آزمایشهای سیفیلیس در جامعه ی کم سیفیلیس

Current Application of
Treponemal Tests in a Σ low-prevalent community!

A bit about the

A.P.L.S. and

Spirochetal Diseases

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A GLANCE @
IMMUNOSEROLOGY

(for the ISCLS)

- **Part 1-** ABAN, 3, 1400

  کلیات ایمونولوژی عفونی و مصونیت بخشی

- **Part 2-** ABAN, 10, 1400

  در باب آزمایشهای سیفیلیس در جامعه کم سیفیلیس

  (کاربردهای امروزی آزمایشهای اسپیروکتی)

- **Part 3-** ABAN, ? , 1400

  ایمونوسرولوژی سایر بیماریهای عفونی!

  (تستهای تشخیصی عفونتهای رایج غیر از کووید)

Our Agenda 2day:

- Brief Microbiology of Spirochetes
- Lab. Dx of Spirochetal Diseases
- More focus on the Serologic Tests for the Lues

- Anti Phospholipid Syndrome

Spirochetes

- The spirochetes are a large, heterogeneous group of spiral, motile bacteria.

- One **family,** Spirochaetaceae, (previousely classified as Treponemataceae) of the **order** Spirochaetales consists of two **genera** whose members are human pathogens:

✓ ***Treponema*** (causing kooft!) *and*

✓ ***Borrelia*** (causing Tab-e Raje'eh)**.**

- *The other family (Leptospiraceae)* includes one genus of medical importance:

✓ ***Leptospira*** (causing Tab-e Shaleezar).

Taxonomy

Phylum
Spirochaetes
Class
Spirochaetia
Order
Spirochaetales
Families
Spirochaetaceae (& Leptospiraceae)
Geneus
Treponema (& Leptospira)

Spirochetes Pathogenic for Humans

Genus	Axial Filaments	Insertion Disks	Size in um (& # of Coils)	Morphology	توصیف میکروسکوپی
Borrelia	30-40	2	**10-20 x 0.5**		مار
Treponema	6-10	1	**6-15 x 0.2 (8-14)**		فنر خودکار
Leptospira	2 (با قلاب)	3-5	**6-20 x 0.1**		نخ گونی عطار

Spirochetes

- Spirochetes are gram-negative, slender corkscrew-shaped bacteria with axial periplasmic flagella wound around a helical protoplasm.
- The bacteria are covered in a membrane called an outer sheath, which may mask bacterial antigens from the host immune response.
- *Treponema pallidum* subsp. *pallidum* *is the microaerophilic spirochete that* causes syphilis.
- Other closely related treponemes cause:
  - **Bejel or endemic syphilis** *(Treponema endemicum) and*
  - **Pinta** *(Treponema pallidum subsp. carateum) and*
  - **yaws** *(Treponema pallidum subsp. pertenue)*
- *T. pallidum subsp. pallidum,* usually referred to simply as *T.pallidum, is too slender to be seen* in Gram stain, but it can be visualized by silver stains and immunofluorescence technique.

A (defective!) table about *Treponema*-Associated Diseases

Bacteria	Associated Disease
T. pallidum	Syphilis
T. pallidum (variant)	Bejel
T. pertenue	Yaws
T. carateum	Pinta

Introduction

Human disease caused by spirochetes typically follows a clinical course that reflects three sequential phenomena:

(1) early, local proliferation of the organisms at the site of entry,

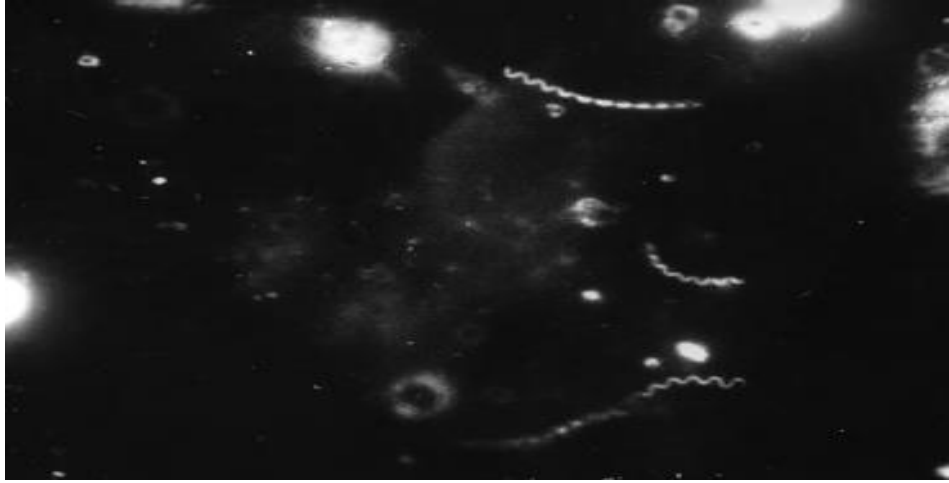
(2) spirochetemia with systemic dissemination, and

(3) persistence of small numbers of microbes at various, often immune, “privileged”sites.

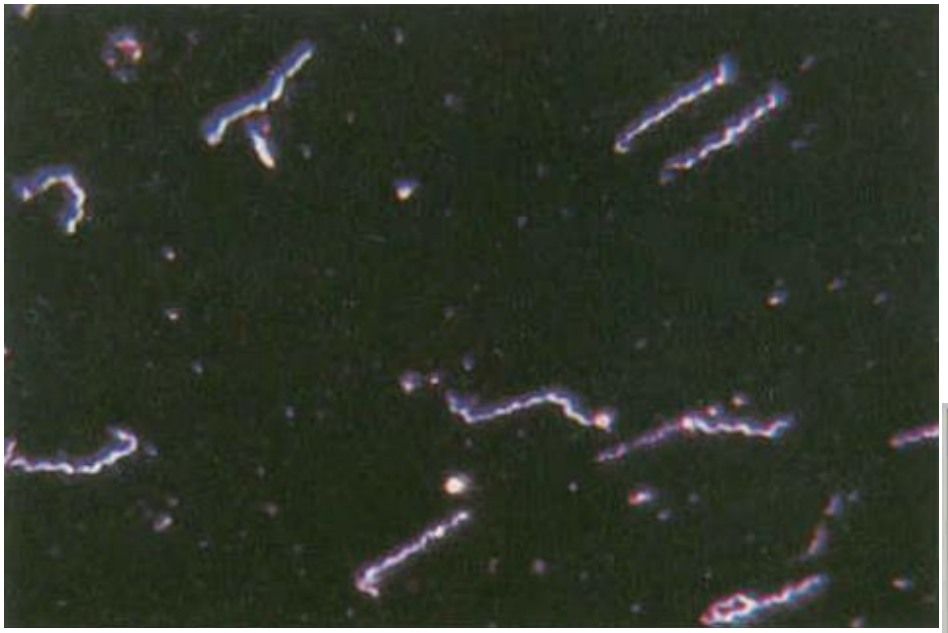
- Direct detection of pathogenic spirochetes is sometimes possible by microbiologic culture, microscopy, or genomic amplification, but diagnosis more often relies upon the demonstration of a patient’s **serologic response** to the offending agent.
- **Serologic detection of syphilis**, by far the most common mode of diagnosis, comprises methods that semi-quantitatively measure **antibody** to various lipoproteins, as well as methods that detect antibody to **treponeme-specific antigens**. Serologic testing algorithms may begin with a **non-treponemal** assay, or with a **treponeme specific** assay (referred to as the ***reverse algorithm***).

In the History

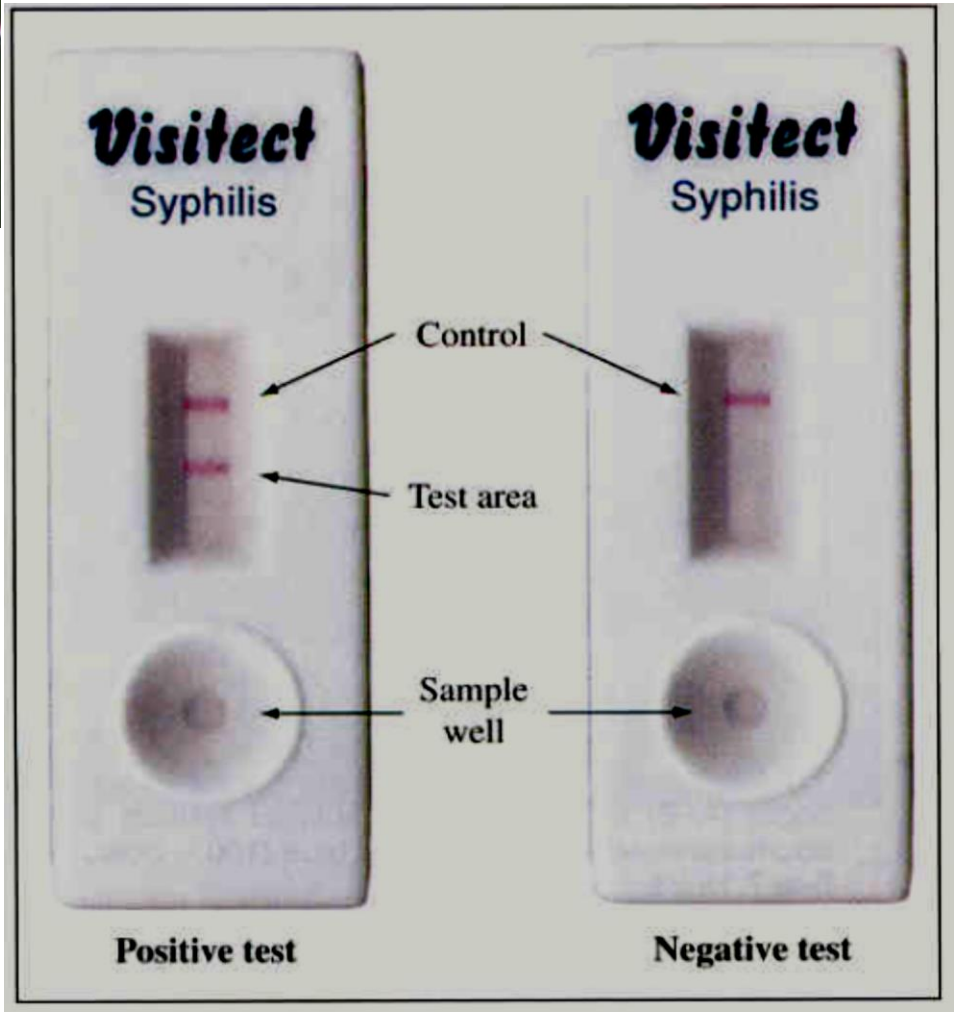
- Over the centuries, Europeans have had to contend with four pox diseases:
  - chickenpox,
  - cowpox,
  - smallpox, and
  - the Great Pox, a disease now known as syphilis.
- The first European epidemic was recorded in the late 1400s, shortly after the conquest of Naples by the French army.
- For decades the disease had various names, but by the 1700s, it had come to be called **syphilis.**
- Syphilis is currently ranked among the top five most reported microbial diseases in the United States.
- Statistics indicate more than 40,000 people are afflicted with the disease annually and the number has been rising since 2000. Twelve million cases are reported each year worldwide.
- Taken alone, these figures suggest the magnitude of the syphilis epidemic, but some public health microbiologists believe for every case reported, as many as nine cases go unreported.



- Syphilis is caused by ***Treponema pallidum***.
  (*trepo = "turn"; nema = "thread"; pallid = "pale"; thus* literally "pale turning thread").
- This spirochete moves by means of endoflagella.
- Humans are the only host for *T. pallidum, so the* organism must spread by direct human-to-human contact, usually during sexual intercourse.
- It penetrates the skin surface through the mucous membranes of the genitalia or via a wound, abrasion, or hair follicle.
- The variety of clinical symptoms accompanying the stages, and their similarity to other diseases, have led some physicians to call syphilis the "great imitator."
- Untreated, the disease can progress through a number of stages.



T. Pallidum in chancre fluid
as seen by DFM
using the 40x objective

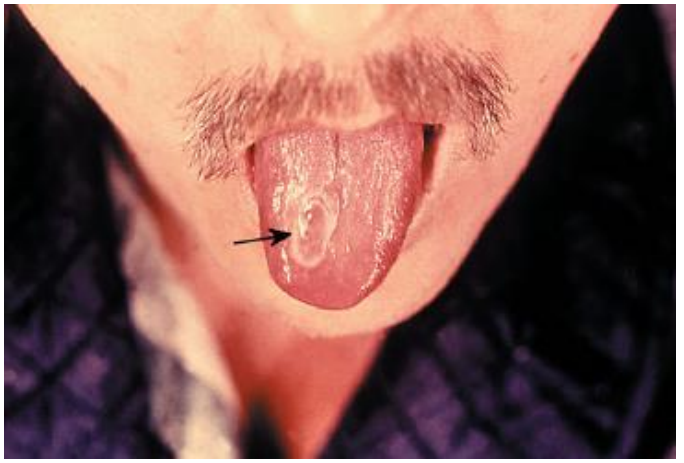


An Immunochromatographic
Rapid POC Test
for syphilis

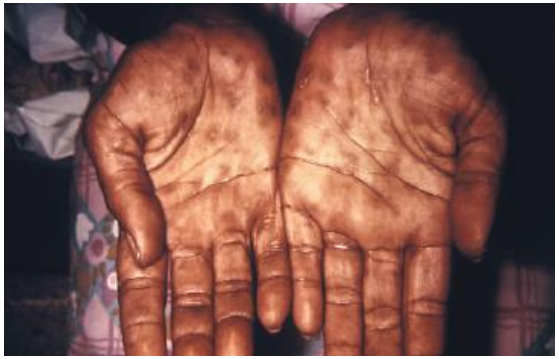
Courses of the 3 or 4 Syphilitic Stages:

