Treponema, Leptospira, Borrelia

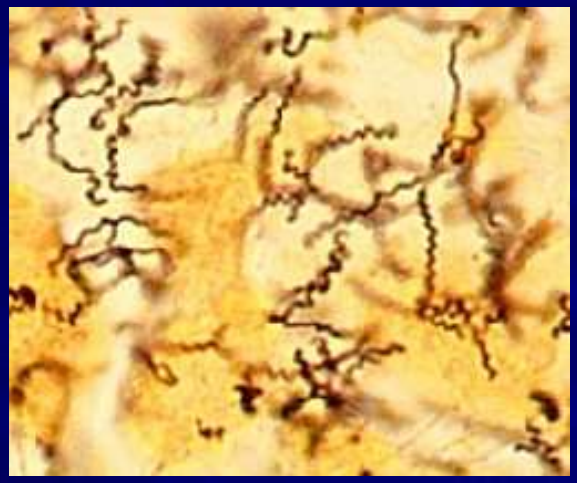
Taxonomy

- Order *Spirochaetales*
- Families *Spirochaetaceae* and *Leptospiraceae*
- *Spirochaetaceae* has two genera *Borrelia* and *Treponema*

Treponema

- *T. pallidum* – obligate human pathogen
- Spirochetes
  - thin (0,18 μm) but long (6-20 μm)
  - coiled – 6 – 14 helices per cell
- Motile – flagella are endoflagella are localised beneath the outer membrane and are at both ends
- Cause of syphilis

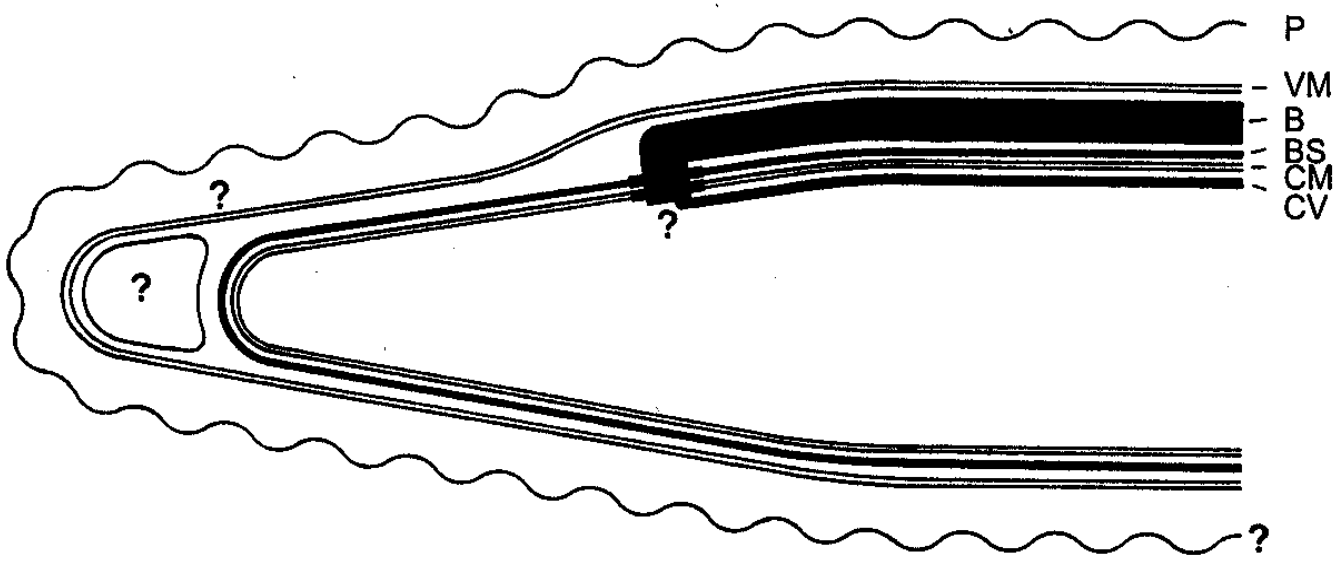
Morphology of *Treponema pallidum*

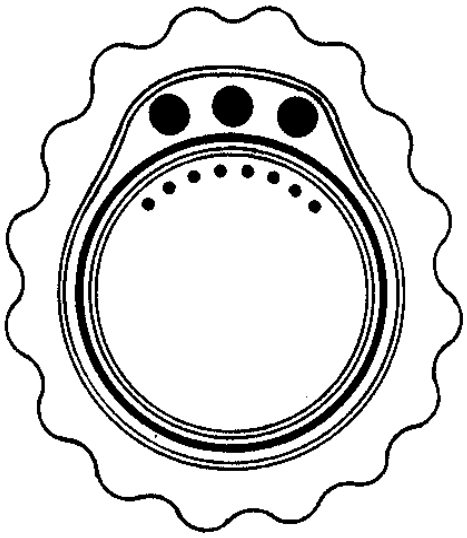


- Too thin (0.1-0.2 μm) to be seen with light microscopy in specimens stained with Gram stain or Giemsa stain
- Motile spirochetes can be seen with darkfield micoscopy
- Cannot be grown in cell-free cultures in vitro
- Do not survive well outside of host
- Care must be taken with clinical specimens for laboratory culture or testing

Mechanisms of pathogenicity

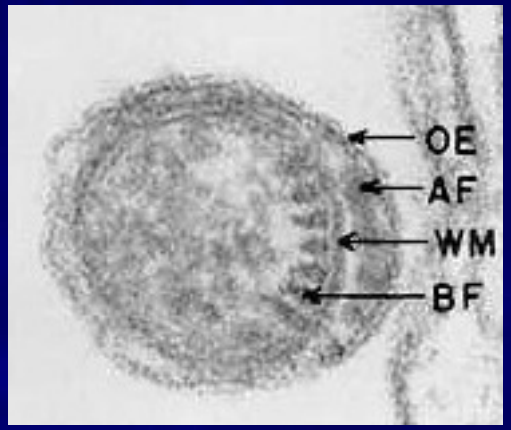
- Treponemes are highly invasive pathogens which often disseminate relatively soon after inoculation.
- Evasion of host immune responses appears to be, at least in part, due to the unique structure of the treponemal outer membrane (i.e., its extremely low content of surface-exposed proteins).
- Although treponemes lack classical lipopolysaccharide (endotoxin), they possess abundant lipoproteins which induce inflammatory processes.





Schematic structure of *T.pallidum:*

- P – capsule slime layaer… antiphagocytic
- VM - outer membrane (OE →) … adherence
- B – flagellum (AF →)
- BS – cell wall(peptidoglycan) (WM →)
- CM – cytoplasmatic membrane
- CV – cytoplasmatic fibers (BF →)



