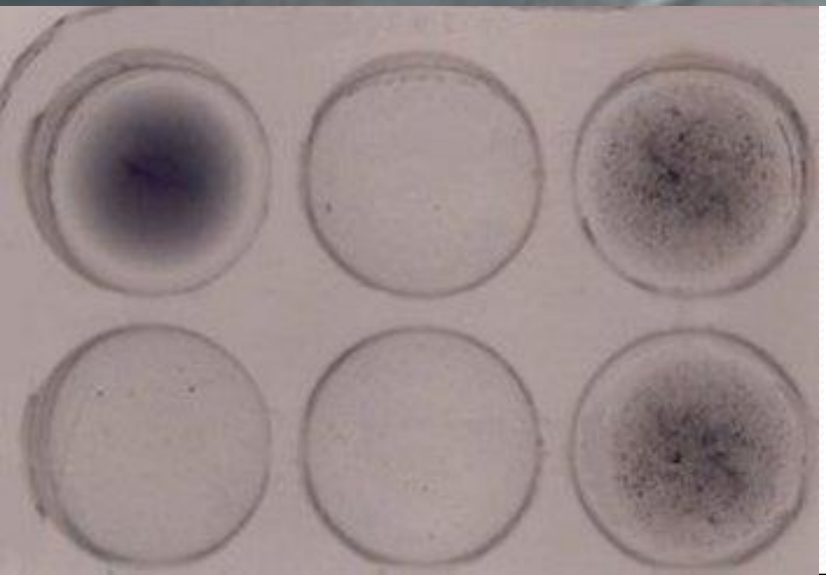
KAHN FLOCCULATION TEST

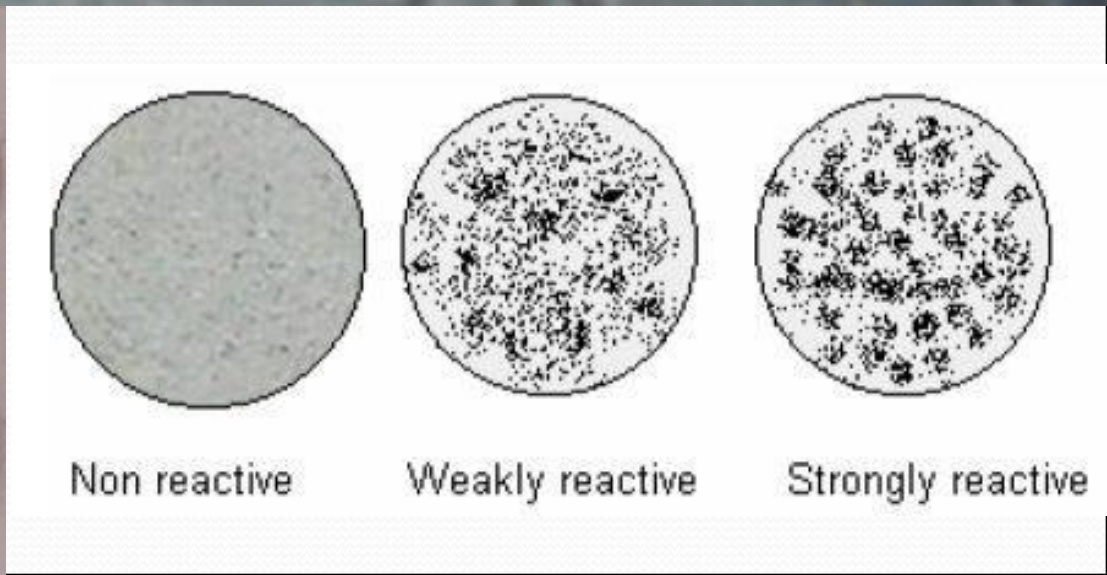
- In 1922, Kahn introduced a flocculation test without complement that could be read macroscopically in a few hours.
- Kahn antigen – alcoholic extract of fresh beef heart with cholesterol.
- On reaction with syphilitic serum, floccules are formed which can be seen with the naked eye.
- Standardization of the tests was difficult.