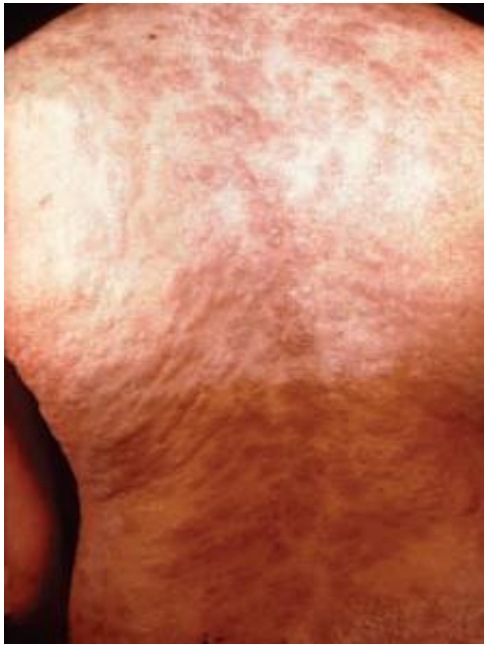
- The **incubation period** for syphilis varies greatly, but it averages about **3 weeks**.
- **Primary syphilis** **is the first stage of the disease**. It is characterized by a lesion, called a **chancre**, which is a painless circular, purplish ulcer with a small, raised margin with hard edges.
- The chancre develops at the site of entry of the spirochetes, often the genital organs. However, any area of the skin may be affected, including the pharynx, rectum, or lips.
- The chancre teems with spirochetes and represents the stage that is **most infectious**.
- Spirochetes are plentiful within the chancre and spread from there throughout the body by hematologic and lymphatic dissemination.
- The chancre persists for **three to six weeks**, and then heals **spontaneously**. However, the infection has not been eliminated, as the spirochetes have spread through the blood and lymph to other body organs.

secondary syphilis



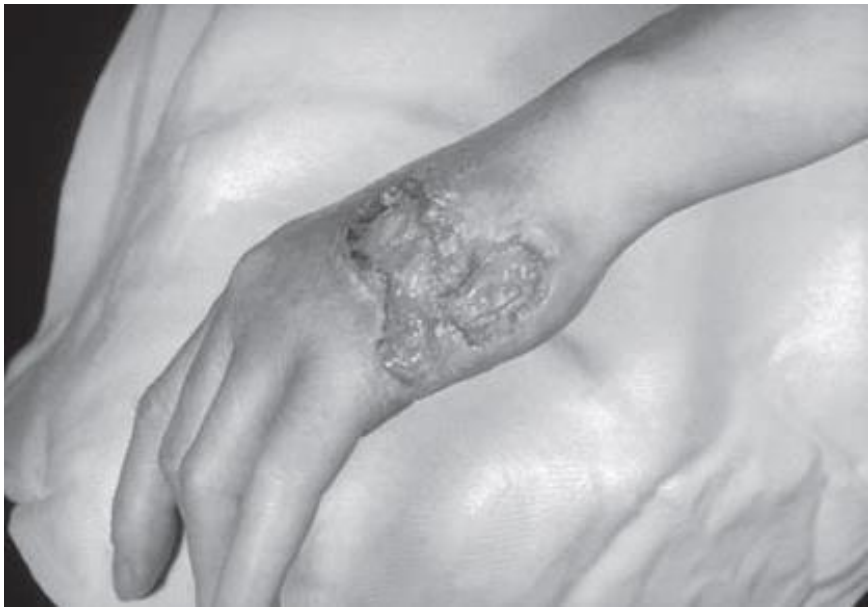
- **Several (2-10) weeks** after the chancre has healed, ~70% of the untreated patients experiences **secondary syphilis.**
- Symptoms include fever and a flu-like illness as well as swollen lymph nodes and condylomas. The **skin rash** that develops, which may be mistaken for measles, rubella, or chickenpox, appears as reddish-brown spots on the palms, soles, face, and scalp.
- Transmission can occur if there are moist lesions.
- Loss of the eyebrows often occurs, and a patchy loss of hair results in“moth-eaten” areas commonly seen on the head.
- Involvement of the liver may lead to jaundice and suspicion of hepatitis.

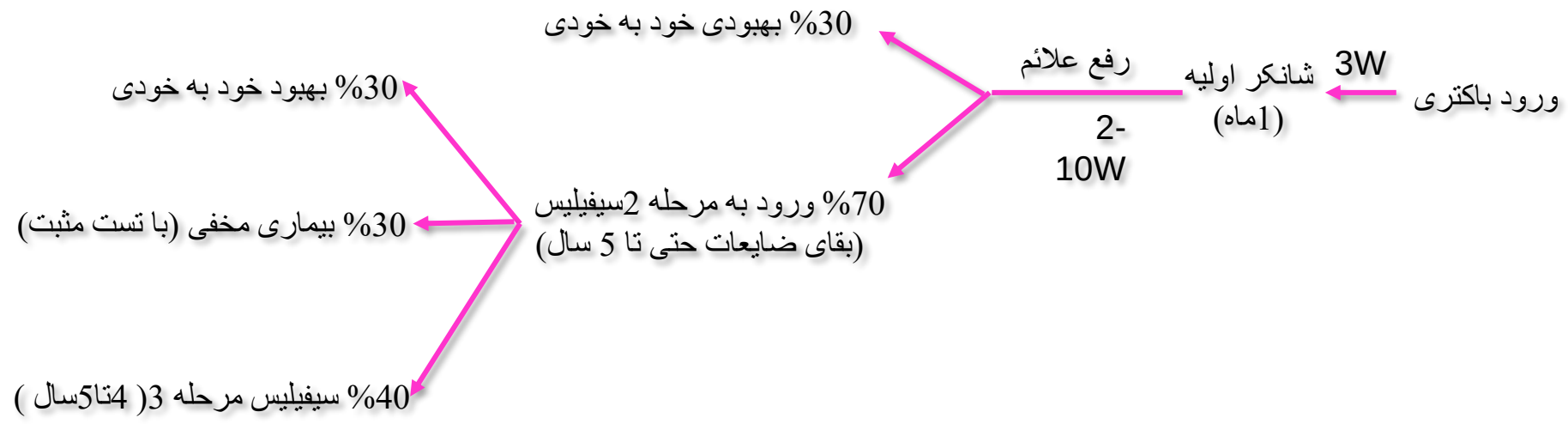




Latent Syphilis; Tertiary syphilis

- In untreated patients, the symptoms resolve after **several weeks**. Most patients recover, but they bear pitted scars from the healed lesions and remain "pockmarked.
- These individuals now enter a **latent period** that can last **several years**.
- Many patients will have **relapses of secondary syphilis** during which time they remain infectious.
- Within **4-5 years**, the relapses cease and the disease is no longer infectious (except in pregnant females).
- **Tertiary syphilis or Late syphilis** (Lues Tarda) occurs in one third of untreated patients, usually after a **latent period** of **5 years or more**. ***(cf. late vs latent!)***
- Patients either remain asymptomatic or slowly progress to the **3rd stage**.
- About 40% of untreated patients eventually develop **tertiary syphilis.**
- **The stage 3 occurs in many** forms, but most commonly it involves the skin, skeletal, or heart and CNS.
- The hallmark of tertiary syphilis is the **gumma,** a soft, painless, gummy noninfectious granular lesion.




ورود باکتری
3W
شانکر اولیه
(1ماه)
رفع علائم
2-
10W
30% بهبودی خود به خودی
70% ورود به مرحله 2سیفیلیس
(بقای ضایعات حتی تا 5 سال)
30% بهبود خود به خودی
30% بیماری مخفی (با تست مثبت)
40% سیفیلیس مرحله 3(4تا5سال)

آزمون ایمونوسرولوژی بیماریهای اسپیروکتال
برای انجمن متخصصین آزمایشگاه
(مبحث دوم- دوشنبه 10 آبان 1400)

1	سوال: با نمونه برداری و آزمایش کدامیک از ضایعات سیفیلیسی زیر احتمال مشاهده ترپونم بسیار کم است؟	
	الف	شانکر
	ب	جوش پوستی
	ج	کوندیلوم
	د	گوم
منبع:	Baily & Scott Diagnostic Microbiology, 2022, ed 15	

آزمون ایمونوسرولوژی بیماریهای اسپیروکتال
برای انجمن متخصصین آزمایشگاه
(مبحث دوم- دوشنبه 10 آبان 1400)

12	سوال: با نمونه برداری و آزمایش کدامیک از ضایعات سیفیلیسی زیر احتمال مشاهده ترپونم بسیار کم است؟	
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Clinical Classification of Syphilis course

Stage	Description	Symptoms and Signs
Acquired		
Primary	Contagious	Chancre (a small, usually painless skin sore), regional lymphadenopathy
Secondary	Contagious Occurs weeks to months after the primary stage	Rashes (which may be confused with those due to several other disorders), sores on mucous membranes, hair loss, fever, many other symptoms
Latent	Asymptomatic; generally not contagious May persist indefinitely or be followed by late-stage disease	**Early latent syphilis** (infection < 1 year duration), sometimes with recurrence of infectious lesions **Late latent syphilis** (infection ≥ 1 year duration), rarely with recurrences; positive serologic tests
Late or tertiary	Symptomatic; not contagious	Clinically classified as benign tertiary syphilis, cardiovascular syphilis, or neurosyphilis (eg, asymptomatic, meningovascular, or parenchymatous neurosyphilis; tabes dorsalis)
Congenital*		
Early	Symptomatic Occurring up to age 2 years	Overt disease
Late	Symptomatic Occurring later in life	Hutchinson teeth, eye or bone abnormalities

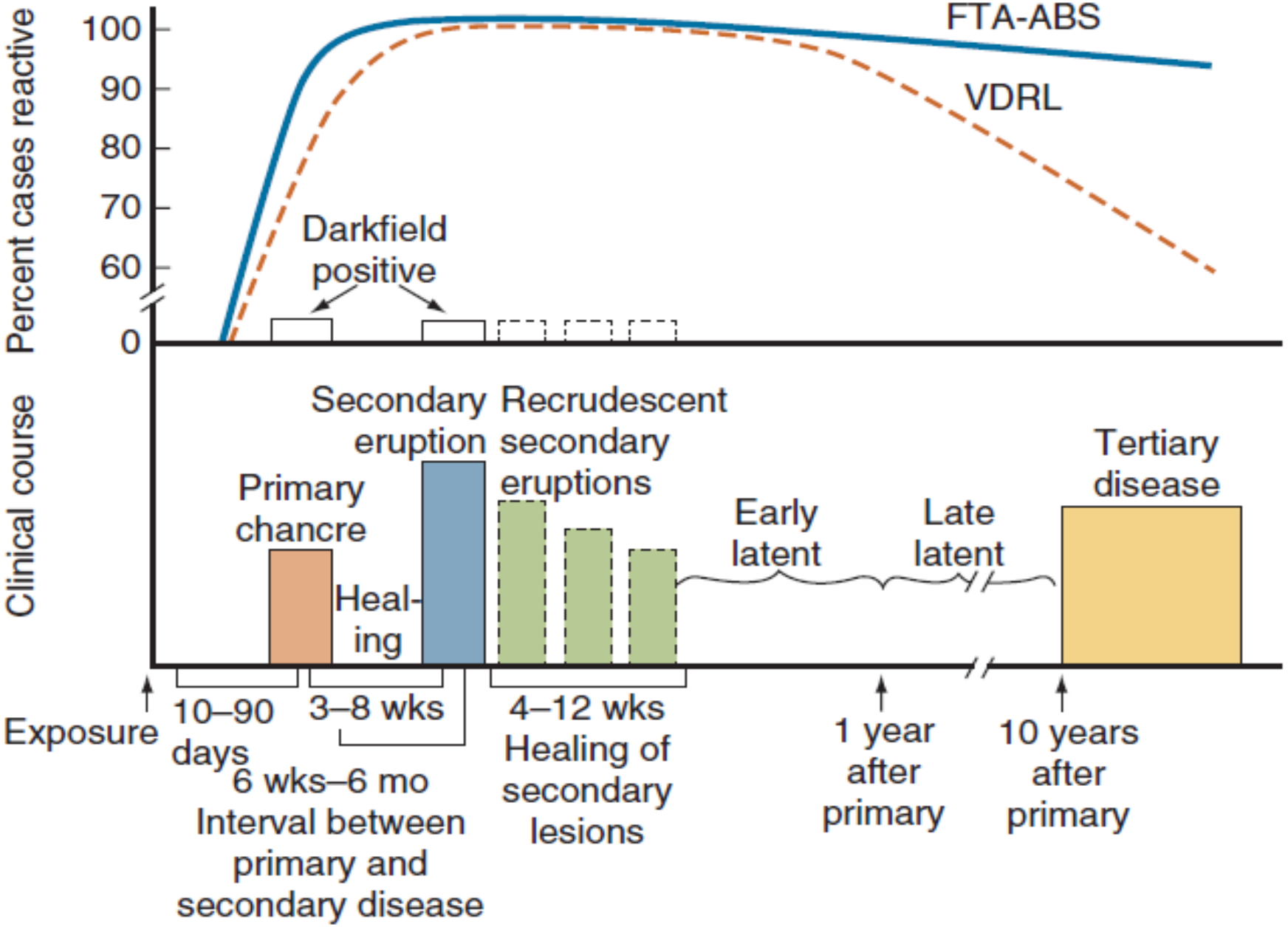
* Can also exist in a permanently latent (asymptomatic) state.