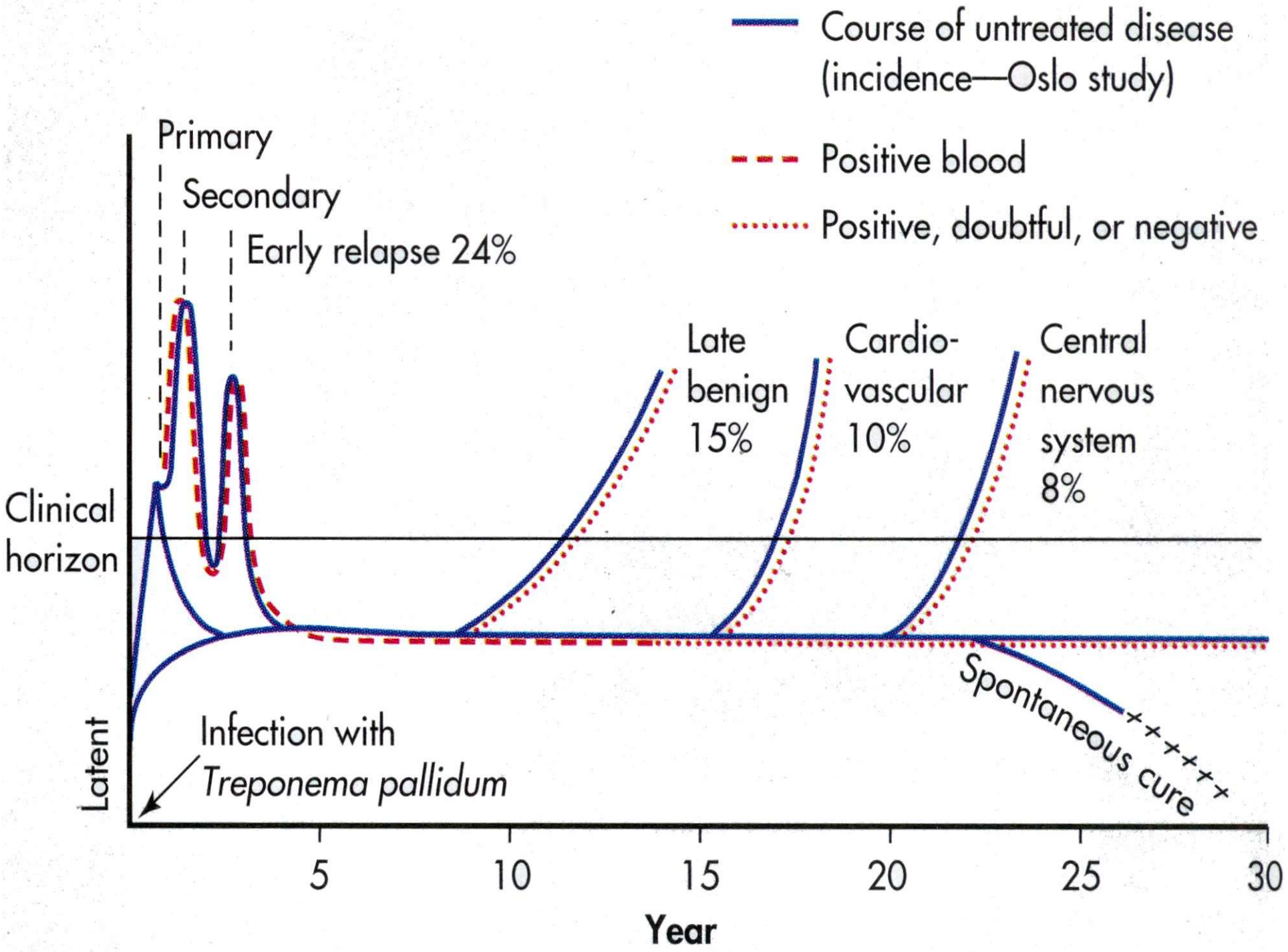
Mechanisms of patogenicity/virulence

- *Treponema pallidum* enters via abraded skin or intact mucous membrane
- Outer membrane proteins promote adherence
- Hyaluronidase may facilitate perivascular infiltration
- Antiphagocytic coating of fibronectin
- Tissue destruction and lesions are primarily result of host's immune response
- The average time between acquisition of syphilis and the start of the first symptom is 21 days, but can range from 10 to 90 days.
- It is not generally possible to contract syphilis through toilet seats, daily activities, hot tubs, or sharing eating utensils or clothing. This is mainly because the bacteria die very quickly outside of the body, making transmission by objects extremely difficult.

Specific epidemiologal properties of *Treponema pallidum*

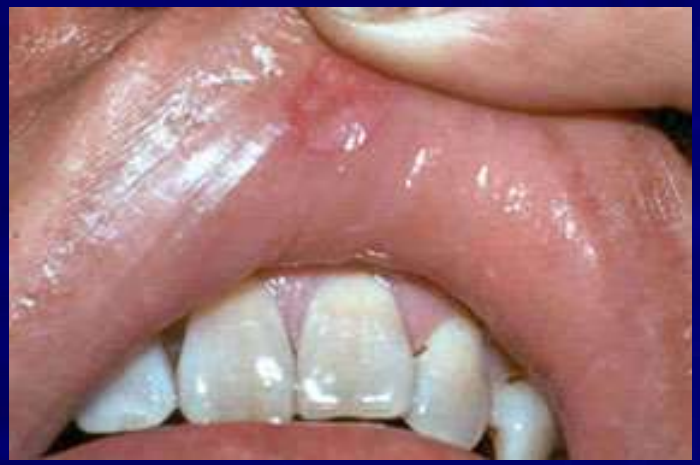
- Transmitted from direct sexual contact or from mother to fetus
- Not highly contagious (~30% chance of acquiring disease after single exposure to infected partner) but transmission rate dependent upon stage of disease
- Long incubation period during which time host is non-infectious
  - Useful epidemiologically for contact tracing and administration of preventative therapy

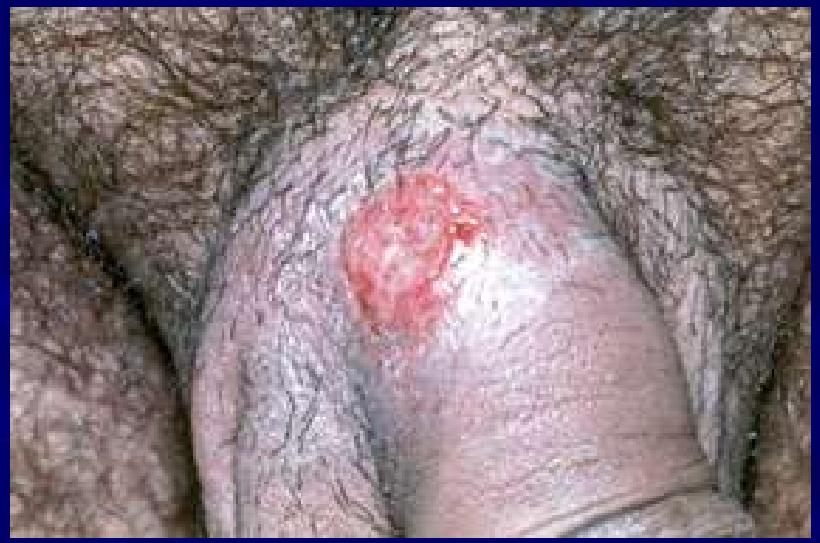
Syphilis, Attributed to Albrecht Dürer (1496)


Course of untreated disease (incidence—Oslo study)
Positive blood
Positive, doubtful, or negative
Primary
Secondary
Early relapse 24%
Late benign 15%
Cardio-vascular 10%
Central nervous system 8%
Clinical horizon
Latent
Infection with Treponema pallidum
Spontaneous cure
++++++
5
10
15
20
25
30
Year

Early syphilis <1-year duration primary stage

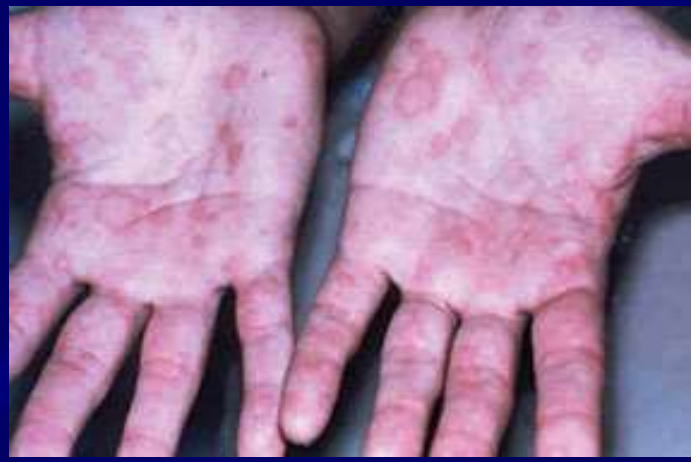
- 10-90 days (usually 3-4 weeks) after initial contact the host mounts an inflammatory response at the point of entry resulting in the hallmark syphilitic lesion, called chancre (usually painless).
- Chancre changes from hard to ulcerative with profuse shedding of spirochetes.
- Heals sponaneously within 3-6 weeks.