VDRL

- Slide micro flocculation test.
- Serum or CSF can be used.
- The basis of the test is that an antibody produced by a patient with syphilis reacts with an extract of ox heart
- Visualized through foaming of the test tube fluid, or "flocculation".
- It therefore detects anti-cardiolipin antibodies.
- Antigen - 0.03% cardiolipin, 0.21% lecithin and 0.9% cholesterol.

- A reactive VDRL is seen in about 50-75% of patients with primary syphilis and 100% in patients with secondary syphilis.
- VDRL test can be quantitated by examining serial dilutions of serum and can be used to follow the course of illness, including the response to therapy.
- A dilution of > 1:8 is suggestive of syphilis.
- VDRL yields reproducible results, can be rapidly performed, acceptable levels of sensitivity and specificity, valuable tool for mass screening




Non reactive
Weakly reactive
Strongly reactive

Biological false positive

Since the test employs a non-treponemal antigen, there are many chances of biological false positive results.

- Pregnancy
- Menstruation
- Repeated blood loss
- Vaccination
- Severe trauma
- Antiphospholipid syndrome
- Drug addiction

- SLE and other collagen vascular disorders
- Hepatitis or any other liver disease
- Malaria, Filariasis,Tuberculosis
- Malignancy
- Tropical eosinophilia
- Lepromatous leprosy
- Infectious mononucleosis

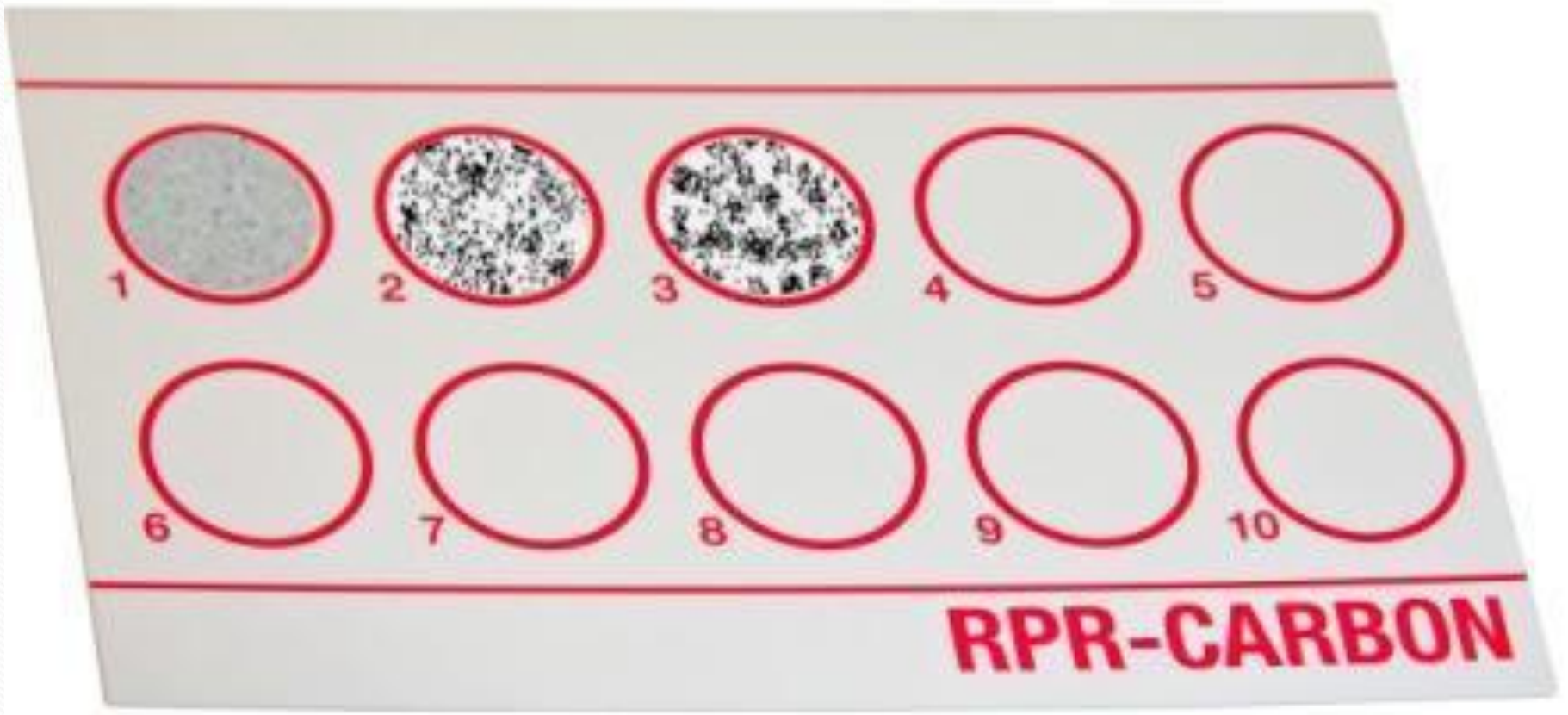
Prozone reactions are false-negative reactions that occur due to interference by high concentrations of target antibodies in a specimen.