These stages may overlap or present atypically in the setting of HIV coinfection

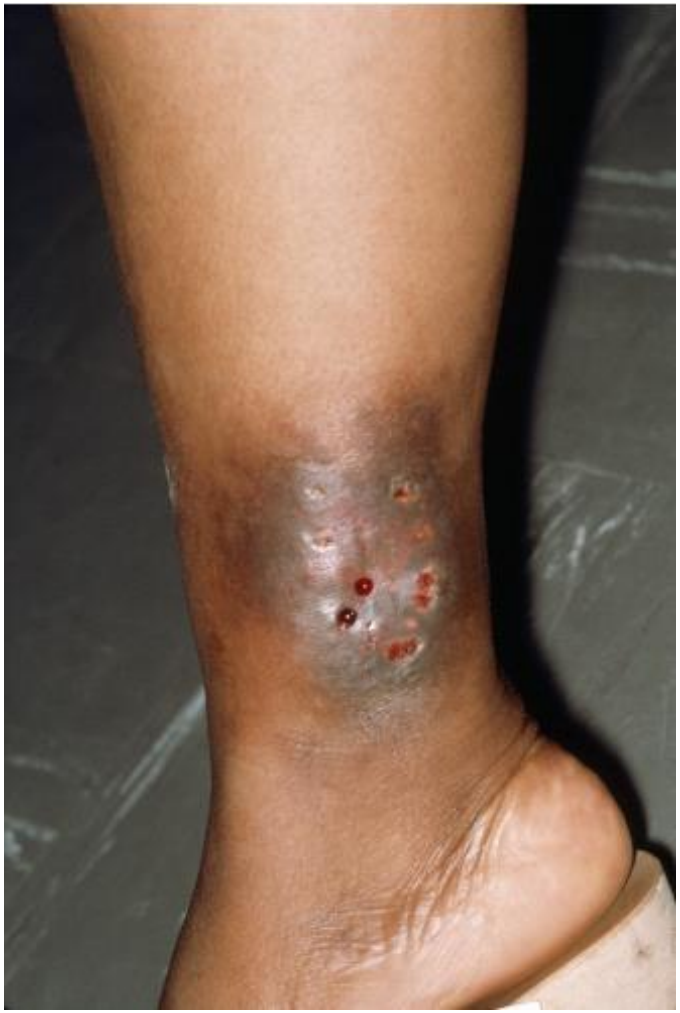
Another sample table for Stages of Syphilis

Phase or Stage	Features and Comments	Test
Incubating phase	The incubation period usually lasts ≈3 wk but can range from 10-90 days.	Laboratory examination
Primary stage	• During the primary stage, a painless chancre develops at the site where the bacteria entered the body. • A person is highly contagious during the primary stage. • The chancre lasts 28-42 days and heals without treatment.	Darkfield examination
Secondary stage	• This is characterized by a rash that appears from 2-8 wk after the chancre develops. • A person is highly contagious during the secondary stage. • A rash often develops all over the body, including palms of the hands and the soles of the feet. The rash usually heals without scarring in 2-12 wk. • Open sores may be present on mucous membranes and may contain pus (condyloma lata). • Symptoms can include nervous system abnormalities.	• RPR or VDRL • TP-PA used to confirm a syphilis infection after another method tests positive for syphilis. It can be used to detect syphilis in all stages, except during the first 3-4 wk. This test is not done on spinal fluid. • FTA-ABS test detects syphilis except during the first 3-4 wk after exposure to syphilis bacteria. It is more difficult to perform and may be used to confirm a syphilis infection after another method tests positive for the syphilis bacteria. It can be done on a sample of blood or cerebrospinal fluid. CSF
Latent (hidden) stage	• If untreated, an infected person will progress to the latent (hidden) stage of syphilis with no symptoms (latent period). • The latent period may be as brief as 1 yr or range from 5-20 yr. • A person is contagious during the early part of the latent stage and may be contagious during the latent period.	
Relapses of secondary syphilis	• About 20%-30% of people with syphilis have a relapse of the secondary stage of syphilis during the latent stage. • A relapse means that the person had passed through the second stage, was symptom-free, then began to reexperience secondary stage symptoms. Relapses can occur several times. • When relapses no longer occur, a person is not contagious through contact. • A woman in the latent stage of syphilis may still pass the disease to her unborn baby and may have a miscarriage, a stillbirth, or give birth to a baby infected with congenital syphilis.	• Nontreponemal tests measure IgM and IgG antibodies. • It is best for testing for reinfection.
Tertiary (late) stage	• Most destructive stage of syphilis • If untreated, the tertiary stage may begin as early as 1 yr after infection or at any time during a person's lifetime. A person may never experience this stage of the illness. • The symptoms of tertiary (late) syphilis depend on the complications that develop—gummata, large sores inside the body or on the skin, cardiovascular syphilis, or neurosyphilis.	• VDRL on cerebrospinal fluid (CSF) with concurrent RPR serum • If RPR is negative and a high index of suspicion for neurosyphilis remains, perform FTA-Abs on serum. • Some patients have nonreactive nontreponemal tests in late neurosyphilis.

The natural course of untreated syphilis

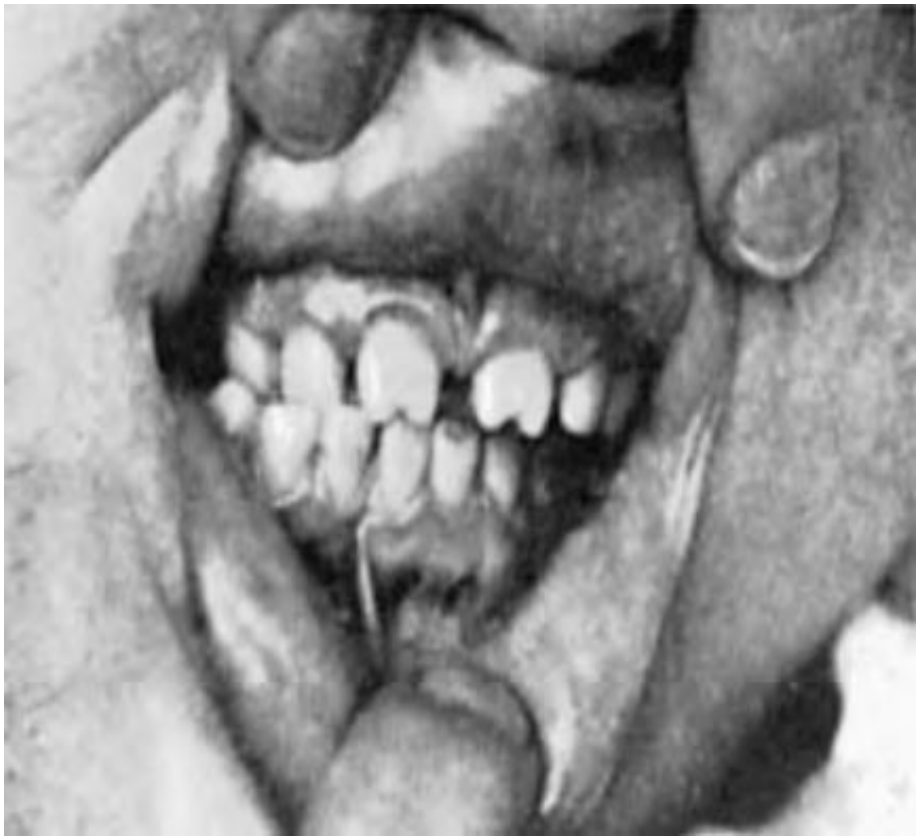


A gumma (as a mass of inflamed tissue) on the leg of a patient with tertiary (late) syphilis

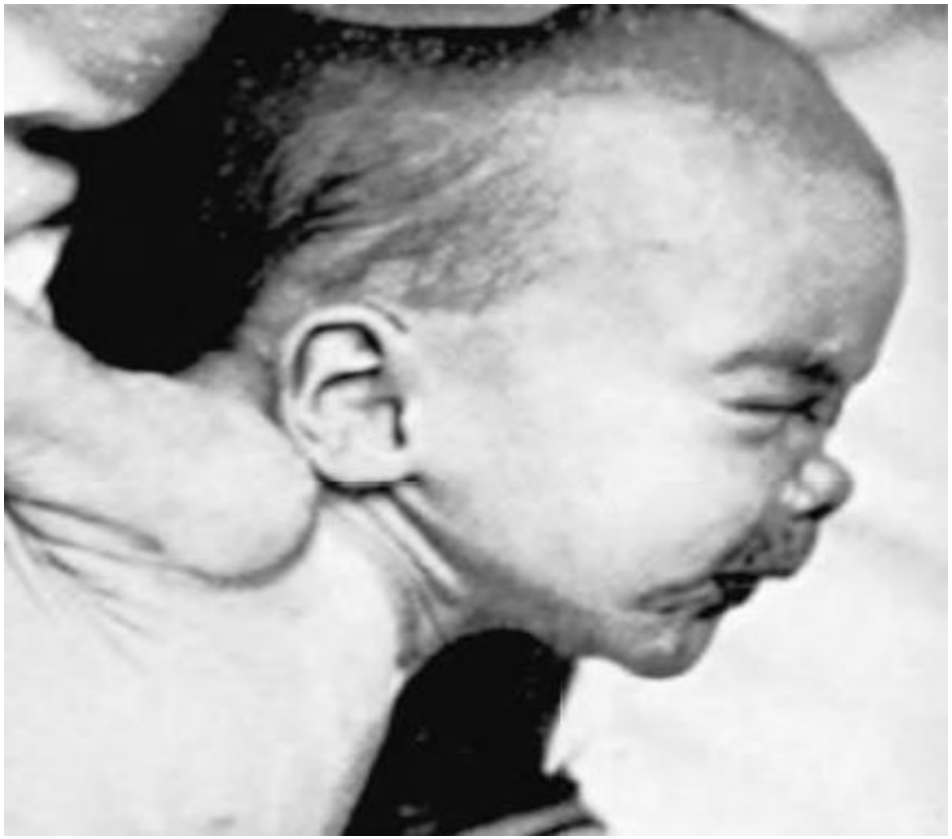


More on Tertiary Syphilis

- In the cardiovascular system, **gummas** weaken the major blood vessels, causing them to bulge and burst; in the spinal cord and meninges, gummas lead to degeneration of the tissues and paralysis; and in the brain, they alter the patient's personality, mental health and judgment. Damage can be so serious as to cause death.
- **Syphilitic gummas** are white-gray and rubbery, occur singly or multiply, and vary in size from microscopic lesions resembling tubercles to large tumor-like masses. They occur in most organs but particularly in skin, subcutaneous tissue, bone, and joints. In the liver, scarring as a result of gummas may cause a distinctive hepatic lesion known as hepar lobatum. On histologic examination, the gummas have centers of coagulated, necrotic material and margins composed of plump, palisading macrophages and fibroblasts surrounded by large numbers of mononuclear leukocytes, chiefly plasma cells.
- Treponemes are scant in gummas and are difficult to demonstrate.
- **Syphilis is a serious problem in pregnant women** because the spirochetes penetrate the placental barrier after the third or fourth month of pregnancy, causing **congenital syphilis in** the fetus. Infection can lead to death (stillbirth).
- Surviving infants develop skin lesions and open sores. Affected children often suffer poor bone formation, meningitis, or **Hutchinson's triad, a** combination of deafness, impaired vision, and notched, peg-shaped teeth.



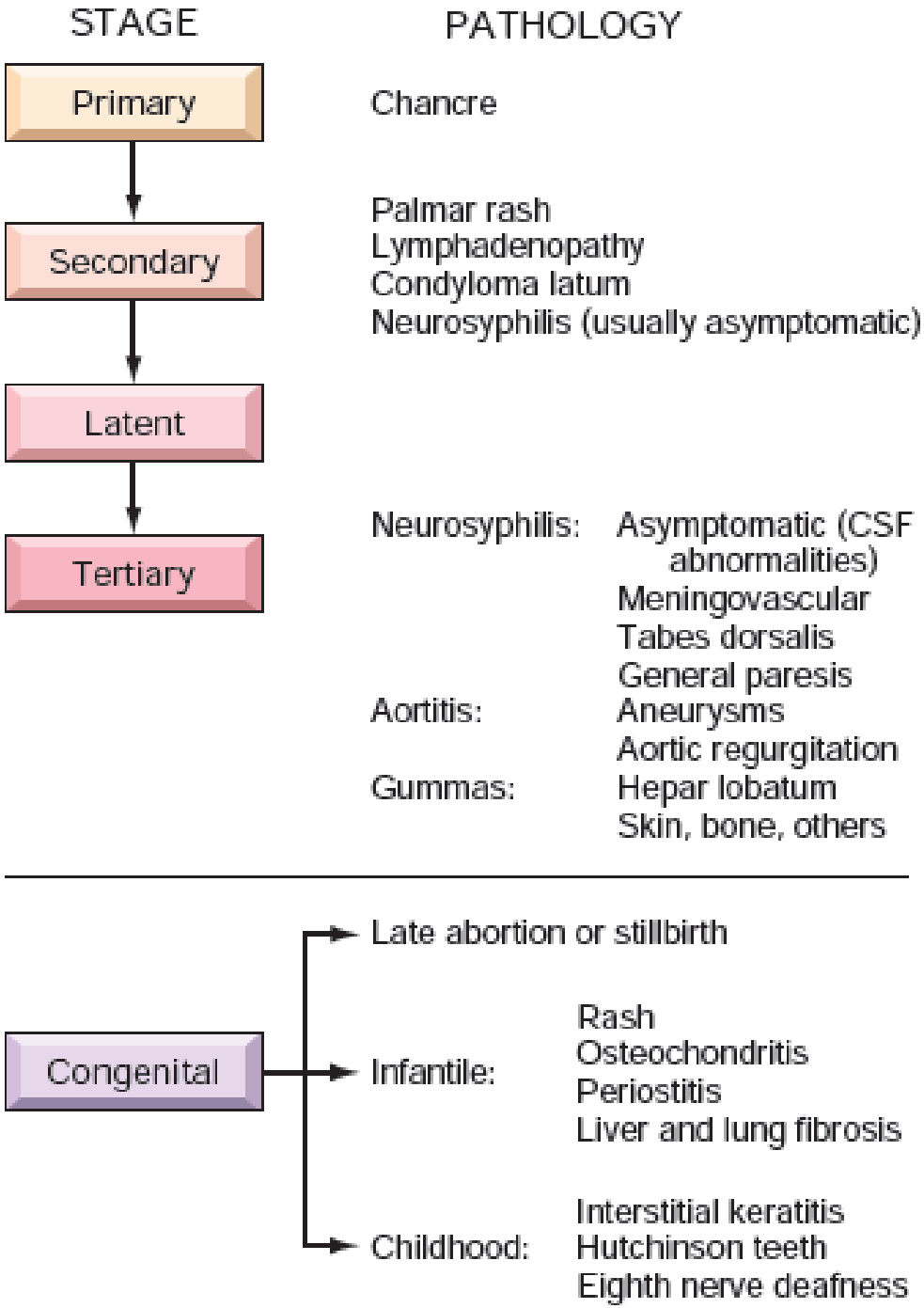
Hutchinson teeth
as a late manifestation of
congenital syphilis