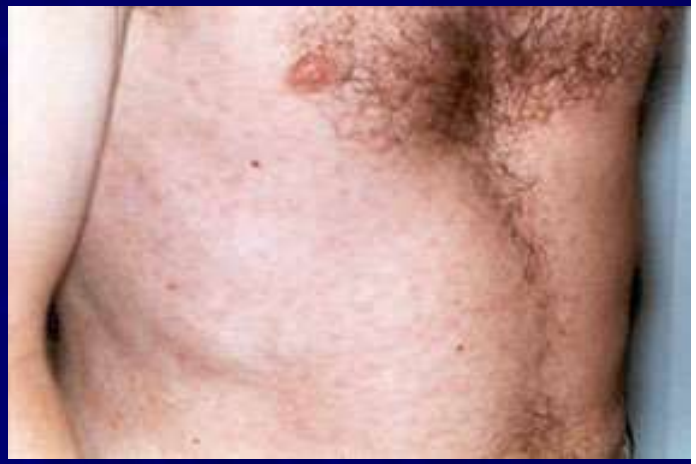


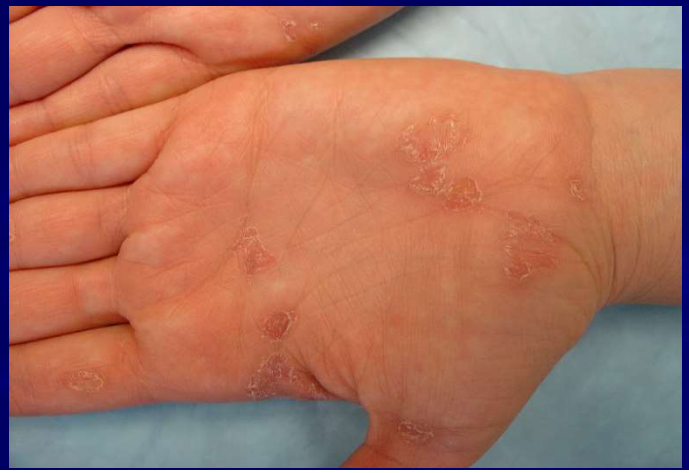


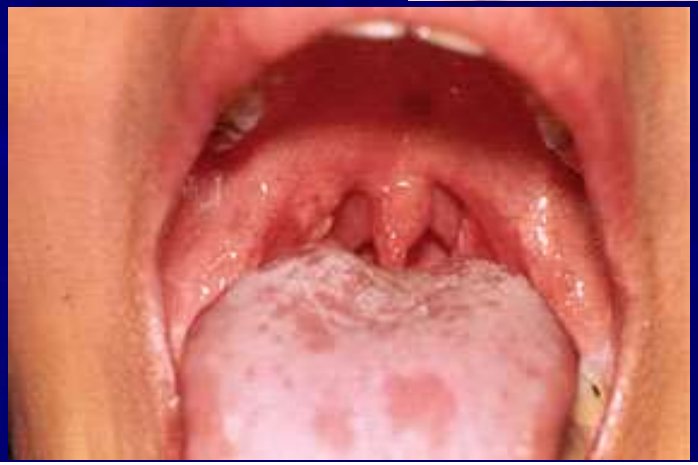
Secondary syphilis

- Secondary disease 2-10 weeks after primary lesion
- Widely disseminated mucocutaneous rash
- Secondary lesions of the skin and mucus membranes are highly contagious
- Raised lesions – condylomata lata (mouth)
- Generalized immunological response
- Relapses can occur in up to 25% of untreated patients