UNHEATED SERUM REAGIN TEST

- Quantitative, microscopic, non treponemal, flocculation test similar to VDRL.
- The VDRL antigen is enhanced by the addition of choline chloride and EDTA.
- So the need for heating serum was eliminated.
- Plasma could also be used an acceptable sample source.

RAPID PLASMA REAGIN TEST

- Rapid Plasma Reagin (RPR) test is a macroscopic Non Treponemal flocculation test , and is a simplified version of the VDRL test.
- The RPR test uses a stabilized suspension of VDRL antigen to which **charcoal particles** are added to aid in the visualization of the test reaction.
- Serum or plasma can be used.
- RPR Teardrop card test and RPR 18mm circle card test are further refinements of this test which are used currently for screening.


1
2
3
4
5
6
7
8
9
10
RPR-CARBON

TRUST

- Toluidine Red Unheated Serum Test (TRUST) is a macroscopic Non Treponemal flocculation test.
- In the TRUST test, particles of **toluidine red** are used in place of the charcoal particles of the RPR test as the visualising agents.
- Serum or plasma can be used.
- Quantitative values allow evaluation of recent infection and response to treatment. Used for screening and follow up of therapy


TRUST
ANTIGEN
2.8 ml
FOR LABORATORY
HARDY
DIAGNOSTICS
Copyright 2009 All rights reserved.

TREPONEMAL TESTS

- Treponemal tests may remain reactive for years with or without treatment
- Treponemal test antibody titres correlate poorly with disease activity.
- Therefore, treponemal tests should not be used to evaluate response to therapy, relapse or reinfection in previously treated patients.
- Treponemal tests do not differentiate venereal syphilis from endemic syphilis (yaws and pinta).
- Treponemal tests are used mainly as confirmatory tests to verify reactivity in nontreponemal tests.

These include :

- T. pallidum Immobilization (TPI)
- Reiter's Antigen CFT
- Fluorescent Treponemal Antibody test (FTA)
- FTA Absorption test (FTA - ABS)
- T. pallidum haemagglutination assay (TPHA)
- T. pallidum Particle Agglutination Assay (TPPA)
- MHA – TP
- PK – TP

TPI

- T. pallidum immobilization (TPI) test
- Antigen - T. pallidum (Nichols strain) grown in rabbit testes.
- It is based on the ability of patient's antibody and complement to immobilize living treponemes, as observed by dark-field microscopy.
- However, the TPI test was complicated, technically difficult, time-consuming, expensive to perform and is not used much now.

REITER's ANTIGEN CFT

- Reiter protein complement fixation test.
- Antigen - prepared from T. phagedenis, the Reiter treponeme, a nonpathogenic organism was used in a complement fixation test.
- High proportion of false-positive reactions.
- Less specific and sensitive than the TPI test.

Fluorescent treponemal antibody (FTA) test

- The FTA procedure uses a 1:5 dilution of the patient's serum in saline solution, reacted with a suspension of Reiter treponeme.
- FITC(fluorescein isothiocyanate) was used as the conjugate, and the test was read under a microscope with a UV light source.
- Nonspecific reactions were encountered in approximately 25% of normal serum specimens.