Saddle nose
in a newborn with
congenital syphilis

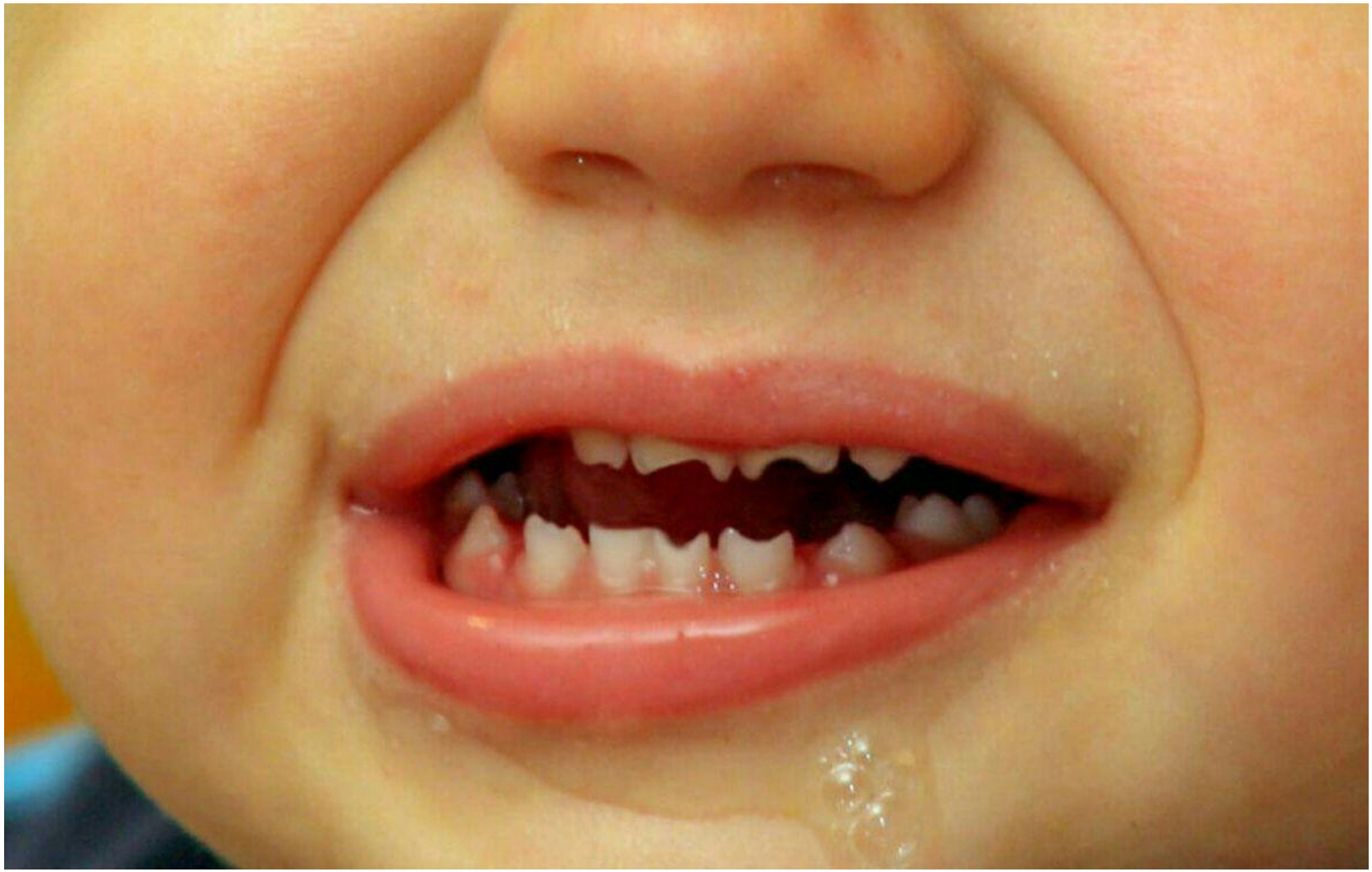
From Nelson Txtbk Paed 2020 ed 21

Protean manifestations of **syphilis**



Hutchinson's teeth

a sign of congenital syphilis



Other Immunological Aspects of the Sigma

- There is **no vaccine for syphilis**, so the cornerstone of syphilis control is:
  - safe sex practices, and
  - the identification and treatment of the sexual contacts of patients.

- Because *T. pallidum* cannot be cultivated on laboratory media, diagnosis in the primary stage depends on the observation of spirochetes from the chancre using fluorescence or dark-field microscopy.

- As the disease progresses, a number of common tests to detect syphilis antibodies becomes useful, including:
  - **The rapid plasma reagin (RPR) test,**
  - **The Venereal Disease Research Laboratory (VDRL) test, and**
  - **Other tests for more specific anti-Treponemal Ab's.**

- **Penicillin is the drug of choice** and a single dose usually is sufficient to cure primary and secondary syphilis, then mitigation of clinical manifestations and lowering some antibody titers.

Other Immunological Aspects of the Sigma (cont.)

- **The immune response** to *T. pallidum* reduces the burden of bacteria and can lead to resolution of local lesions **but does not reliably eliminate** the systemic infection.
- Superficial sites of infection (**chancres and rashes**) have an intense inflammatory infiltrate that includes **T cells, plasma cells and macrophages** that surround the bacteria.
- The infiltrating CD4+ T cells are **TH1 cells** that may activate macrophages to kill the bacteria.
- Treponeme-specific **antibodies** are detectable and these activate **complement** in the lesion and opsonize the bacteria for phagocytosis by **macrophages**.
- In many patients, the organism persists despite these host responses.
- A series of duplicated genes, termed *T. pallidum* repeats (tprA to tprL, etc), which encode homologous membrane proteins that may impart the means of antigenic variability and immunologic escape.
- A protein in the outer membrane of *T. pallidum, **TprK**,* accumulates structural diversity during the course of infection through gene conversion (recombination) between silent donor sites and the ***tprK*** gene and this might contribute to **antigenic diversity** that allows the organism to persist.

Common Serologic Tests for Syphilis

- **Serology** remains the mainstay of diagnosis of syphilis. Serologic tests include nontreponemal antibody tests and antitreponemal antibody tests.

- **Nontreponemal tests** measure antibody to cardiolipin, a phospholipid present in both host tissues and *T. pallidum.* These antibodies are detected in the **RPR and VDRL tests**.
- **Treponemal antibody tests** measure antibodies that specifically react with *T. pallidum and not other treponemes.*
- These include the **FTA_absorption test**, the dangerous **TPI (Nelson)**, the *T. pallidum* MHA or Particle Agglutination and ELISA. The use of these tests is complex because of differences in the antibody responses they measure and imperfections in the tests.
- In the diagnosis of **congenital syphilis**, having a means to differentiate between **passive transplacental** transfer of maternal antibody to the fetus and production by the fetus of **active endogenous fetal** antitreponemal antibody would be most helpful in the **T.O.R.C.H. (and not ToRCH!)** series.
- Because antibodies of the **IgM class** do not cross the placenta, detection of IgM antibody in the umbilical/ fetal or neonatal serum would indicate antibody production by the fetus because of **active fetal infection**.
- No IgM assay currently is commercially available that is sufficiently sensitive and specific to recommend for routine use in the evaluation of infants born to mothers with syphilis. The fluorescent treponemal antibody that measures IgM antitreponemal antibodies, the IgM-FTA-ABS test, has been associated with false-positive and false-negative results.
- **False negative** results may occur due to the blockade of antigenic epitopes of the test materials by the presence of maternal IgG. Occasional **false-positive** IgM-FTA-ABS tests occur because of the presence of an IgM anti-IgG antibody or rheumatoid factor.
- For these reasons, the CDC has recommended that the IgM-FTA-ABS test be **suspended** for diagnostic testing of neonates, and the test is available only as a provisional test.

Nontreponemal and Treponemal Assays

Nontreponemal Screening Assays	Treponemal Confirmatory‡ Assays
T. pallidum (RPR)*	FTA-ABS
T. pallidum (VDRL)†	*T. pallidum* particle agglutination (antibody to TP-PA) *T. pallidum* antibody IgM by ELISA *T. pallidum* antibody, IgG by Immunoblot *T. pallidum* antibody, IgG by indirect fluorescent antibody (IFA)§

*Serum or CSF with reflex to titer.
†Serum or CSF with reflex to titer.
‡If nontreponemal screening assay is positive.
§CSF.

دانشگاه علوم پزشکی
و خدمات بهداشتی درمانی تهران
معاونت پژوهشی
اداره چاپ و انتشارات

چهل سال تحقیق

در بیماریهای شایع عفونی ایران

(انفکسیو ایمونولوژی)

تالیف

دکتر غلامرضا نظری

استاد دانشگاه علوم پزشکی تهران

۱۳۸۰

چاپ دوم

دانشگاه علوم پزشکی
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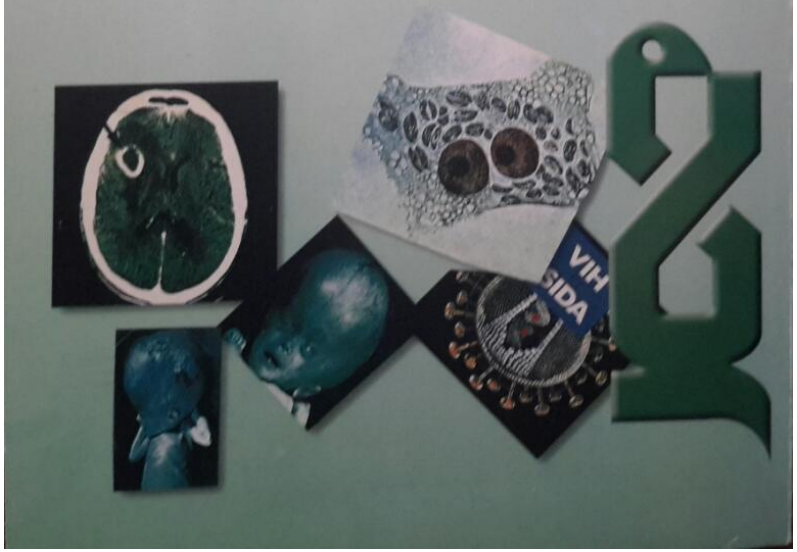
استاد دانشگاه علوم پزشکی تهران

۱۳۸۰

چاپ دوم



استاد دکتر غلامرضا نظری

شناسنامه علمی

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بیماریهای مقاربتی

سیفیلیس ، سوزاک و اورتریتهای دیگر فراوان دیده می‌شدند، درمان آنها مشکل و طولانی و گاهی نتیجه قطعی حاصل نمی‌شد. آنتی بیوتیک و جود نداشت و پنی سیلین در اواخر جنگ به ایران وارد شد و پس از آن بود که با به کار گرفتن آن از شدت این بیماریها کاسته شد.

آبله

واکسن ضد آبله در انستیتو پاستور ایران به مقدار زیاد تهیه می‌شد و واکسیناسیون اجباری بود ولی به علت نبودن برنامه های جدی مبارزه با آن ، آبله همه جا وجود داشت. این بیماری یا می‌کشت و یا بیمار را آبله رو می‌کرد. در زمان جنگ جهانی دوم ، دو نفر مبتلا به آبله وارد بندر انزلی شدند. چون طی چند سال نقشهٔ جامع بندر انزلی و جزایر اطراف آن را تهیه کرده بودیم و به کمک و همت پزشکان شهر ۷۰ نفر پرستار را آموزش داده بودیم ، از شادروان پروفسور بالتازار[1] رئیس انستیتو پاستور ایران ۵۰ هزار دوز واکسن آبله در خواست کردم که فوراً فرستاد. ۷۰ پرستار داوطلبانه طبق نقشه در بندر و جزایر به خانه های مردم که در خانه های خود را به روی آنان گشوده و از آنان استقبال کرده بودند ، رفتند و در مدت یک هفته از صبح تا غروب به اهالی بندر و جزایر واکسن زدند. تا هنگام ایجاد مصونیت عمومی فقط ۱۴ نفر مبتلا شدند و پس از آن دیگر آبله دیده نشد ، در صورتیکه در شهرها و روستاهای دیگر استان گیلان آبله فراوان دیده می‌شد. این امر به وضوح نشان داد که اگر بهداری برنامه دقیق و پیگیر و همه جانبه ای تهیه می‌کرد و بوسیله کارکنان متعهد خود با این بیماری به مبارزه می‌پرداخت مسلماً سرایت آبله در ایران متوقف می‌شد. در صورتیکه هشت سال بعد در یک مسافرت علمی‌که به همراه دانشجویان پزشکی برای مبارزه با مالاریا رفته بودیم ، در روستاهای دشت آبی قزوین دو کودک مبتلا به آبله دیدیم و از آنها عکس برداری کردیم. مسئول واکسیناسیون که ساکن همان محل بود به محض اطلاع از ورود ما

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۱ - Baltazard

آنها مسائل جدیدی در موسسات مربوط مطرح خواهد کرد).

ترپونماتوزها

در طول نیم قرن با آزمایش های سرولژی کلاسیک سیفیلیس، کمک‌های زیادی به تشخیص این بیماری و عوارض آن به عمل آمد ولی به علت جواب‌های غیر اختصاصی، اشتباهات زیادی در تشخیص وجود داشت که گاهی باعث ایجاد ناراحتی های اجتماعی و درمانی می‌شدند.

در سال ۱۹۴۹ نلسن و مایر TPI (بی حرکت کردن ترپونم) را که اولین تست اختصاصی در تشخیص ترپونماتوزها بود ارائه دادند و به‌طور قطع و برای همیشه موضوع مثبت های غیر اختصاصی (به اصطلاح بیولژیک) را حل کردند ، و در نتیجه از تجویز داروهای ضد سیفیلیس به کسانی که به علت جواب های نادرست سرولژیک بی جهت تحت درمان قرار می گرفتند، جلوگیری به عمل آمد .

توکسوپلاسموز

این بیماری که در مدت بیش از ۴۰ سال پس از کشف انگل بوسیله شارل نیکل[1] و مانسو[2] فقط موارد محدودی از آن تشخیص داده شده بود ، باکشف تکنیک بسیار جالب و حساس و اختصاصی (دای تست سابین و فلدمن) در ۱۹۴۸ تحولی عظیم در تشخیص این بیماری به‌وجود آمد که باعث گسترش تحقیق در این بیماری در سراسر جهان شد.

بیماریهای اتوایمیون

پس از نیم قرن تحقیق و بحث به وسیله دانشمندان، بالاخره از ۱۹۵۶ به بعد منجر به کشف علل ایجاد بیش از سی بیماری شد مانند : بیماری شوگرن ، لوپوس اریتماتوی منتشر، هپاتیت مزمن فعال، بیماری هاشیموتو و .....

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1 . Charles Nicolle 2 . Manceau

مجلات علمی پاریس چاپ شد.متاسفانه همکار صمیمی و وظیفه شناس و متعهد ما در حین انجام وظیفه در پشت میز آزمایشگاه آلرژی به علت سکته قلبی درگذشت.

سلولهای B و T

دکتر احمد مسعود پس از گرفتن دکترای داروسازی از دانشگاه تهران عازم لیون فرانسه شد، در مراجعت روش های مربوط به تشخیص سلول های T و B را با خود به ارمغان آورد. در بخش ایمونولژی دانشکده دستگاههای لازم برای کار تحقیق را تهیه کردیم. که با موافقت رئیس سازمان انتقال خون در آن سازمان به تحقیق پرداخت.

هموگلوبین های غیر طبیعی

برای تحقیق هموگلوبین‌ها یکی از پزشکان بخش ایمونولوژی با انجام الکتروفورز خون بیماران را جهت تشخیص نوع هموگلوبین بهزینه بخش با هواپیما برای پروفسور لهمن[1] در بیرمنگام انگلستان فرستاد. در نتیجه چند هموگلوبین تازه در ایران کشف شد بعداً با دستگاه امین اسید آنالایز اهدائی دولت ژاپن تحقیق ادامه یافت. پروانه دانشمند در این تحقیقات همکاری می‌کرد.