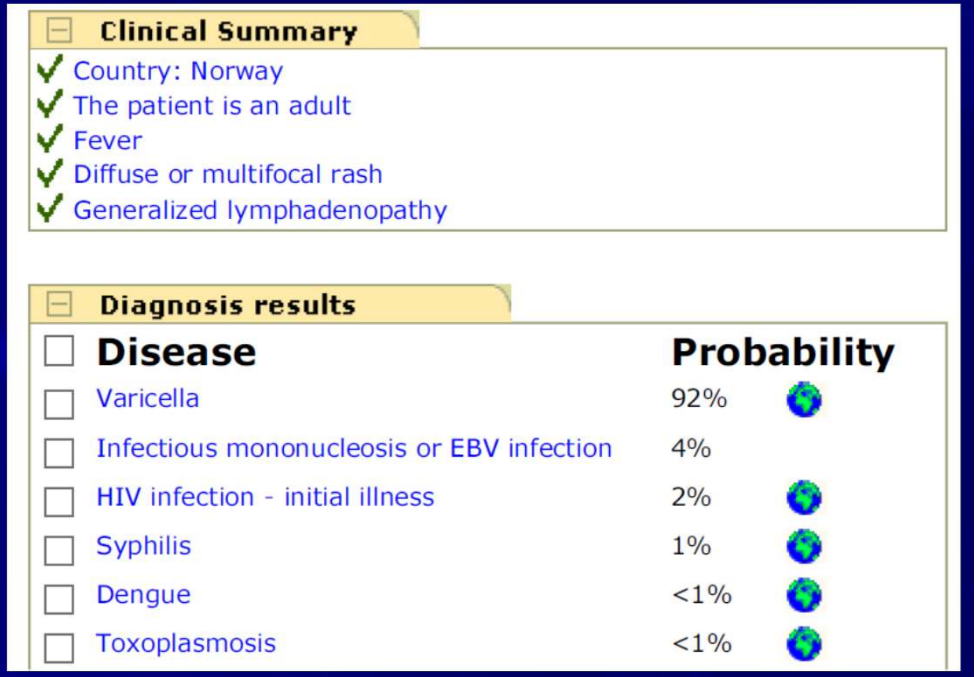


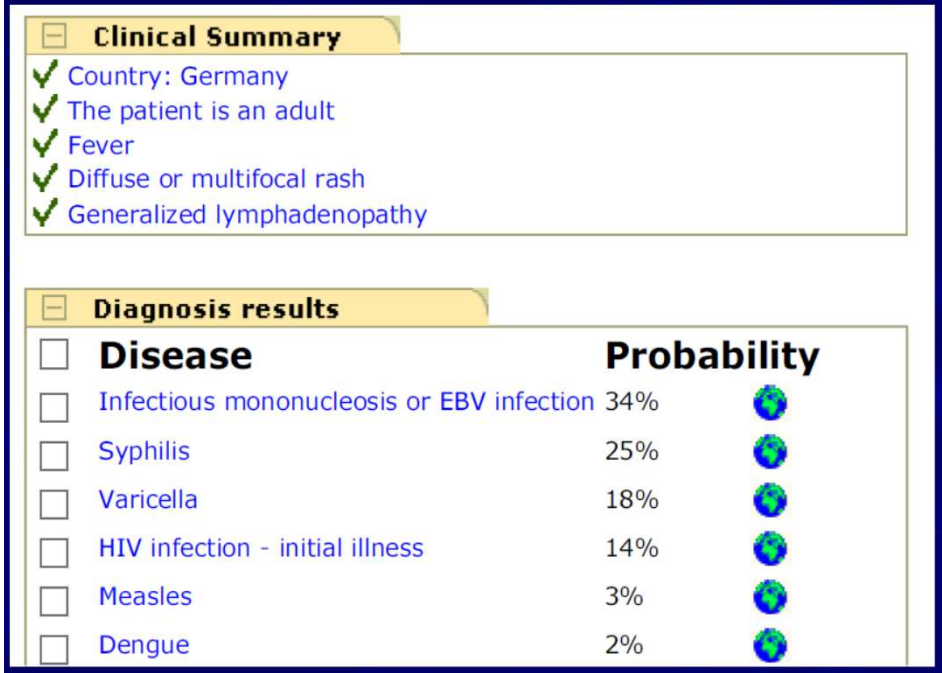


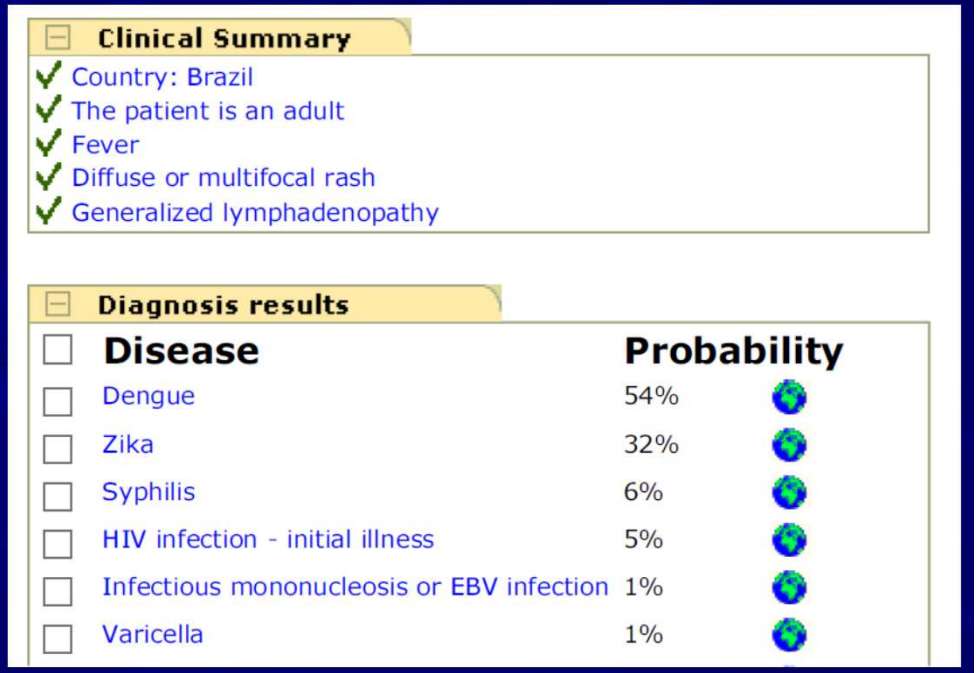


Syphilis is often called "the great imitator" - syphilis rash sometimes can take on the form and look of many other common skin problems (psoriasis, tinea, measles, varicella). However, over 90% of patients present rash, which is almost always characteristic. Secondary lesions of the skin and mucus membranes are highly contagious.

Calculations from GIDEON database.







Other symptoms of secondary syphilis

- Fever, swollen lymph glands, sore throat, patchy hair loss, headaches, weight loss, muscle aches, and fatigue.
- The acute symptoms usually resolve after three to six weeks
- The symptoms of secondary syphilis will go away with or without treatment. However, aprox. one third of patient without treatment will progress tertiary stage of syphilis.
- About 24% of people may present with a recurrence of secondary symptoms.
- Many people who present with secondary syphilis (40–85% of women, 20–65% of men) do not report previously having had the classical chancre of primary syphilis.

Syphilis & HIV infection

- Approximately half of men who have sex with men (MSM) with primary and secondary (P&S) syphilis were also living with HIV.
- In addition, MSM who are HIV-negative and diagnosed with P&S syphilis are more likely to be infected with HIV in the future.
- Genital sores caused by syphilis make it easier to transmit and acquire HIV infection sexually. There is an estimated 2- to 5-fold increased risk of acquiring HIV if exposed to that infection when syphilis is present.
- Furthermore, syphilis and certain other STDs might be indicators of ongoing behaviors and exposures that place a person at greater risk for acquiring HIV.

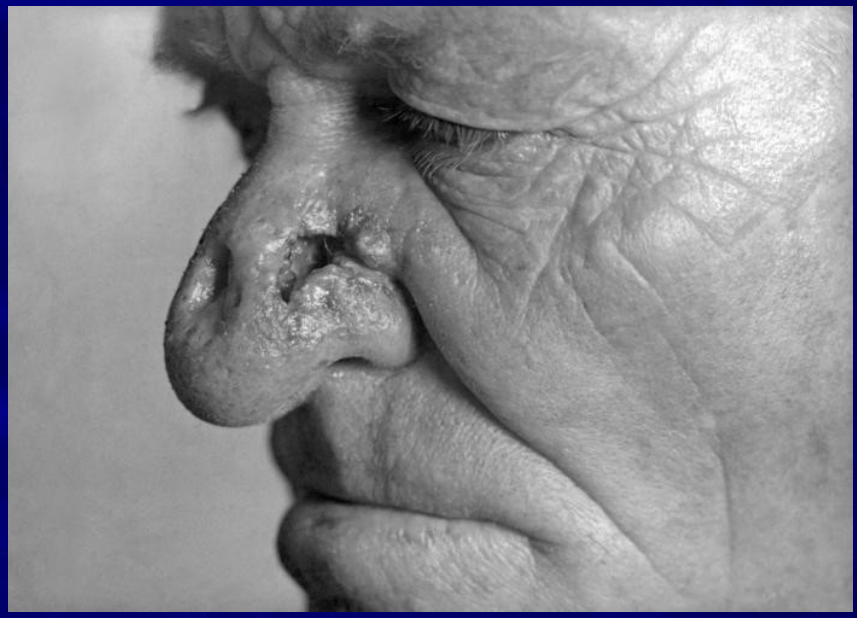
Terciary of Late syphilis

- Late syphilis develops in about 1/3 of untreated cases following an extended period of latency (usually years to decades)

- Late syphilis is characterized by chronic inflammatory lesions (gummas) of skin or bone (even organs)
- Central nervous system and spinal cord involvement
  - dementia, seizures, wasting, etc.
- Cardiovascular involvement
  - disease of aortic valve and thoracic aorta - aneurysma

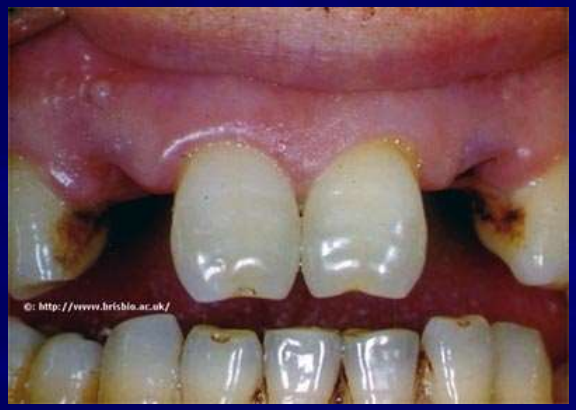
Pathophysiology of tertiary syphilis

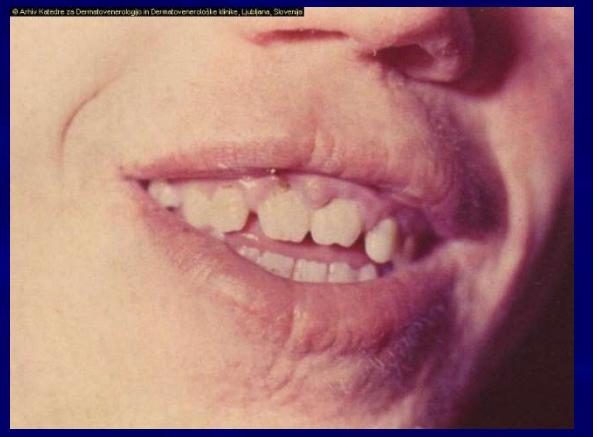
- Gummas are rubbery tumor-like growths that are most likely to involve the skin or long bones but may also develop in the eyes, mucous membranes, throat, liver, or stomach lining. Gummas are increasingly uncommon since the introduction of antibiotics for treating syphilis. Benign late syphilis is usually rapid in onset and responds well to treatment.
- Patients with tertiary syphilis cannot infect others with the disease. It is thought that the symptoms of this stage are a delayed hypersensitivity reaction to the spirochetes.