- To eliminate these false-positive reactions, the test was modified by diluting the patient's serum 1:200, the FTA-200 test.

- The FTA – 200 is highly specific but not very sensitive.

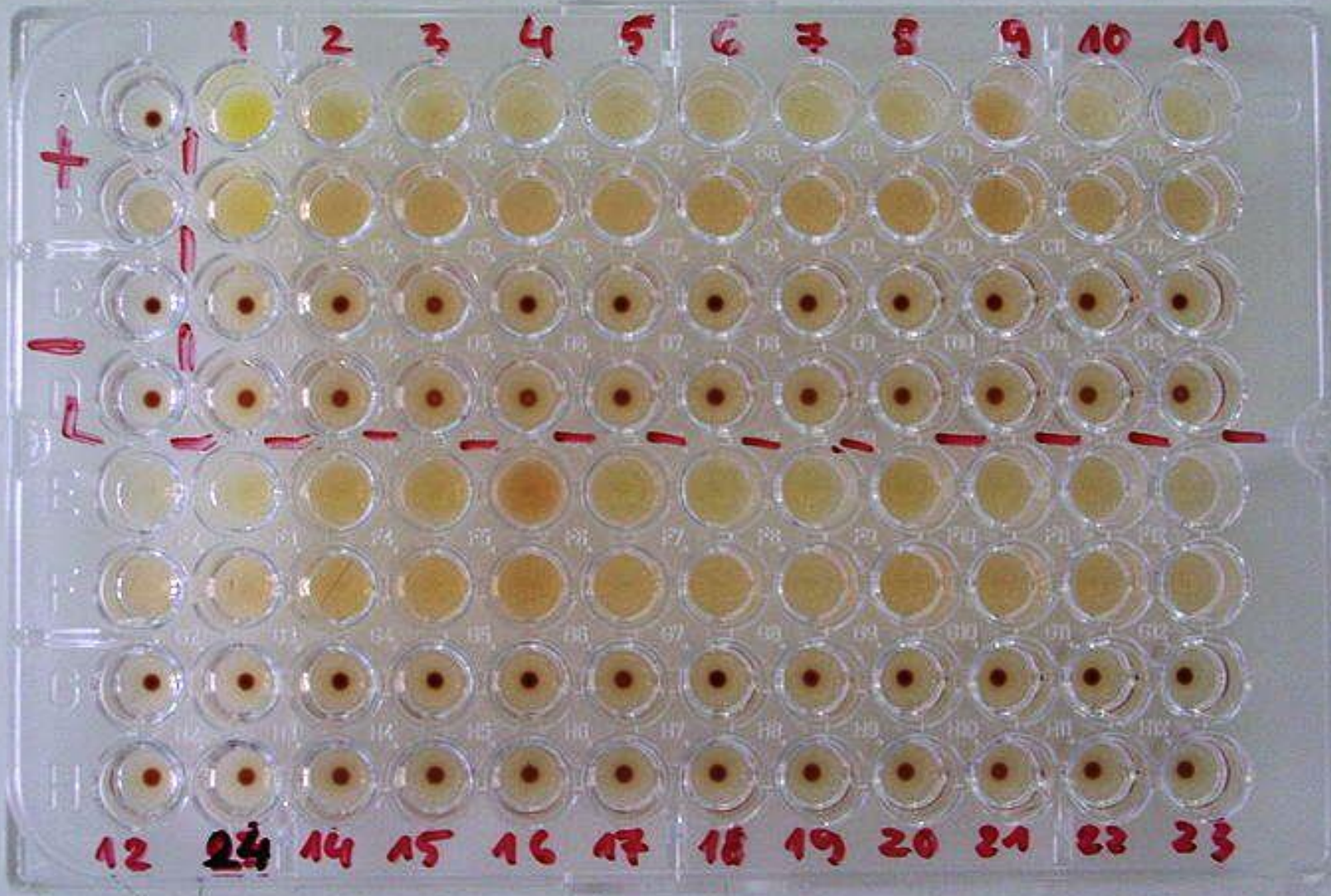
FTA absorption (FTA-ABS) test

- Generally regarded as the gold standard test for confirming diagnosis.
- FTA-ABS is the most sensitive test in all stages of syphilis.
- The patient's diluted serum (1:5) is added to the Reiter antigen and "group" treponemal antibodies are absorbed leaving behind "species specific" antibodies in the serum.