میکرسکپ الکترونیک

دکتر ح. فرزادگان همکار میکرب‌شناسی، در گروه قبل از مراجعت به ایران مطالعات زیادی با میکرسکپ الکترونیک در امریکا انجام داده بود. در سال ۱۳۵۵ به رئیس وقت دانشگاه تهران پیشنهاد کردم یک میکرسکپ الکترونیک برای گروه میکربشناسی و ایمونولژی خریداری کند تا تحت نظر دکتر فرزادگان برای تحقیق مخصوصاً در بیماریهای ویروسی و آموزش آن به پزشکان و متخصصان و دانشجویان، مورد استفاده قرار گیرد. رئیس دانشگاه موافقت نکرد زیرا برای فردی از دانشکدهٔ دیگر که وابسته به مقامات بالا بود دستور خرید داده بود. دکتر فرزادگان تحقیق در HBs Antigen را در دهندگان خون در سازمان انتقال خون انجام و روش آزمایش را به کارمندان آن سازمان آموزش داد و از ایران رفت و اکنون در امریکا مشغول تحقیق در بارهٔ بیماری ایدز می باشد.

تر پونماتوز[2]

از بدو تاسیس بخش سرولژی در دانشگاه تهران در سال ۱۳۱۸ دکتر حسن میردامادی، آزمایش فیکساسیون کمپلمان (واسرمن) و آزمایش های فلوکولاسیون

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۱. Lehman

۲. Treponematose

برای آزمایش FTA مجبورند ترپونم مرده را با ارسال ارز از خارج وارد کنند. همین امر باعث شد که اغلب اوقات آنتی‌ژن ترپونمی وجود ندارد و آزمایشگاه‌های کشور نمی‌توانند به‌طور مرتب جوابگوی بیماران مشکوک به ترپونما توز و پزشکان معالج آنها شوند. علاوه بر تعطیل‌شدن آزمایش TPI (تست نلسن و مایر)، آزمایش FTA نیز اکثراً در دسترس بیماران نیست.

اخیراً با فعالیت دکتر عباس زمانی آقای دکتر آذرنوش رئیس انستیتو پاستور ایران موافقت کردند که پاساژ ترپونم را در انستیتو پاستور انجام دهیم، برای اینکار از دکتر ابل در انستیتو فورنیه پاریس درخواست کردم سوش زندهٔ ترپونم نیکلس را در بیضهٔ خرگوش در ترموس محتوی کاربوگلاس ۷۰- درجه برای ما ارسال دارند. پاساژ ترپونم را در بخش حیوانات آزمایشگاهی واقع در مجتمع تولیدی و تحقیقاتی انستیتوپاستور با همکاری ویکتوریا امیری انجام و خوشوقتم از این که مجدداً ترپونم را در دسترس آزمایشگاهها و پزشکان ما قرار دادیم.

از بین تحقیقات جالب با این روش اینک محض نمونه و بطور اختصار فقط چند مورد زیر را می نویسم:

۱- تحقیق در دهندگان خون در سال های ۵۴، ۵۵، ۵۶ که موضوع پایان نامه ویکتوریا امیری قرار گرفت. در خون ۱۲۸۰۸۴ نفر که با آزمایش RPR در سازمان انتقال خون ایران ۳۴۵ مثبت نشان داد (۱/۰۵٪) آزمایش TPI در سرم‌های RPR مثبت با احتساب کل آزمایش شدگان فقط ۰/۶۵٪ مثبت شد بنابراین فقط همین عده قطعاً مبتلا به سیفیلیس بودند. ۰/۴٪ بقیه با وجود RPR مثبت سیفیلیس نداشتند در نتیجه از تجویز دارو به آنها جلوگیری شد.

۲- در مورد ازدواج: زنی گریه کنان به آزمایشگاه TPI مراجعه کرد، یک برگ جواب آزمایش به امضای تکنیسین یک آزمایشگاه را ارائه داد. تکنیسین مزبور خارج از وظیفهٔ خود و بر خلاف کلیهٔ موازین پزشکی اظهار نظر کرده و نوشته بود (V.D.R.L از لحاظ سیفیلیس مثبت است) در نتیجه دفتر خانه به استناد آن برگ آزمایش، مانع ازدواج آن زن شده بود سرم زن مزبور را مورد آزمایش قرار دادیم، آزمایش V.D.R.L مثبت شد ولی آزمایش TPI منفی بود مقدار IgM سرم مزبور را تعیین کردیم که خیلی زیاد تر از طبیعی بود، با

توجه به نتیجه آزمایش ها به دفتر خانه نوشتم که تست نلسن بطور قطع ابتلا به سیفیلیس را رد کرده است و جواب مثبت V.D.R.L غیر حقیقی و به علت زیاد بودن مقدار IgM می باشد زن مبتلا به سیفیلیس نیست و تفسیر غلط از طرف شخصی بی اطلاع و بدون صلاحیت در یک آزمایشگاه به عمل آمده است. و زن می تواند ازدواج کند. با نوشتن این گواهی خوشبختانه ازدواج آن زن انجام گرفت.

۳- در خون هائی که آزمایش فیکساسیون با آنتی ژن های بیماری های مختلف جواب مثبت می داد، تست نلسن منفی ما را راهنمائی کرد که تحقیقات خود را ادامه داده و نتایج بسیار جالب زیر را بدست آوردیم:

اینک شرح حال یک زن و شوهر را می نویسم:

زن و شوهر جوانی از مسافرت خارج برگشتند و به وسیله یکی از اساتید دانشگاه تهران به ما معرفی شدند، زن یک بار آبستن شد که منجر به سقط جنین گردید، آزمایش V.D.R.L و واسرمن هر دو نفر در دو آزمایشگاه مختلف جواب مثبت داد، با سرم های آن زن و شوهر، آزمایش نلسن به عمل آوردیم، هر دو سرم منفی بودند بنابراین مسلماً مبتلا به سیفیلیس نبودند.

تحقیقات را ادامه دادم، در سرم ها جداگانه با آنتی ژن های لیستریا، توکسوپلاسما، گونوکک، (گونورآکسیون)، کیست هیداتیک آزمایش فیکساسیون کمپلمان به عمل آوردیم، همه جواب ها مثبت بودند. باید توجه داشت با چنین سرم هائی اگر با هر یک از آنتی ژن های فوق به تنهائی آزمایش انجام می گرفت مشکوک به همان بیماری شده و بی جهت بیمار تحت درمان همان بیماری قرار می گرفت. در صورتیکه مبتلا به هیچیک از بیماریهای مزبور نبودند با تعیین IgM خون آنها معلوم شد ازدیاد آن در سرم زن و شوهر جواب های غیر اختصاصی داده اند. در یکی از مسافرت هایم به پاریس پرفسور مارگریت فور[1] در انستیتو پاستور از من خواست سرم های مثبت بیولژیک را جهت مطالعه برایش به پاریس بفرستم زیرا در آن زمان در اکثر مراکز پژوهش کشورها در این باره تحقیق می کردند. وقتی بررسی‌های فوق را شرح دادم از خواستهٔ خود صرف نظر کرد.

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۱. Marguerite Faure

بررسی شد و ثابت گشت رآژین سرم ها که موثر در آزمایش فیکساسیون کمپلمان و V.D.R.L می باشد به علت انجماد و خروج از انجماد زود خراب می شود، در حالیکه این عوامل در آنتی بادی های موثر در آزمایشهای FTA و TPI کمتر تاثیر دارند. از طرف دیگر مسلم شد که حرارت باعث ازدیاد فلوکولاسیون و در نتیجه موجب ایجاد مثبت های کاذب می گردد ولی سرما برعکس از فلوکولاسیون می کاهد.

سازمان بهداشت جهانی برای محفوظ نگهداشتن سرم ها از خرابی، جعبه های مخصوص محتوی ازت مایع را که از (۱۵۰-) تا (۱۹۶-) درجه می باشند تهیه کرده است و سرم ها را باید در آن قرار داد و با هواپیما به مراکز مطالعاتی فرستاد.

آیاتهیه وسائل فوق به‌مقیاس یک‌کشوروهمچنین حمل‌آنهابا هواپیماازنقاط دورافتاده به مرکز آن هم درکشورهای جهان سوم امکان پذیر است ؟

در مطالعات اپیدمیولژیک از طرف سازمان بهداشت جهانی، مقایسه جواب های آزمایش V.D.R.L با آزمایشهای FTA و TPI نشان داد که منحنی FTA و TPI نزدیک به هم و تقریباً شبیه به یکدیگرند در حالیکه برحسب سنین مختلف منحنی V.D.R.L به کلی با آنها متفاوت است و مخصوصاً در سنین کم این اختلاف به طرز فاحشی نمودار می گردد. پس از مطالعات عمیق در خون میلیون ها نفر و اثبات نارسائی آزمایش V.D.R.L در مبارزه صحیح با ترپونماتوز، سازمان بهداشت جهانی آزمایش FTA را به جای آن برگزید.

در ایران نیز ماگرفتار مشکلات فوق هستیم. برای رفع آن اندیشیدم مطالعات خود را در برنامهٔ زیر قرار دهم:

۱- علاوه بر تهران از نواحی گرمسیر آلوده به سیفیلیس و بژل مانند خوزستان خون تهیه کنیم.

۲- از نوک انگشت یا لاله گوش بر روی رندل هائی که در ایران تهیه می‌شود خون بگیریم.

۳- رندل ها را در درجات حرارت مختلف (مخصوصاً با در نظرگرفتن هوای نواحی گرمسیر) در مدت های کم و بیش طولانی، خارج از یخچال نگهداری کنیم.

۴- سرم ها را به طور استریل تهیه کرده و در یخچال قرار دهیم.

۵- آزمایش FTA کانتیتاتیف بر روی سرم را در زمان های مختلف به عمل آوریم.

مقایسه نتایج آزمایش های سرولژی کـلاسیک و ایـمونوفلوئرسانس (FTA)

در کشور ما سالهاست آزمایش V.D.R.L مورد استفاده و آمارهای به‌دست آمده برای سیفیلیس نتیجه همین آزمایش می باشند ولی به علت جواب های مثبت غیر حقیقی مخصوصاً در خون هائیکه از نقاط دور دست گرفته و به مرکز ارسال می شود به علت آلوده شدن خون و سرم آمارها با حقیقت وفق نمی‌دهند، به این جهت یکی از تحقیقاتی کـه در سـال ۱۳۴۸ بـه عـمل آوردم، مـقایسه نـتایج آزمـایش‌های سـرولژی کـلاسیک بـا ایمونوفلوئرسانس درخون روستائیان و ساکنین نقاط دور دست کشور بود.

دکتر بزرگ آرامش از طرف انستیتو تحقیقات بهداشتی و دانشکده بهداشت برای مطالعات اپیدمیولژیک در بیماریهای مختلف عازم استان خراسان بود، با تحویل ۱۰۰۰ رندل به دکتر آرامش پیشنهاد کردم که در شهرها و روستاهای آن استان از ۱۰۰۰ نفر خون در لوله و روی رندل بگیرند، ایشان مجموعاً از ۸۱۱ نفر خون در لوله و روی رندل تهیه کردند که تحت آزمایش V.D.R.L و C.F (کولمر M.C.F) و ایمونوفلوئرسانس FTA 200 قرار دادیم. مبنای تحقیق آزمایش V.D.R.L بود و روش های دیگر را با آن مقایسه کردیم (جدول ۱).

V.D.R.L	C.F
مثبت ۶۴	مثبت ۱۴ AC ۱۳ منفی ۳۷
مثبت ضعیف ۷۴	مثبت ۱۲ AC ۱۲ منفی ۵۰

ج - اگر آمار را از روی FTA 200 در سرم تهیه کنیم ۳۳ مورد مثبت داشتیم (۴/۰۷٪) یعنی تعداد جواب مثبت FTA 200 از $\frac{1}{4}$ تعداد مثبت های V.D.R.L نیز کمتر است.

د - در مقایسه این آزمایش روی سرم و رندل باید متذکر شد که جواب FTA 200 روی رندل هائی که «هشت ماه در حرارت معمولی بین ۲۵ و ۳۸ درجه نگهداری شده‌اند» با سرم همان اشخاص «که در یخچال نگهداری شده‌اند» تطبیق می‌کرد طبق جدول ۲ در پنج آزمایش جواب کاملاً یکسان فقط در یک رندل یک +کمتر از سرم جواب داده بود.

هـ- از لحاظ اپیدمیولژی اگر V.D.R.L را راهنمای مبارزه با ترپونماتوز قرار دهیم به علت مثبت غیر حقیقی زیادی که در دهات مختلف نشان داده شد مبارزه مشکلتر و هزینه زیادتر خواهد شد چون برای مبارزه معمولاً اکیپ های سیار با توجه به آمار آن به مردم نواحی که آلوده تشخیص می‌دهند پنی سیلین تزریق می کنند.

در تحقیقات ما آمار FTA 200 کمتر از یک چهارم V.D.R.L بود و در بعضی دهات ابداً بیمار مبتلا به سیفیلیس وجود نداشت، بنابراین با به کار بردن FTA روی رندل اولاً تشخیص صحیح خیلی عملی تر و دقیق تر انجام می گیرد. ثانیاً عده زیادی از مردم بدون دلیل تحت درمان قرار نمی گیرند. مقالهٔ مربوط به این تحقیق را در اسفند ۱۳۴۷ در مجله طب عمومی سال هشتم صفحات ۱۳۸ - ۱۴۱ منتشر و در آخر مقاله توجه مسئولان مبارزه با سیفیلیس را به این مطالب جلب کردم.

پس از انتشار این مقاله یکی از مسئولان مبارزه با بیماری فوق به من تلفن کرد و به جای اظهار خوشوقتی گله کرد که چرا آمار مبتلایان را کم نشان دادم و اصرار داشت که این مقاله را به سازمان بهداشت جهانی نفرستم، پس از تحقیق معلوم شد که ادارهٔ مربوط همیشه استان خراسان را به علت تعداد زیاد زائران و مسافرین (منطقهٔ آلوده به سیفیلیس) به سازمان بهداشت جهانی معرفی می کرد و برای دریافت پنی سیلین آمار مبتلایان آن سال را ۴۶٪ گزارش کرده بودند و از اینکه نتیجه تحقیق ما کذب گزارش آنها را نشان می داد با دست پاچگی بلافاصله اکیپ سیار به استان خراسان فرستادند و دستور تزریق پنی سیلین دادند و هنوز چند هفته نگذشته بود به من اطلاع دادند که براساس آزمایش به عمل آمده

برای مقایسه: همه سرم ها را با روش‌های V.D.R.L و CF , FTA کانتیتاتیف و T.P.i , FTA - ABS آزمایش می کردیم. آزمایش های V.D.R.L , CF گاهی جواب های مثبت و منفی غیر حقیقی نشان می‌دادند. در این موقع از FTA استفاده می‌شد موارد مثبت غیر حقیقی دیده شد FTA - ABS به کار بردیم (FTA کمتر از $\frac{1}{200}$ ارزش تشخیصی ندارد) و (FTA - ABS $\frac{1}{50}$ برای تشخیص کافی است) و در موارد مشکوک در آزمایش های فوق از تست TPI برای قضاوت نهائی کمک می گرفتیم. (تحقیق فوق را سرور اقدس وکیل موضوع پایان نامه خود قرار داد (اردیبهشت ۱۳۴۷).