Congenital syphilis

- Congenital syphilis results from transplacental infection
- Early congenital syphilis (onset, <2 years)
  - stillbirth, fulminant infection, osteochondritis, hepatosplenomegaly, CNS involvement, anemia
- Late manifestations of congenital syphilis (persistent, > 2 years)
  - Interstitial keratitis, deafness, bone and tooth deformities (Hutchinson trias), neurosyphilis





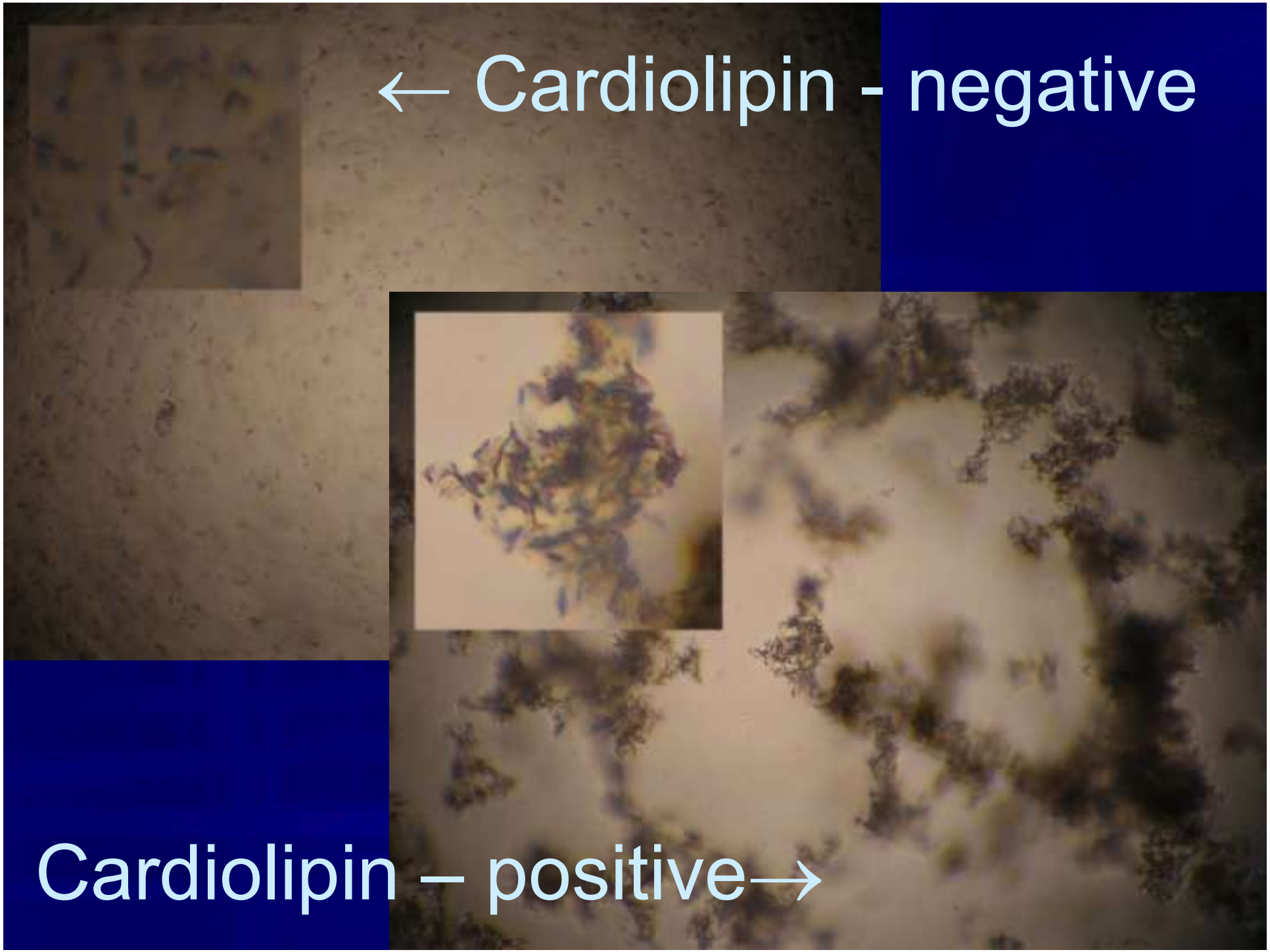
Laboratory diagnostics of syphilis

- Clinical samples – blood, serum, CSF, tissue samples
- Direct methods
  - Darkfield microscopy
  - Direct fluorescent antibody test (DFA-TP)
  - PCR – detection of neurosyphilis from CSF/serum; skin lesions

- Indirect methods – Serologic test - „Gold standard“ for diagnosis
  - Non-treponemal – detect reaginic antibodies that react with phospholipin cardiolipin – used for screening
  - Treponemal - use *T. pallidum* antigens and detect *T. pallidum* antibodies

Non-Treponemal Tests:
Advantages

- NONTREPONEMAL ANTIGEN TESTS. Nontreponemal antigen tests are used as screeners. They measure the presence of reagin (IgM or IgG antibodies), released from damaged host cells or treponemes.
- A sample of the patient's serum is mixed with cardiolipin and cholesterol. If the mixture forms clumps or masses of matter, the test is considered reactive or positive. The serum sample can be diluted several times to determine the concentration of reagin in the patient's blood.
- Flocculation tests – VDRL or rapid plasma reagin (RPR) test


← Cardiolipin - negative
Cardiolipin – positive→

Non-Treponemal Tests:
Limitations

- Results are subjective
  - Intra- and Inter-laboratory variability
- Non-specific
  - False positive results can result from other infectious or non-infectious conditions (autoimmune diseases, pregnancy, etc)
    - EBV, Lupus, etc.
- Limited sensitivity in early/primary syphilis and in late/latent syphilis
- Low throughput
  - Problematic for high volume laboratories

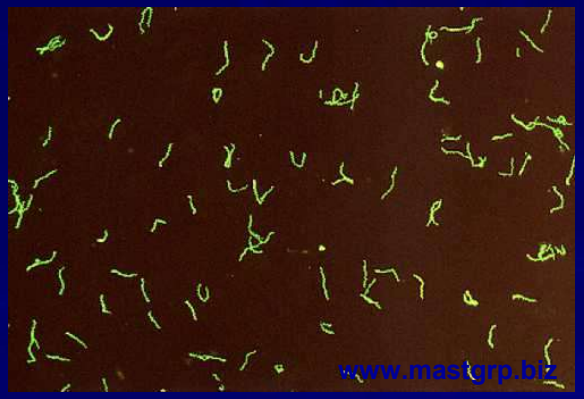
Serologic Tests for Syphilis:
Treponemal Assays

- To distinguish true or flase-positive nontreponemal tests results and to diagnose late syphilis
- Principle:
  - Infection leads to production of specific antibodies directed against *T. pallidum*
- Treponemal tests detect IgG or total IgM/IgG antibodies directed against *T. pallidum*

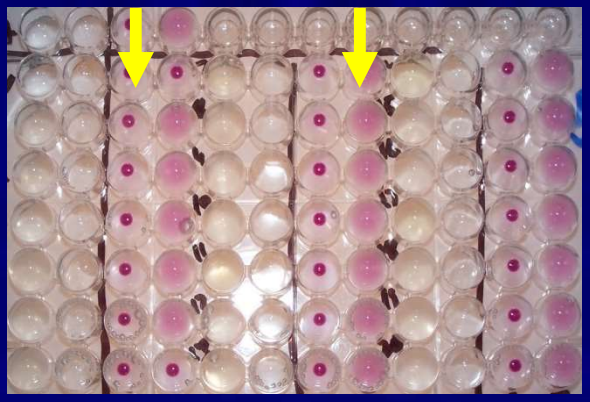
Serologic Tests for Syphilis:
Treponemal Assays

- Fluorescent treponemal antibody (FTA-ABS)
- ***Treponema pallidum* particle agglutination (TP-PA)**
- Enzyme Immunoassay (EIA)