- Results are reported as reactive, reactive minimal, nonreactive, or atypical fluorescence
- It is a subjective test and difficult to standardize.
- Less than 1% false positives are due to HIV, SLE, RA or old-age.
- The FTA-ABS double staining test is a modification of the FTA-ABS test using a double staining procedure with the addition of a contrasting counterstain.

T. pallidum haemagglutination test.

- The most appropriate test for confirming diagnosis.
- It is an indirect haemagglutination assay.
- Antigen – formalinized, tanned, erythrocytes sensitized with ultrasonicated material from T. pallidum (Nichols strain).
- The presence of treponemal antibody in the patient's serum is detected by the indirect agglutination of the sensitized erythrocytes and the subsequent formation of a mat of erythrocytes upon their settling.


1 2 3 4 5 6 7 8 9 10 11
+
−
12 13 14 15 16 17 18 19 20 21 22 23

- Results are reported as reactive, nonreactive, or inconclusive.
- Specificity - 99%
- Biological False Positive in some cases of Leprosy.
- If gelatin particles are used instead of erythrocytes, test is called T. pallidum particle agglutination assay (TPPA).
- Microhaemagglutination assay for antibodies to T. pallidum (MHA-TP) uses reagents for a microvolume haemagglutination test.
- Haemagglutination Treponemal test for Syphilis (HATTS) is another variant.