تذکـــر:

هـــزینهٔ آزمـــایش FTA-ABS خـــیلی بـــیشتر از FTA است و در مـطالعات اپیدمیولوژیک از FTA استفاده می‌شود. اگر FTA منفی باشد احتیاج به FTA-ABS نیست زیرا آنتی‌بادی وجود ندارد تا جذب بشود. در موارد مثبت اگر FTA کانتیتاتیف انجام شود و تیتر خیلی بالا باشد سیفیلیس مسلم است بنابراین باز احتیاج به جذب نیست. در حقیقت «فقط در سرم‌های مثبت با تیتر کم» مثل ۴۰۰ و ۸۰۰ حتماً باید FTA-ABS انجام داد. با توجه به مطالب فوق در مواردی که کیت FTA-ABS کمیاب و یا نایاب می‌گردد آزمایشگاه‌ها و پزشکان برای تشخیص سیفیلیس دچار مشکلی نخواهند شد چون تعیین از روی تیتر

FTA { ۱۶۰۰ ≤ مثبت قلمداد می‌شود
۸۰۰ ≤ باید FTA-ABS انجام شود.

نتیجه عملی و جالبی که از انجام چهار روش به موازات هـم بـه‌دست آوردم

اصولاً در بیماری سیفیلیس تاریخ پیدایش آنتی بادی های مختلف که در برابر هر روش پاسخ می دهد متفاوت می باشد:

آنتی بادی ضد ترپونم پاسخ دهنده به FTA خیلی زود در آخر هفته اول بعد از بروز شانکر و راژین ها در V.D.R.L اواخر هفته دوم و در CF اواخر هفته سوم و ایموبیلیزین خیلی دیر در آخر هفته چهارم ظاهر می شود.

بنا بر این فرمول زیر را در انجام آزمایش های سرولژی سیفیلیس ارائه دادم :

تاریخ آزمایش	نوع آزمایش
در آخر هفته اول	FTA
" " دوم	V.D.R.L , FTA
" " سوم	CF , V.D.R.L , FTA
" " چهارم	TPI , CF , V.D.R.L , FTA

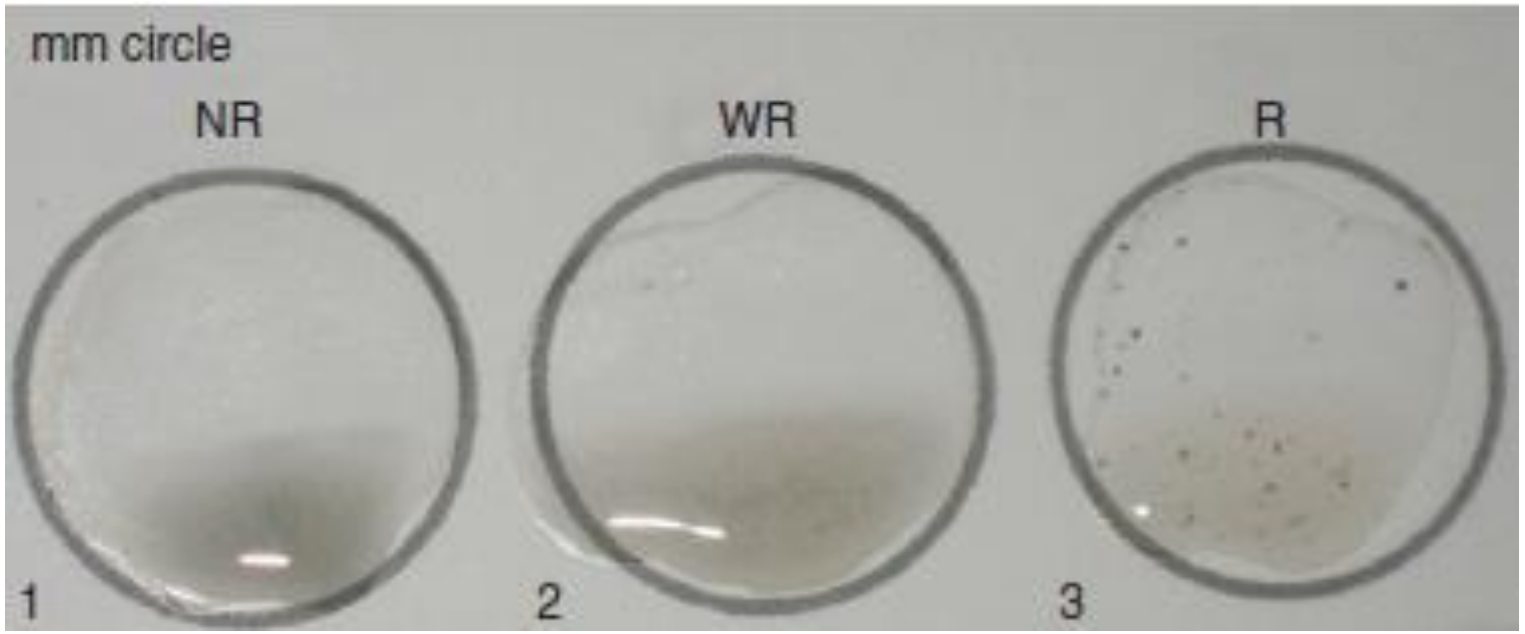
هر نوع تغییر در تاریخ انجام آزمایش ها باعث اشتباه در تشخیص می گردد ، با همین فرمول پیشنهادی من، تحقیق در سیفیلیس را در سطح وسیع انجام دادم و مسائل بسیار مهمی را توانستم حل کنم .

شرح حال یک زن و شوهر مبتلا :

یک زن جوان آبستن به علت زخم روی زبان به پزشک مراجعه کرد . ابتدا مشکوک به سرطان زبان می شوند ولی یک پزشک متخصص او را مورد معاینه قرار داده مشکوک به شانکر سیفیلیس می شود و زن و شوهر را به بخش ایمونولژی معرفی می کند . در زن و شوهر هر چهار آزمایش را به موازات هم انجام دادیم که نتیجهٔ آن به شرح زیر است :

	FTA	V.D.R.L	CF	TPI
زن	$\frac{1}{800}$	مثبت	++++	٪۰
شوهر	$\frac{1}{1600}$	مثبت	++++	٪۵۵

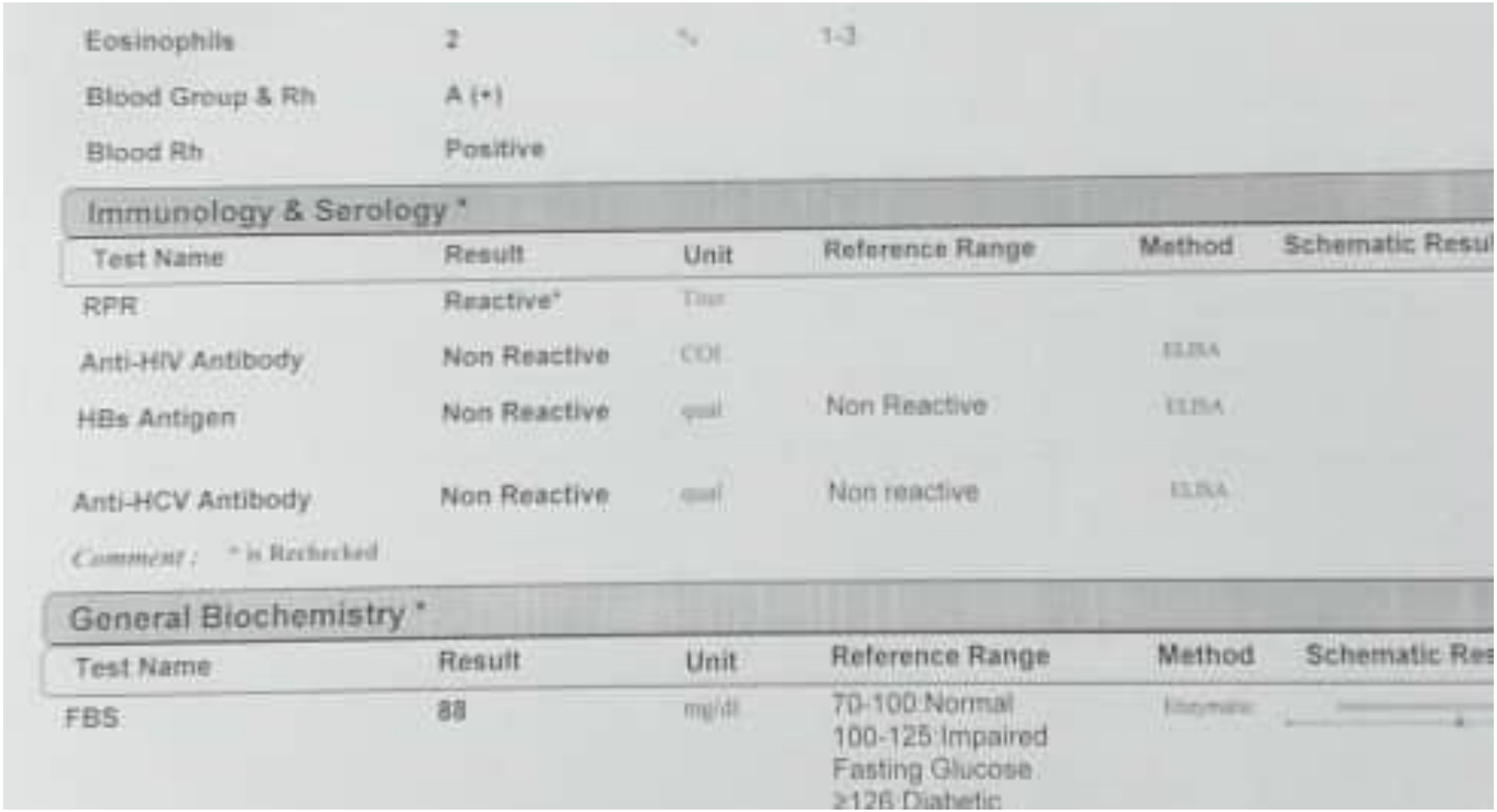
از روی این جدول مسلم شد که شوهر مبتلا به سیفیلیس بود ولی از زن خود پنهان و او را مبتلا کرد ، به شوهر تشخیص بیماری را گفتم ، انکار کرد و بر خلاف میلش ازاو معاینهٔ



RPR (Rapid plasma reagin) **test card** showing (from Left to Right): nonreactive, weakly reactive, and strongly reactive serum samples (well 1 to well 3) with their respective agglutination patterns. Do **NOT** use POSITIVE or NEATIVE terminology!

- **Lab NOTE:** Non-treponamal Testing on CSF should be strictly limited to **VDRL**, rather than RPR.
- Diagnosis of neurosyphilis, ususally requires a positive serum treponemal test in addition to a positive VDRL on CSF.
- The VDRL applied to CSF is highly specific for the diagnosis of neurosyphilis but is insensitive.
- It is negative in 22%–69% of patients with active neurosyphilis, and its sensitivity in inactive neurosyphilis may be as low as 10%.
- By contrast, the CSF FTA-ABS is reported to have high sensitivity and negative predictive value for neurosyphilis, but is not specific. Some experts advocate using this as a means of excluding the diagnosis.

Your Action?!?



Eosinophils	2	%	1-3
Blood Group & Rh	A (+)		
Blood Rh	Positive		

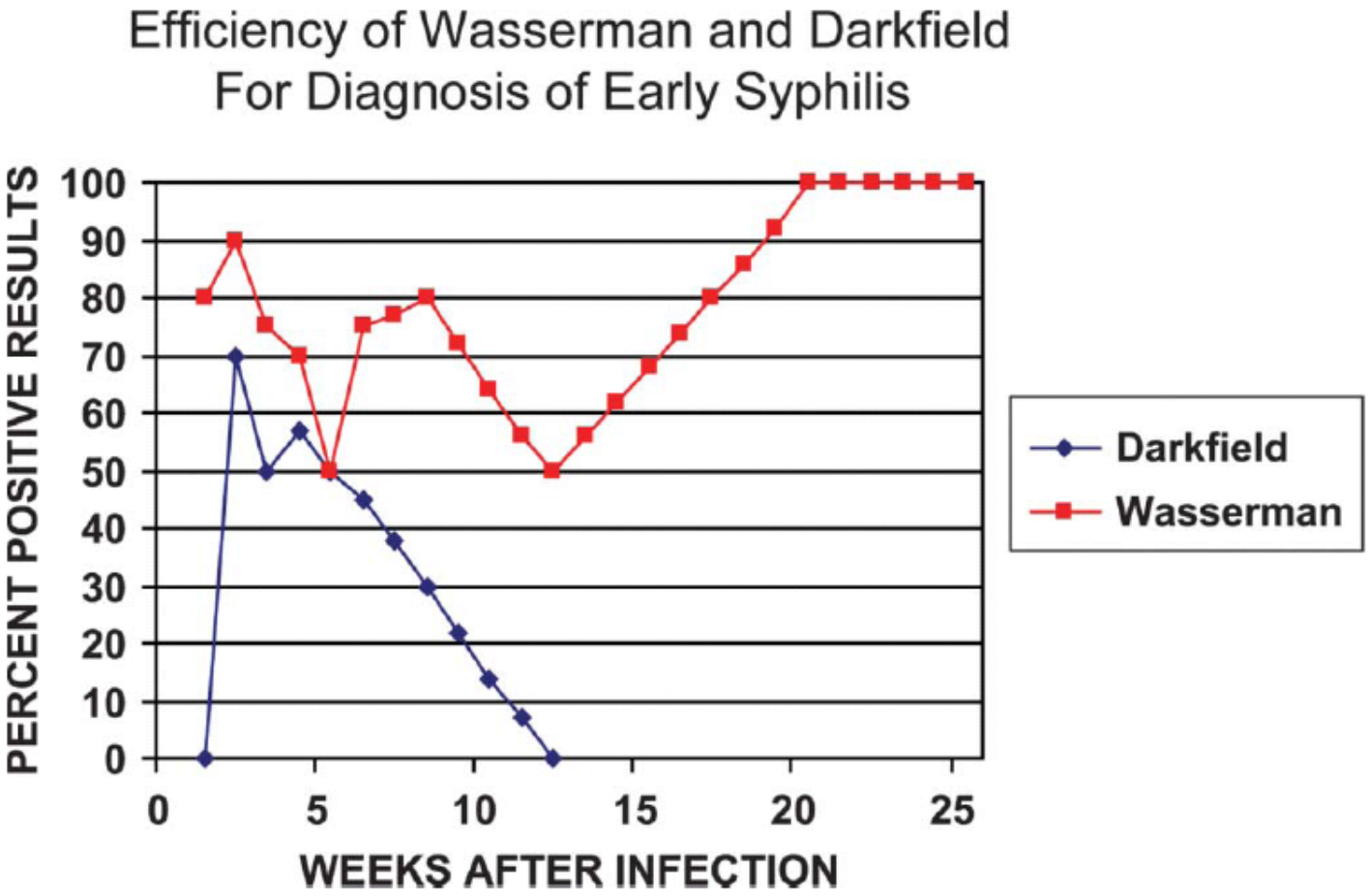
Immunology & Serology *

Test Name	Result	Unit	Reference Range	Method	Schematic Resul
RPR	Reactive*				
Anti-HIV Antibody	Non Reactive	COI		ELISA	
HBs Antigen	Non Reactive	qual	Non Reactive	ELISA	
Anti-HCV Antibody	Non Reactive	qual	Non reactive	ELISA	

Comment : * is Rechecked

General Biochemistry *

Test Name	Result	Unit	Reference Range	Method	Schematic Res
FBS	88	mg/dl	70-100 Normal 100-125 Impaired Fasting Glucose ≥126 Diabetic	Enzymatic	



- Dark-Field Examination of the chancre may be more specific but less sensitive compared to the serologic reaction in the early weeks of syphilis.
- More specific serologic tests for syphilis may be negative for up to 10 days after formation of the chancre.
- The occurrence of seronegative syphilis during active infection has important implications for diagnosis, not only for the patients but also for seronegative mothers giving birth to newborns with congenital syphilis.