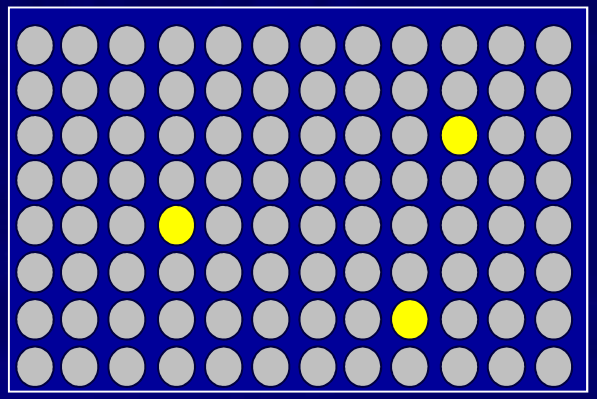
FTA-ABS



TP-PA



Conventional EIA



Yellow wells = positive

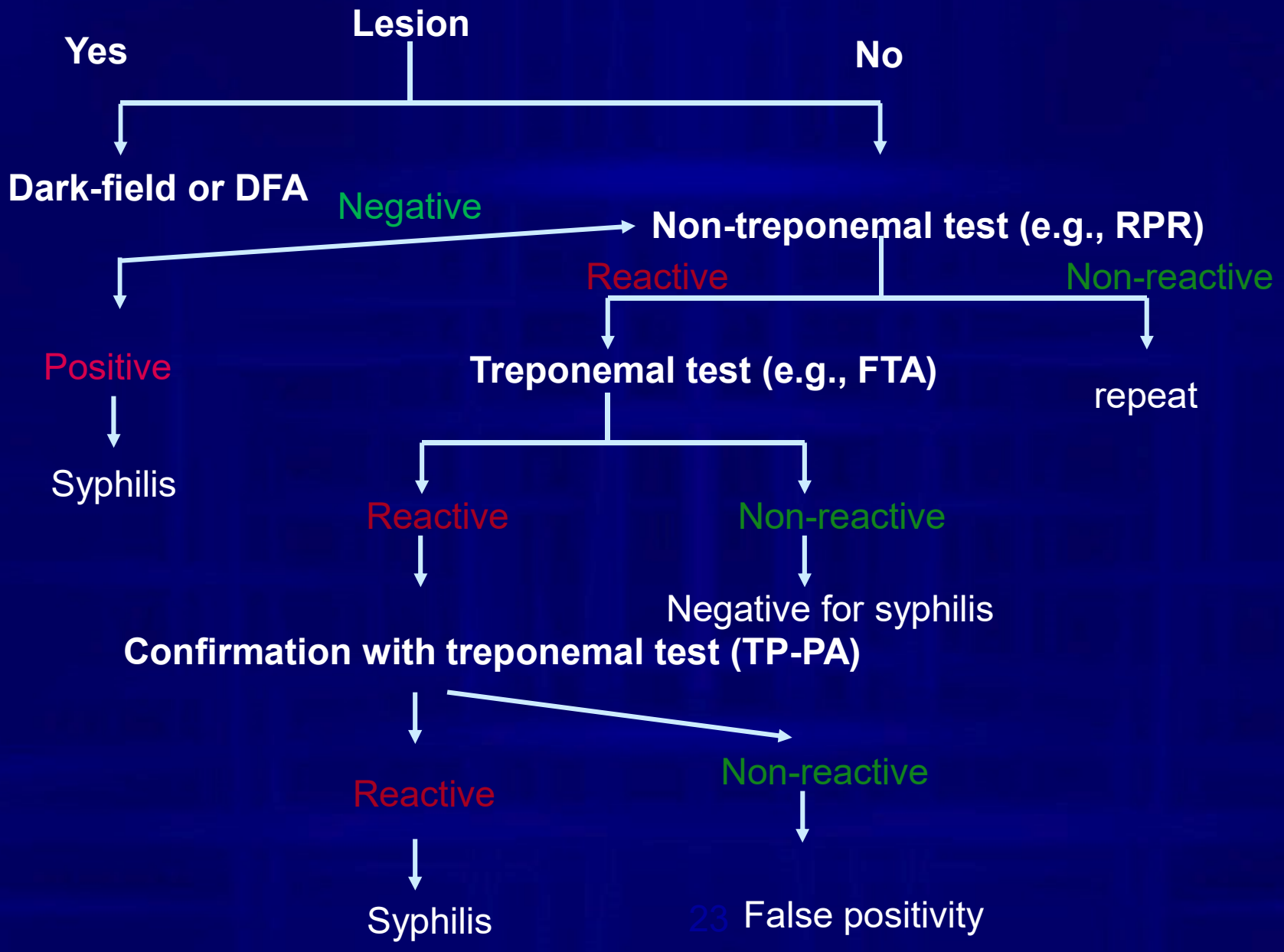
Treponemal Assays:
Advantages

- High Specificity
- Possibly higher sensitivity during early and late syphilis stages compared to non-treponemal tests
- Newer Methods
  - Objective result interpretation
  - Automation option
  - High throughput
  - High reproducibility/precision

Treponemal Assays:
Limitations

- Remain positive despite treatment
  - **Cannot** be used to monitor response to therapy
- Conventional Methods
  - Subjective interpretation requiring technician expertise to read
- Newer Methods
  - Expensive instrumentation
  - Higher cost/test

Routine screening scheme for early syphilis



Treatment for syphilis

- The first-line treatment for uncomplicated syphilis remains a single dose of intramuscular benzathine benzylpenicillin

- For neurosyphilis, due to the poor penetration of benzathine penicillin into the central nervous system, those affected are given large doses of intravenous penicillin for a minimum of 10 days.

- After appropriate treatment, syphilis does not recur. However, having syphilis once does not protect a person from becoming infected again. Even following successful treatment, people can be reinfected. Patients with signs or symptoms that persist or recur or who have a sustained fourfold increase in nontreponemal test titer probably failed treatment or were reinfected. These patients should be retreated.

Borrelia

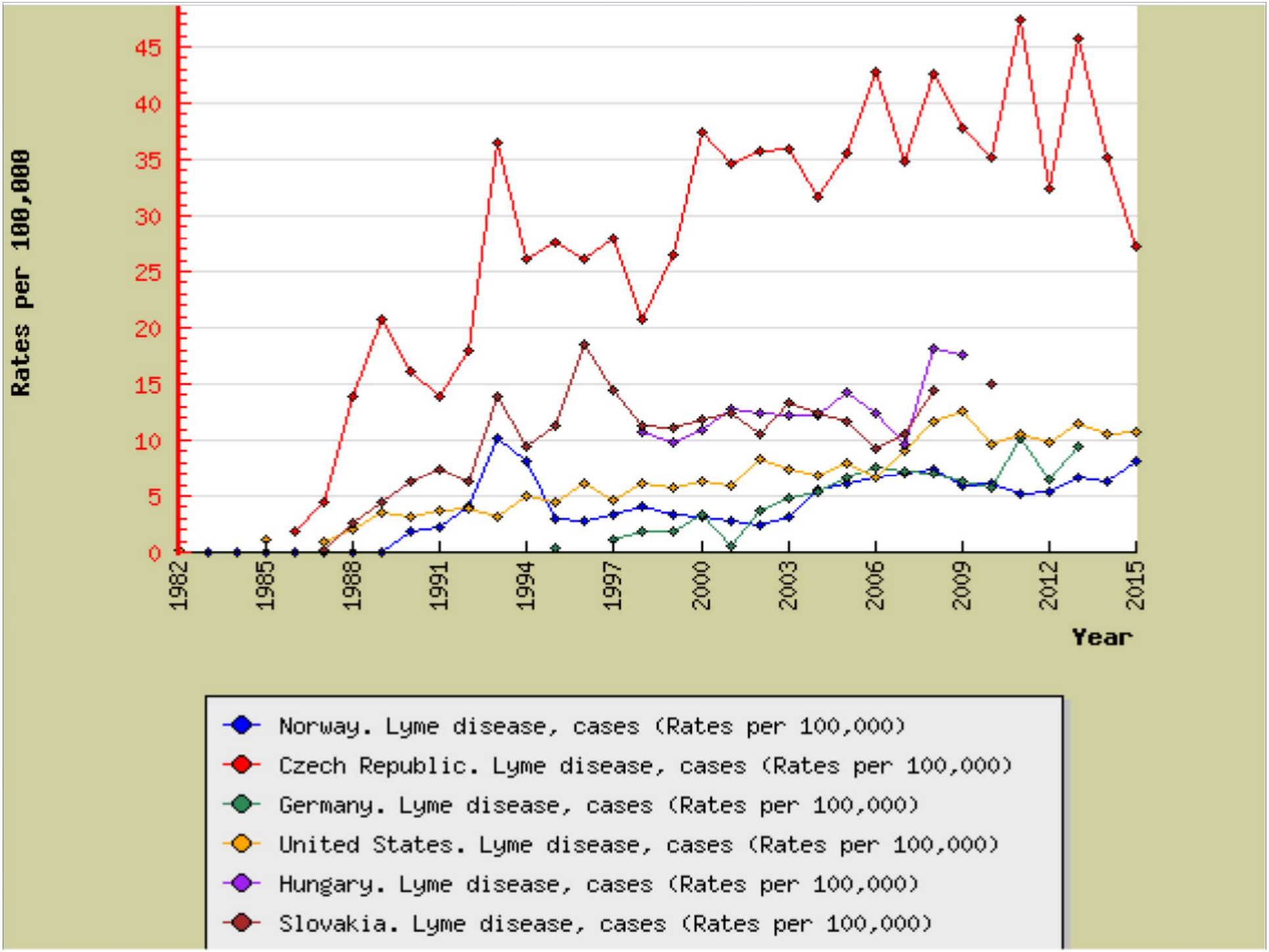
- Borreliae are similar in length (8 – 30 μm) but wider (0,2 – 0,5 μm) than other human-pathogenic spirochetes (treponemes and leptospires)
- Highly motile – corkscrew motility, endoflagella (7 – 20 per terminus)
- Borreliae grow slowly under microaerophilic or anaerobic conditions