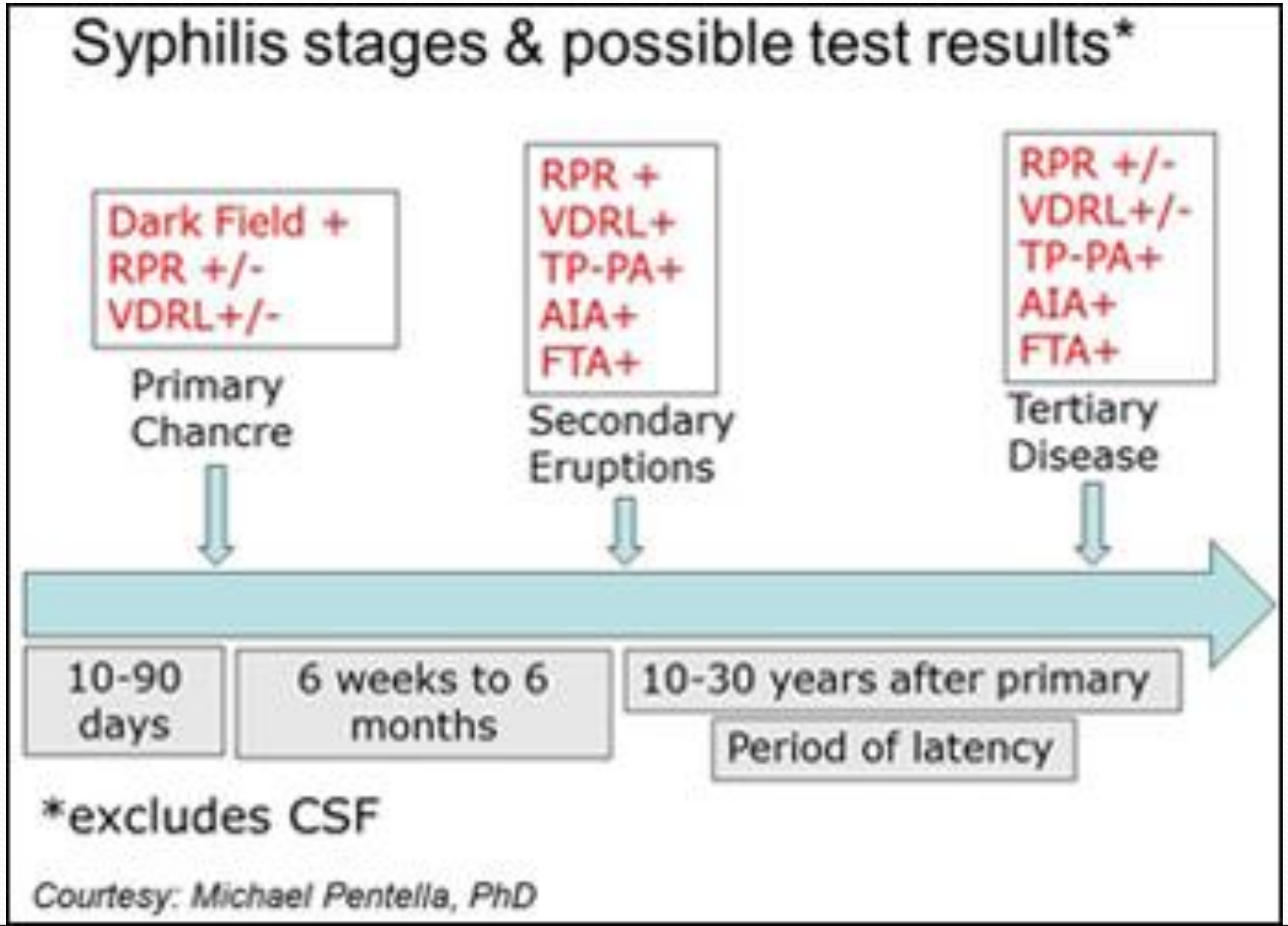
PK- T. pallidum (PK-TP) test

- PK-TP is a new haemagglutination test which has achieved provisional status.

- The PK-TP reagent is composed of chicken erythrocytes which have been fixed and then sensitized with components of sonicated T. pallidum.

Enzyme Immuno Assay

- A number of treponemal EIA tests are now available:
  - Captia Syphilis M
  - Captia Syphilis G
  - Captia select Syph-G
  - SpiroTek syphilis test
  - Enzygnost Syphilis
- These are newer tests with provisional status which are being used now in a number of laboratories.


Syphilis stages & possible test results*
Dark Field +
RPR +/-
VDRL+/-
Primary Chancre
RPR +
VDRL+
TP-PA+
AIA+
FTA+
Secondary Eruptions
RPR +/-
VDRL+/-
TP-PA+
AIA+
FTA+
Tertiary Disease
10-90 days
6 weeks to 6 months
10-30 years after primary
Period of latency
*excludes CSF
Courtesy: Michael Pentella, PhD

- Congenital Syphilis
- Jarisch Herxheimer Reaction

	VDRL/RPR	FTA-ABS	TPHA
Primary	70-80%	85-100%	65-85%
Secondary	100%	100%	100%
Tertiary	60-70%	95-100%	95-100%

Borrelia

- Gram negative, 5-30μ *long*, 0.3-0.7μ wide

Relapsing Fever

- Louse borne
- B recurrentis, exclusive human pathogen
- Human louse is vector

- Tick borne
- Rodents and mammals are hosts

B recurrentis

- Antigenic variation- relapses
- Relapses of fever
- transmitted transovarially
- Soft Ticks are vectors- Ornithodorus