Standard Serologic Tests for Syphilis

Type	Test	SENSITIVITY BY STAGE OF INFECTION (%)				Specificity (%)
		Primary	*Secondary*	*Latent*	*Late/Tertiary*	
Nontreponemal						
Extracts of tissue (cardiolipin-lecithin-cholesterol)	VDRL	78	100	96	71	98
	RPR	86	100	98	73	98
Treponemal						
Treponema pallidum	MHA-TP[a]	76	100	97	94	99
	FTA-ABS	84	100	100	96	97
	EIA	100	100	100	-	99
	CIA	98	100	100	100	99

*MHA-TP has been replaced with *Treponema pallidum* particle agglutination (TP-PA) test

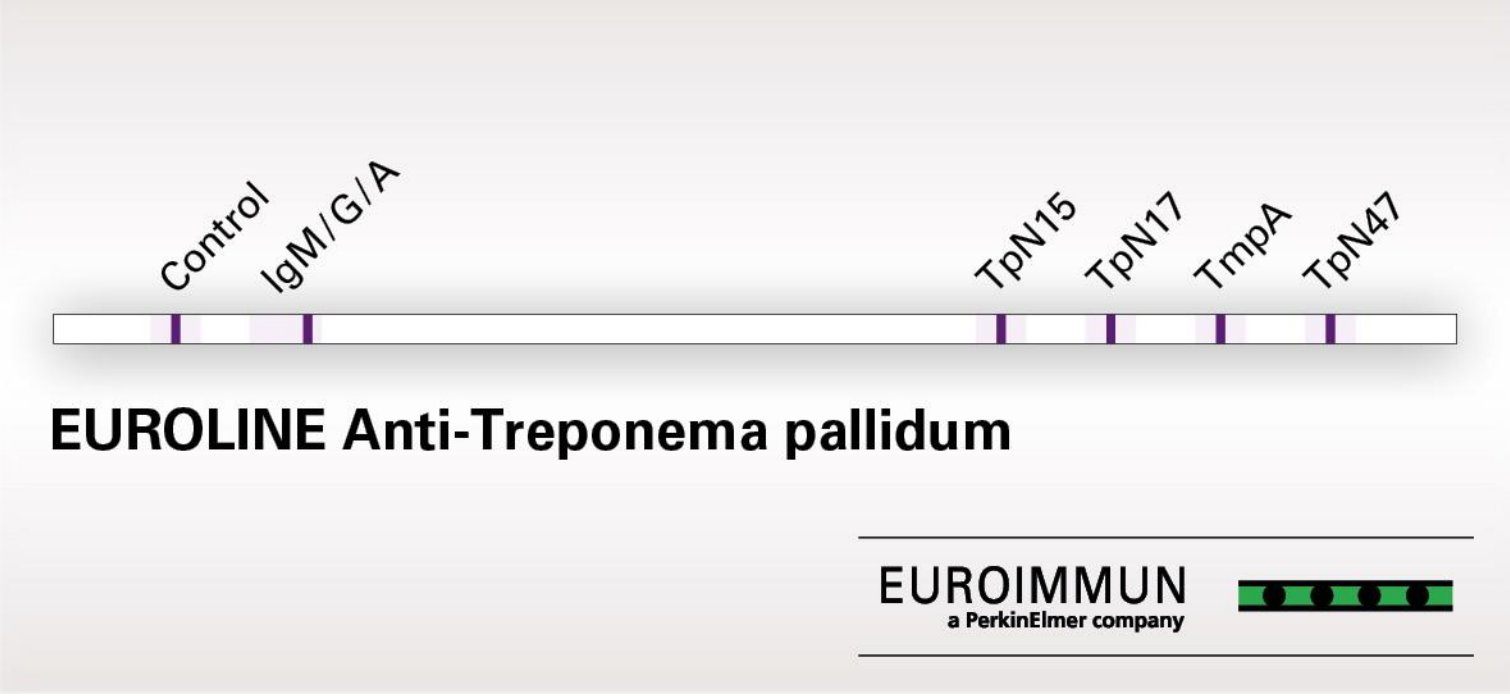
Sensitivities of Serologic Tests for Syphilis in Different Stages of Disease with an overall **Specificity**

From Feigin & Cherry's Txtbk Paed Inf Dis 2019 ed 8

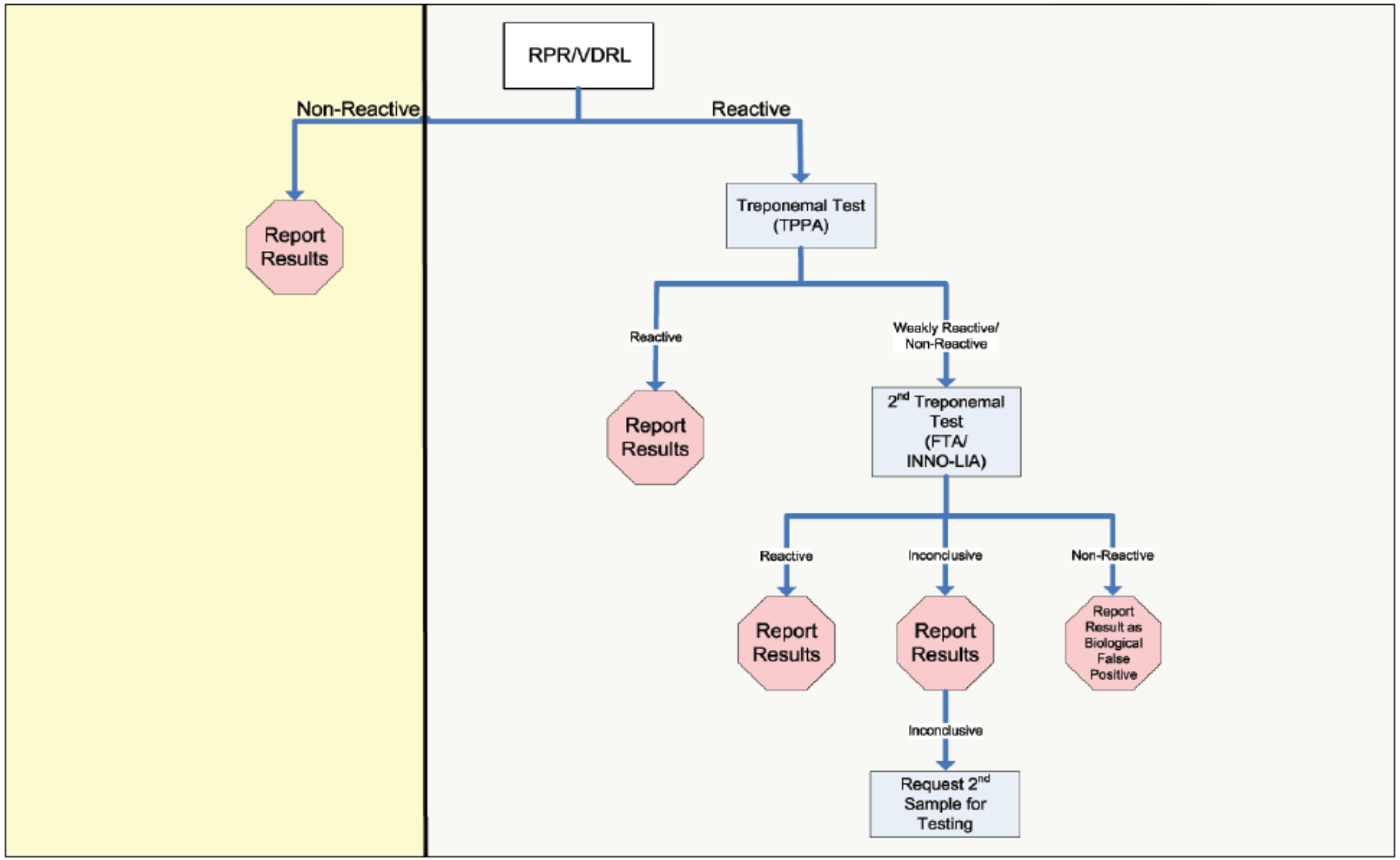
The first diagnostic blood test for syphilis was the:

a. VDRL
b. Wassermann
c. RPR
d. Colloidal gold

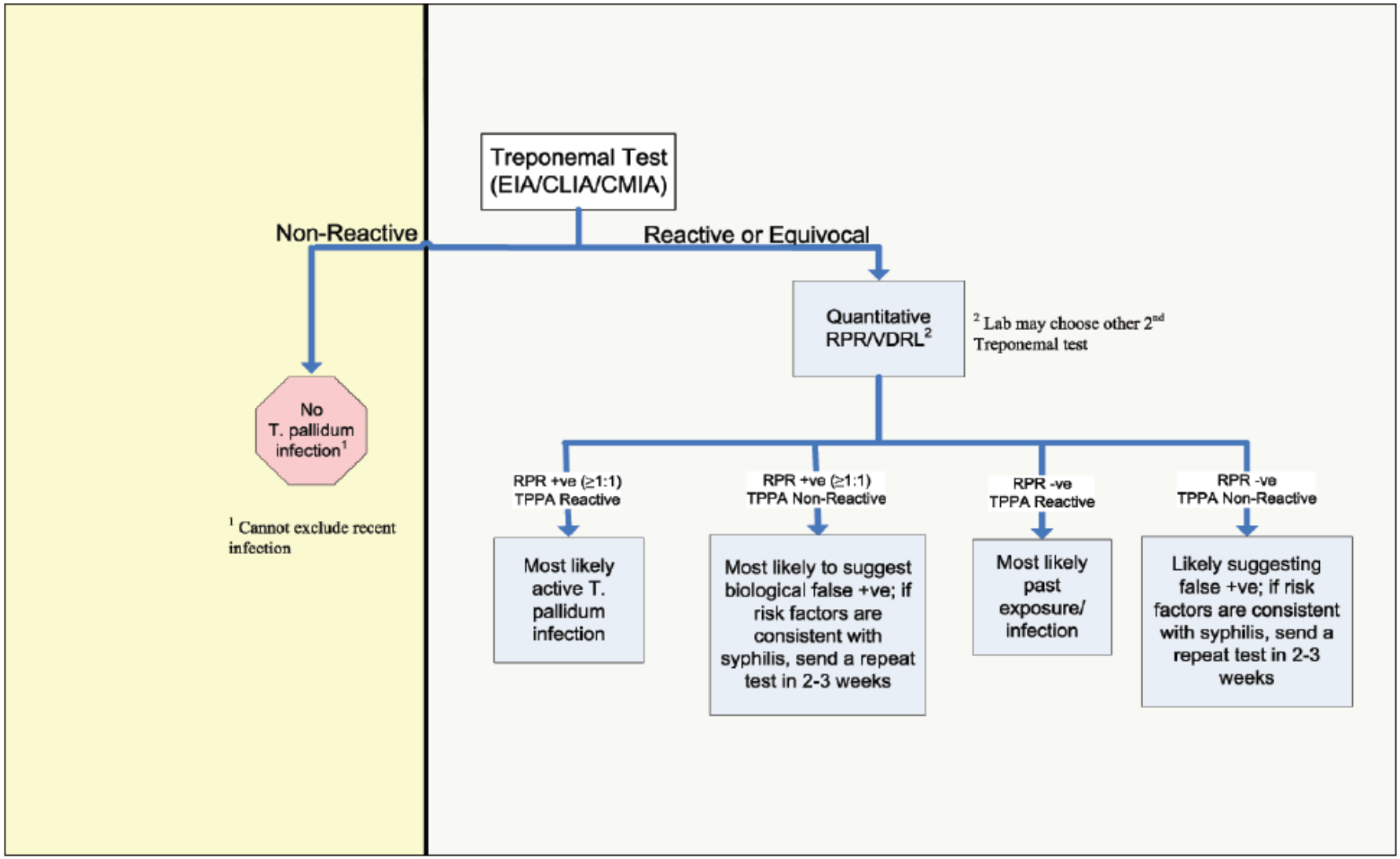
Some other tests R also currently used 4 Syphilis Dx
especially in
Reverse Sequence Syphilis Screening