Borrelia

Beware differencies in epidemiology:

-Relapsing fever – source of infection is human, it is epidemic disease (- *B. recurrentis*), vector is human body louse

- Lyme disease – source of infection are small rodents, is is endemic disease (- *B. hermsii, B.burgdorferi*) , vector is *Ixodes* tick

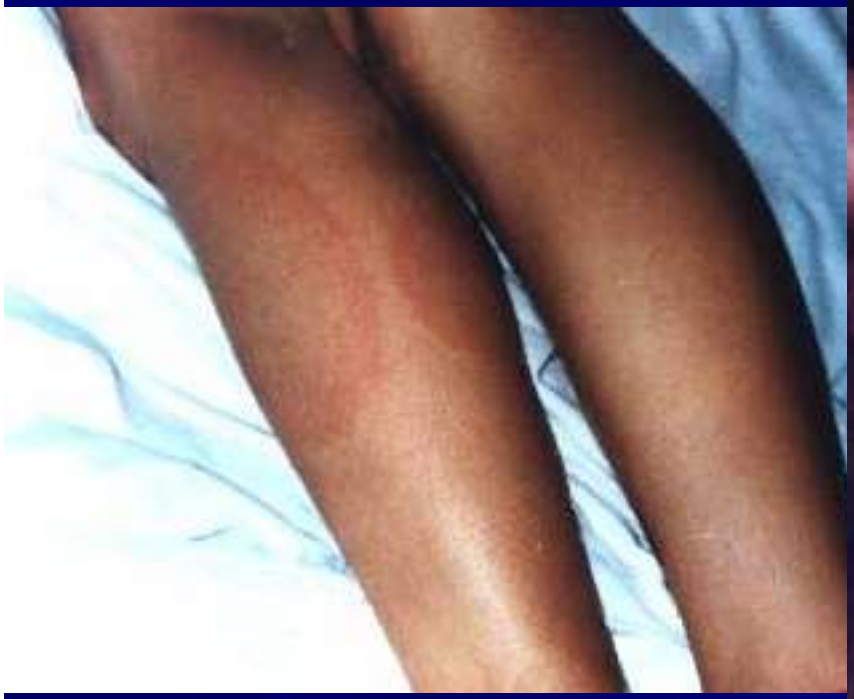

Rates per 100,000
0
5
10
15
20
25
30
35
40
45
1982
1985
1988
1991
1994
1997
2000
2003
2006
2009
2012
2015
Year
Norway. Lyme disease, cases (Rates per 100,000)
Czech Republic. Lyme disease, cases (Rates per 100,000)
Germany. Lyme disease, cases (Rates per 100,000)
United States. Lyme disease, cases (Rates per 100,000)
Hungary. Lyme disease, cases (Rates per 100,000)
Slovakia. Lyme disease, cases (Rates per 100,000)

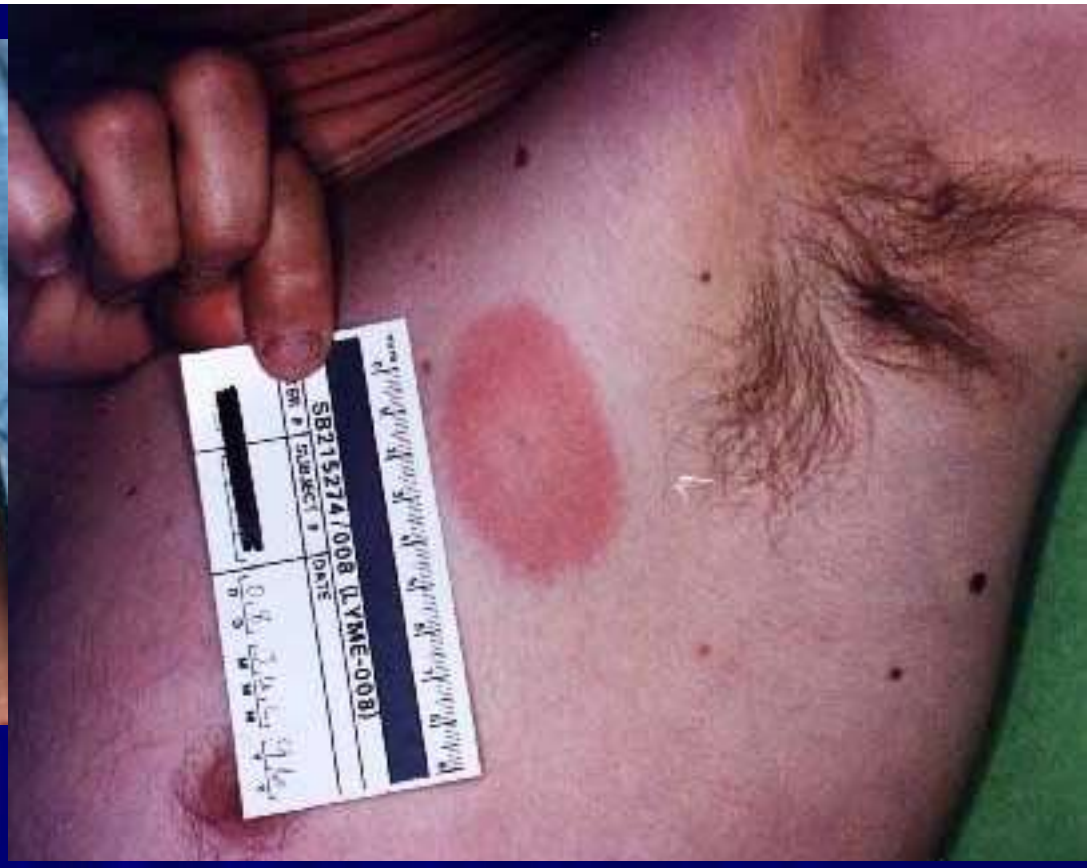
Lyme disease

- *Borrelia burgdorferi* sensu stricto – North America
- *Borrelia garinii* – Europe (CNS isolates)
- *Borrelia afzelii* – Europe (skin isolates)
- The term used to collectively describe all three genospecies is *Borrelia burgdorferi* sensu lato
- Genome of borreliae is composed of small linear chromosome (1,000 kb) and 21 plasmids (linear and circular)
- It has low G+C content (30mol%)
- Lacks mosts cellular biosynthetic pathways but has a number gener that encode putative lipoproteins (essential for life cycle of spirochetes)
- Major outer surface proteins **OspA**, **OspB, and OspC** (encoded by plasmid) – play role in diversity and immune evasion of Lyme disease borreliae