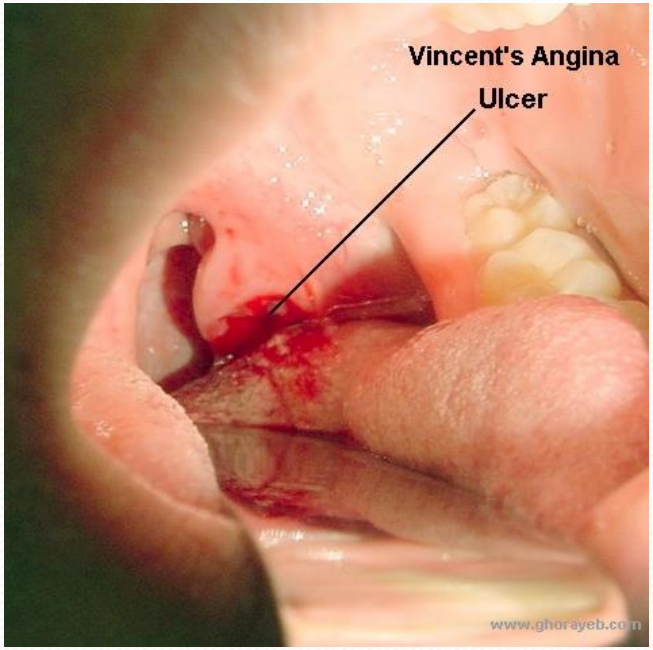
Lab D

- Microscopy
- Mice inoculation
- Serology with Proteus OXK

- T/t- Tetracyclines, Chloramphenicol

B vincenti

- 5-20μ long, Gram negative, 3-8 coils
- Normal mouth commensal
- Assoc with Vincents Angina- Ulcerative gingivostomatitis



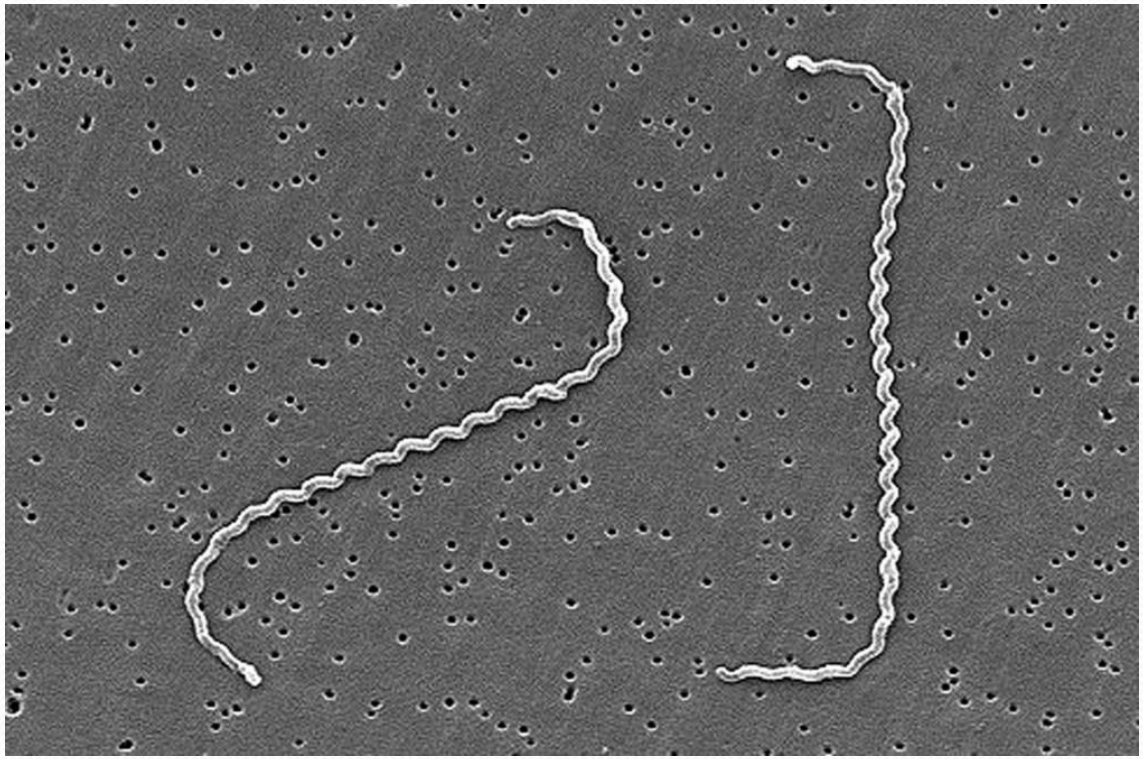
Lyme disease

- B burgdorferi
- Ixodid ticks
- 3 stages-localized infection, disseminated infection, persistant infection
- Modified Kellys medium (BSK)

- Lab D- isolation,serology (elisa, IF) culture

Leptospira

- 6-20 μ long, 0.1μ thick, tightly coiled, ends are hooked



Culture

- Liquid media- enriched with rabbit serum
- Solid media- Korthof, Stuart, Fletchers,
- EMJH
- Aerobic and microaerophilic
- Embryonated eggs- CAM
- Animal- Guinea pig

Ag & Classification

- Genus specific somatic Ag

- Classification based on surface Ags

- 2 species
- L interrogans
- L biflexa

Pathogenecity

- Weils Disease- Fever with hepatorenal damage
- Icteric and non icteric clinical types
- Leptospires persist in kidneys

Lab Diagnosis

- Specimen- blood,urine
- Microscopy- blood, urine
- Culture- blood , CSF, urine
- Animal inocultion
- Serological diagnosis-
- Broadly reactive /genus specific tests- ag from non pathogenic L biflexa Pantoc 1 strain
- Type specific tests-Macroscopic agglutination tests, Microscopic agglutination tests (MAT)
- Animal-
- Water examination

Prophylaxis & Treatment

- Rodent control, disinfection of water, protective clothing
- Penicillin, tetracyclines
- Doxycycline for prophylaxis

	Treponema	Borrelia	Leptospira
Size	6-14X0.2µ	8-30X0.2-0.5µ	6-20X0.1µ
Spirals	1µ width, 1µ amplitude, ends pointed	3µ width, 1-2µ amplitude, loose	0.5µ width and amplitude, tightly coiled, hooked ends
No of Endoflagella	3-4 at each pole	15-20 at each pole	1 at each pole
Motility	Flex ext,translatory, cork screw, bending	Flex ext,translatory, cork screw	Rotation,farward backward, bending, flex
Staining	Giemsa, Silver	Gram negative	Giemsa, Silver
Mode of Infection	Sexual	Ticks and lice	Water cont with rodent urine
Incubation period	10-90 d	2-14 d	7-14 d
Pathogenecity	Syphilis, bejel, yaws, pinta	Relapsing fevers, lyme disease	Weils disease

THANK YOU

- **132.** A 30-year-old male patient was seen by the emergency service and reported a 2-week history of a penile ulcer. He noted that this ulcer did nothurt. Which one of the following conclusions/actions is most valid?
- a. Draw blood for a herpes antibody test
- b. Perform a dark-field examination of the lesion
- c. Prescribe acyclovir for primary genital herpes
- d. Even if treated, the lesion will remain for months
- e. Failure to treat the patient will have no untoward effect, as this is a self-limiting infection

- **133.** The laboratory reports that the Venereal Disease Research Laboratory (VDRL) test performed on the above patient is reactive at a dilution of 1:4 (4 dils). The patient also reports to you that he has recently been diagnosed with hepatitis A. Which one of the following actions would be most appropriate?
- a. Report this patient to the health department, as he has syphilis
- b. Order a confirmatory test such as the fluorescent treponemal antibody test
- (FTA)
- c. Repeat the VDRL test
- d. Order a rapid reagin test (RPR)
- e. Perform a spinal tap to rule out central nervous system syphilis

- **134.** In the above patient, which one of the following test combinations
- for syphilis is most appropriate?
- a. FTA-Abs (IgG)/FTA-Abs (IgM)
- b. RPR/FTA-Abs
- c. RPR/culture of the lesion
- d. VDRL/RPR
- e. *Treponema pallidum* hemagglutination (TPHA)/microhemagglutination-*Treponema pallidum* (MHTP) tests

- **135.** Assume that the patient absolutely denied any contact, sexual or otherwise, with a person who had syphilis. Assume also that both the RPR and the FTA Abs were positive on this patient. Which one of the following tests could be used to show that this patient probably does not have syphilis?

- a. VDRL
- b. Quantitative RPR
- c. *Treponema pallidum* immobilization (TPI) test
- d. Frei test
- e. MHTP test