Lyme disease

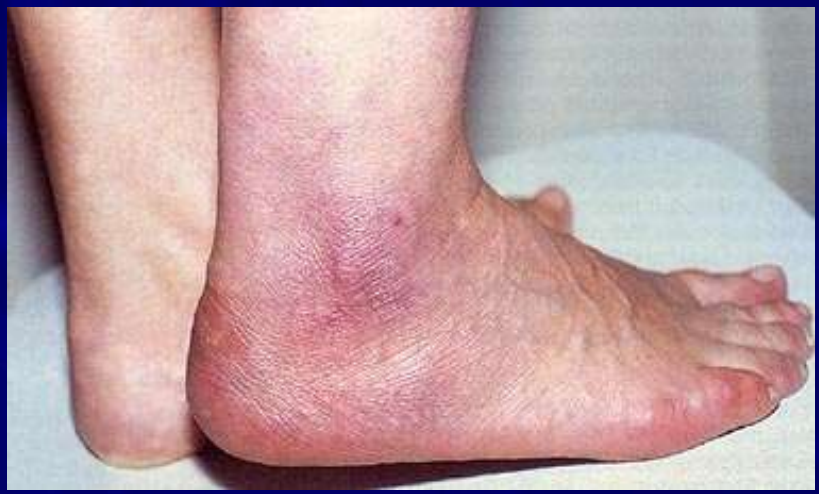
- Stage one (early infection). The early stage of Lyme disease is often characterized by a distinctive, expanding red rash that usually develops at the site of the tick bite. This rash, known as **erythema migrans**, is seen in 60-90% of infected individuals (it is important to remember that the converse is true: no rash is ever observed in 20-40% of the cases)
- Stage two (dissemination stage) occurs days to weeks following infection. At this stage the spirochetes spread hematogenously to additional body tissues. One or more of the following symptoms and signs may be noted:
  - fatigue
  - chills and fever
  - headache
  - muscle and joint pain
  - Meningitis/facial nerve palsy
  - Carditis
  - Borrelial lymphocytoma
- Stage three (late disease). Some symptoms and signs of Lyme disease may not appear until weeks, months, or years after a tick bite.
  - Lyme arthritis (mono or oligoarticular – knee)
  - Acrodermatitis chronica atrophicans
  - Chronic neuroborreliosis – parapareses/tetrapareses





Erythema migrans ↑→

Acrodermatitis chronica atrophicans →

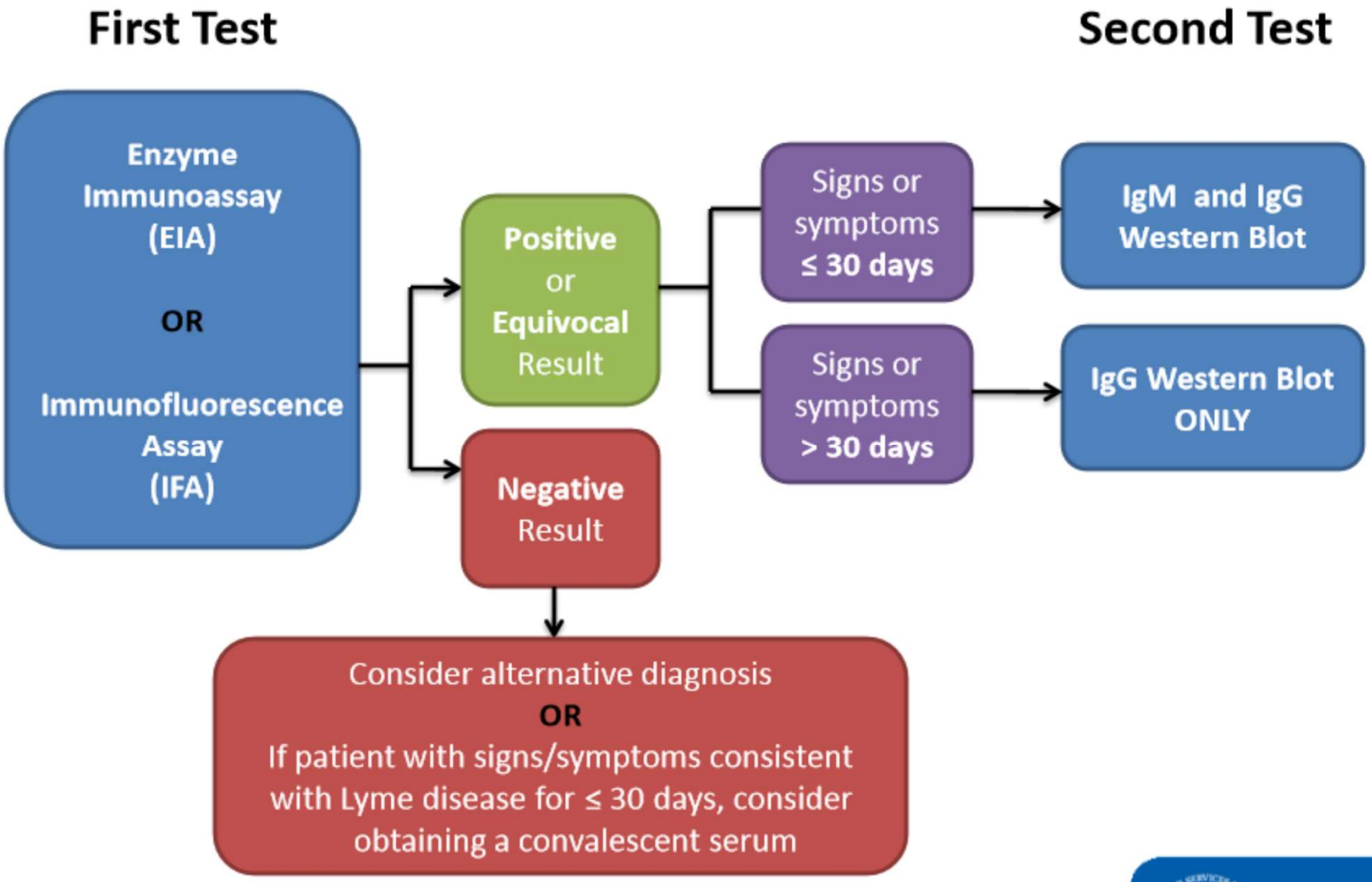


Borrrelial lymphocytoma→

Laboratory diagnostics of Lyme disease

- Clinical samples – blood/serum, CSF, synovial fluid, skin biopsy
- Diagnosis rely on antibody detection to OspC and/or direct pathogen detection (PCR)
- **Two-step approach in serodiagnosis**
  - The first step uses a testing procedure called “EIA” (enzyme immunoassay) or rarely, an “IFA” (indirect immunofluorescence assay). If this first step is negative, no further testing of the specimen is recommended. If the first step is positive or indeterminate (sometimes called "equivocal"), the second step should be performed.
  - The second step uses a test called an immunoblot test, commonly, a “Western blot” test. Results are considered positive only if the EIA/IFA and the immunoblot are both positive.
- Detection of intrathecally produced antibodies – diagnosis of neuroborreliosis

Two-Tiered Testing for Lyme Disease



Antibiotic therapy

- Choise of antibiotic, dosage, route, and duration of therapy rely on stage of disease
- I. Stage (erythema migrans) – oral treatment with amoxicillin or doxycyklin
- II. + III. Stage – disseminated infections (including neuroborreliosis) benzylpenicillin, ceftriaxon

Leptospira

- Spiral-shaped bacteria with hooked ends
- Obligate aerobes, optimal growth temperature is 28 to 30°C on enriched medium (fatty acids, vitamins B, ammonium salts)
- Two species:
  - *L. interrogans* – all pathogenic species
  - *L. biflexa* – saprophytic strains
- Divided to serovars (more than 200 serovars of *L. interrogans*) grouped to serogroups

Leptospira - epidemiology

- Ubiquitous, free-living in water or associated with renal infections of animals
- Leptospirosis is widespread zoonosis
- Source of infection
  - Direct or indirect contact with the urine of infected animal
- Disease is seasonal (summer peak), higher in warm climate regions
- Rodents are reservoirs – infections is transferred to domestic farm animals, dogs, and humans
- Human infections – occupational (farmers, veterinarians..), recreational (water sports)

Leptospira – clinical significance

- Portal of entry – skin abrasions or conjunctiva
- Majority of infections are subclinical
- **Biphasic disease:**
  - Septicemic phase (1 week)
  - Immune phase – excrection of leptospires in the urine
- **Typical signs:** febrile illness of sudden onset, headache, myalgia, abdominal pain, conjunctival suffusion, aseptic meningitis (25% of cases)
- 5 – 10% of patients develop icteric form (Weil's disease)
- **Complications** – renal failure, pulmonary hemorrhage, arrythmias
- Mortality rate between 5 – 15%

Laboratory diagnosis

- Clinical specimens: blood, CSF, peritoneal fluid, urine, serum
- Direct tests: microscopy (dark-field), PCR
- Serological testing: MAT – agglutination of patients sera with killed leptospiral serovars – detection in dark-field microscopy or IgM EIA tests

- Therapy: benzylpenicillin