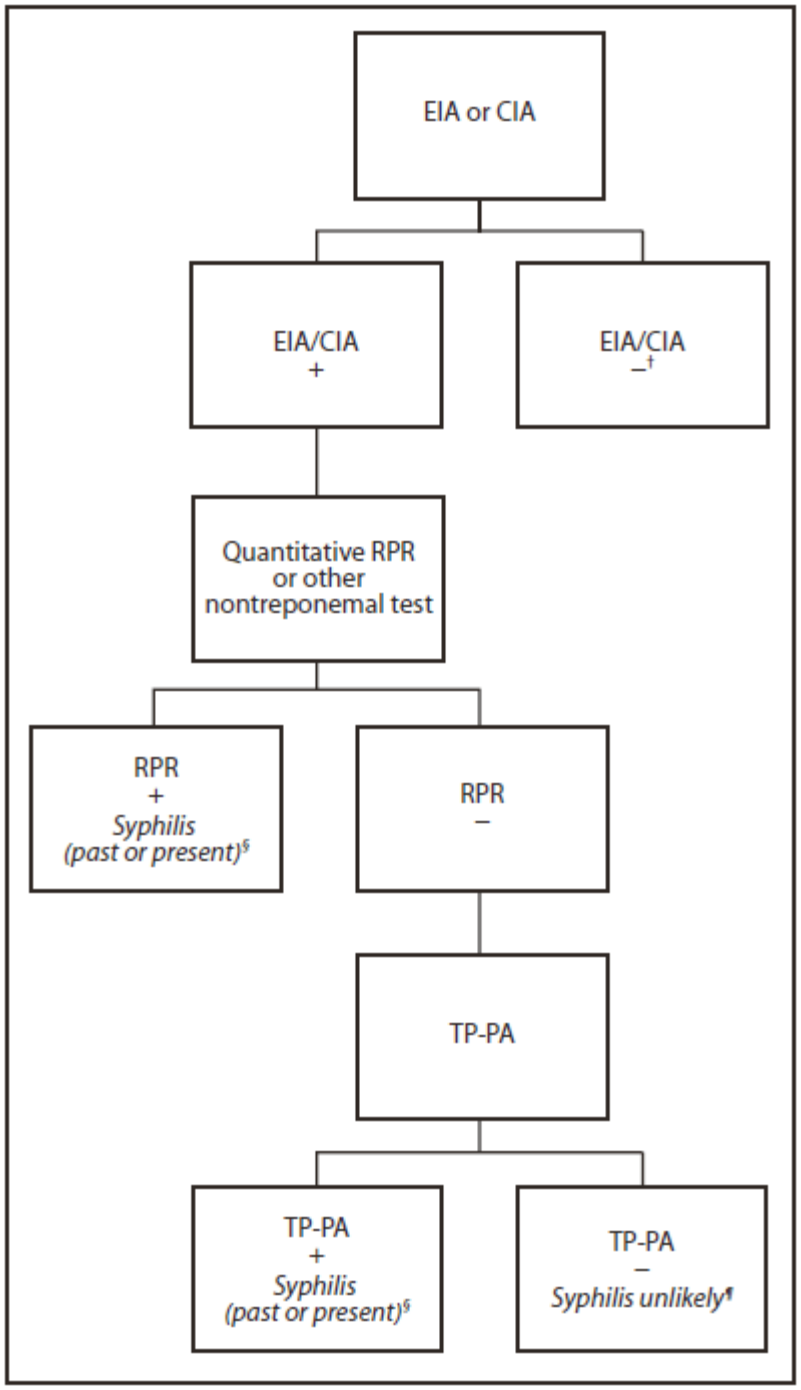


Syphilis Screening by Traditional algorithm (non-treponemal tests first)



Syphilis Screening by Reverse algorithm (treponemal tests 1st)





Recommended algorithm for

reverse sequence syphilis screening

(treponemal test screening followed by nontreponemal test confirmation)

Despite these recommendations for reverse sequence screening, some authorities continue to recommend the traditional algorithm (with reactive nontreponemal tests confirmed by treponemal testing).

† If incubating or primary syphilis is suspected, treat with benzathine penicillin G 2.4 million units intramuscularly in a single dose.

§ Evaluate clinically, determine whether treated for syphilis in the past, assess risk for infection, and administer therapy.

¶ If at risk for syphilis, repeat RPR in several weeks.

Comparison between Treponemal tests using Recombinant Antigens

Manufacturer	Test name or platform	Test format	Specimen	Treponemal antigens included	Antibodies detected
Abbott, USA	Architect Syphilis TP	Two-step sandwich chemiluminescent microparticle assay	30 µL serum or plasma	TpN15, TpN17, TpN47	IgG/IgM
Biokit, Spain	Bioelisa Syphilis 3.0	Microplate EIA	50 µL serum	P15, p17, p47	IgG/IgM
Bio-Rad, USA	BioPlex	Multiplex flow immunoassay	5 µL serum	r15kD, r17kD, r47kD	IgG
Bio-Rad, USA	BioPlex	Multiplex flow immunoassay	5 µL serum	r17 kD, r47 kD	IgM
Innogenetics, Belgium	INNO-LIA	Line immunoassay	10 µL serum or plasma	TpN15, TpN17, TpN47, TmpA	IgG
Diasorin, Italy	Liaison	One-step sandwich chemiluminescent assay	70 µL serum or citrated plasma or heparinized plasma	TpN17	IgG/IgM
Diesse Diagnostica, Italy	Enzy-Well Syphilis IgG	Microplate EIA	20 µL serum or plasma	TpN15, TpN17, TpN47	IgG
Euroimmun, Germany	Anti-Treponema pallidum Screen ELISA (IgG/IgM)	Microplate EIA		p15, p17, p47 and TmpA	IgG/IgM
Euroimmun, Germany	Anti-Treponema pallidum ELISA (IgG)	Microplate EIA		p15, p17, p47 and TmpA	IgG
Euroimmun, Germany	Anti-Treponema pallidum Euroline- WB	Western blot		p15, p17, p45, p47	IgG
Euroimmun, Germany	Anti-Treponema pallidum Euroline- WB	Western blot		p15, p17, p45, p47	IgM
Phoenix Airmid, Canada	TrepSure Anti-Treponema EIA Screen	Microplate EIA	100 µL serum or citrated plasma	TpN (not specified)	IgG/IgM
Siemens, Germany	ADVIA Centaur	One-step chemiluminescent assay	100 µL serum or plasma (EDTA, heparinized, citrate)	Tp15 and Tp17	IgG/IgM
Siemens, Germany	Immulite 2000	One-step chemiluminescent assay	100 µL serum or heparinized plasma	Tp17	IgG/IgM
Trinity Biotech, Ireland	Captia Syphilis TA	Microplate EIA	50 µL Serum or plasma	TpN15, TpN17, TpN47	IgG, IgM and IgA

CASE STUDY

Hx & PE:

- A 19-year-old woman comes to youe center with pain in the right side of her pelvis and a slight temperature.
- She has a history of two episodes of chlamydial cervicitis and herpes simplex vulvitis.
- Physical examination reveals abundant mucopurulent cervical discharge and a painless genital lesion. The patient also has some swelling of her inguinal lymph glands.

Laboratory Data:

- @ the outset of the W/U a stat pregnancy test is ordered. It is positive.

Questions & Discussins:

1- What other Lab Tests would U expect 2 B ordered?

2- Could this Pt have Sigma?!

3- If Syphilis is suspected, what tests should B ordered?

4- Any other point?!

5- Is there risk of congenital infection in this woman's unborn child?

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Screening testing for syphilis can include:
a. Gram stain for *T. pallidum*
b. RPR test
c. Treponemal-MHA-TP
d. FTA-ABS

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- Physical examination reveals abundant mucopurulent cervical discharge and a painless genital lesion. The patient also has some swelling of her inguinal lymph glands.

Laboratory Data:

- @ the outset of the W/U a stat pregnancy test is ordered. It is positive.

Questions & Discussins:

1- What other Lab Tests would U expect 2 B ordered?

2- Could this Pt have Sigma?!

3- If Syphilis is suspected, what tests should B ordered?

4- Any other point?!

5- Is there risk of congenital infection in this woman's unborn child?

CASE STUDY

Hx & PE:

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Diagnostic Testing

T. pallidum cannot be cultured in vitro, and the diagnosis relies on either **direct visualization of the organism or serologic testing.**

In **primary syphilis**, the chancre should be evaluated for spirochetes with **dark-field microscopy or DFA testing.** Both are highly dependent on adequate sample collection and operator skill.

Serologic testing and treatment for presumptive primary syphilis should be performed if a suspicious lesion is present.

Nontreponemal diagnostic tests (e.g., RPR, Venereal Disease Research Laboratory [VDRL]) are **sensitive in immunocompetent patients, but are not specific**.

False-positive results may occur during pregnancy, in IV drug users, or in the presence of other diseases, such as other spirochete infections, tuberculosis, and autoimmune disorders.

False-negative results may occur very early in infection.

In advanced HIV disease, false-negative results or delayed appearance of seropositivity may be seen due to late antibody production.

Very high antibody concentrations may interfere with RPR/VDRL testing and result in a false-negative test (prozone effect). The prozone effect is more common in patients with HIV!! Titers tend to correspond to disease activity.

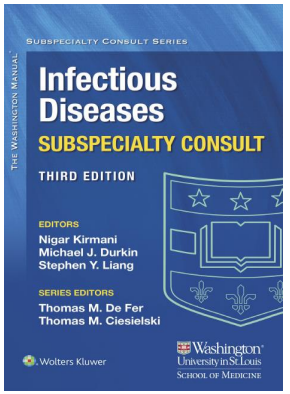
Patients with a history of treated syphilis may have low titers indefinitely. A fourfold increase in titer is indicative of reinfection.

A positive RPR or VDRL should be followed by a confirmatory treponemal-specific test, such as fluorescent treponemal antibody absorption (FTA-ABS), *T. pallidum* particle agglutination assay (TP-PA), or enzyme immunoassay (EIA).

Some laboratories are conducting EIA for initial screening as part of a "reverse screening algorithm."

A positive EIA should be followed with RPR with titer.

All patients with syphilis should be tested for HIV.



دکتر محمد حسن مُرشد

تلفن (منزل) ۷۵۵۸۱۵۵
تلفن (مطب) ۷۵۵۸۱۸۴

VDR۸ [redacted]

آزمایشگاه معتمد

دکتر منوچهر معتمد

شماره دفتر آزمایشگاه ۵۴۵۳

سال ۱۳۵۴/۴/۲۵

پزشک معالج

نام بیمار [redacted]

مواد فرستاده شده

مقصود آزمایش

Réactions humorales du sang.

نتیجه :

Réactions d'hémolyses :

Réaction de Bordet-Wassermann classique Nettement positive.

Réaction de Hecht modifiée ..... Nettement positive.

Réactions de floculations :

Réaction de Kahn ..... Nettement positive.

Réaction de Meinicke .. M. K R. II ..... Nettement positive.

Observation :

آزمایشگاه تشخیص طبی

دکتر نوری نژاد

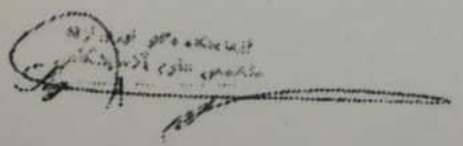
متخصص دوره عالی علوم آزمایشگاهی

Date 18/5/49

Name of Patient Mr. [redacted]

Ex. VDRL

Results. Posetive



آزمایشگاه معتمد

شماره ۲۰۱

سنه ۴۳/۶/۲۲

دکتر محمد حسن عافی

استاد و رئیس آزمایشگاه دبیر دانشکده پزشکی

نام پزشک معالج

نام بیمار [redacted]

Analyse Complète des Urines

CARACTERES GENERAUX

Quantité en 24 h.	X.
Couleur	Jaune ambré.
Aspect	Limpide.
Dépôt	Néant.
Densité	1025

EXAMENS CHIMIQUES

Réaction	Acide.	
Acidité en $So^4 H^2$	1 gr.80	par litre.
Urée	23 gr.30	" "
Phosphates	2 gr.10	" "
Chlorures	20 gr.-	" "
Albumine	Néant.	
Sucre	"	
Acétone	"	
Urobiline	"	
Bilirubine	"	
Sang	"	
Pus	"	

EXAMENS MICROSCPIQUES

Cylindres	ABSENCE.
Cristaux	quelques cristaux d'urate de soude.
Cellules	Assez nombreuses cellules épith.du vagin.
Hématies	Absente.
Les cocytes	Rares leucocytes.

OBSERVATION

Examen du sang pour le séro-diognostic du lues — آزمایش خون برای تشخیص سفلیس

par les procédés : — باطریقه‌های :

1°) Réaction de Bordet - Wassermann : — ۱) آزمایش بورده واسرمان

a) Classique — الف : آزمایش کلاسیك

b) Hecht - Levaditi — ب: آزمایش هشت لوادیتی

} Moyennement positives ++

2°) Réaction de Kahn — Nettement positive +++ — ۲) آزمایش کان

3°) Réaction de Meinicke M. K. R. 2 — Moyennement positive++ — ۳) آزمایش مایتیکه M. K. R. ۲

4°) V. D. R. L. — Nettement positive +++ — ۴) آزمایش V. D. R. L.

اگر یك یا دو از آزمایشهای فوق مثبت باشد مخصوصاً درشخصی که سابقه ابتلا نداشته باشد مشکوك تلقی میشود وباید آزمایش را تجدید نمود بخصوص بعد از زرا کتیواسیون

Observation — ملاحظات :

.....

.....

مدیر آزمایشگاه

۱۰۲۸۷-۱۷

LABORATOIRE CHIMIQUE
ET BACTERIOLOGIQUE

TOUSSI

Dr. A. VARTANY

Prof. à la Fac. de Med.

Téhéran, Av. Hafez No. 181 (Cinéma Iran inclus)

Tel. { Laboratoire 4-24-28
Domicile 4-91-99

Date 196

آزمایشگاه شیمیائی و میکروب‌شناسی

طوسی

دکتر آ. وارتانی

استاد دانشکدهٔ پزشکی

تهران خیابان حافظ چهارراه (یوسف آباد) - ۱۶۱

تلفن { آزمایشگاه ۴-۲۴-۲۸
منزل ۴-۹۱-۹۹

تاریخ 15 7 2 ۱۲٤

شماره تجزیه E.2.435/2

نام پزشک معالج آقای دکتر ~~Mr.le.Dr.H.Morched~~

نام بیمار ~~Mr.~~ [redacted]

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تاریخ ۲ ... ۱۳۴۲

شماره تجزیه ... B. 1428

نام پزشک معالج آقای دکتر ... Dr. le Dr. Mohsen

نام بیمار [redacted]

LABORATOIRE
Dr. R. ZOMORRODI

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Entre Carfour de College et Villa
à Côté de Rue Sarchar,
Tehran, Iran
Teleph. 43904

آزمایشگاه دکتر زمردی
تهران - خیابان شاهرضا
بین چهارراه کالج و ویلا
جنب کوچه سرشار - کاشی ۲۵٤
تلفن ٤۳۹۰٤

No. ۵۵۳۰ شماره

Date ۱۳۵۱/۳/۱۱ تاریخ

Nom de Malade [redacted] نام بیمار

Medecin traitant Dr. M. H. Moreled M.D. نام پزشک

Examen de Sang آزمایش

Resultat نتیجه

Reaction de B-wassermann classique آزمایش واسرمن کلاسیک

Reaction de V.D.R.L. positive + آزمایش وی. دی. ار. ال

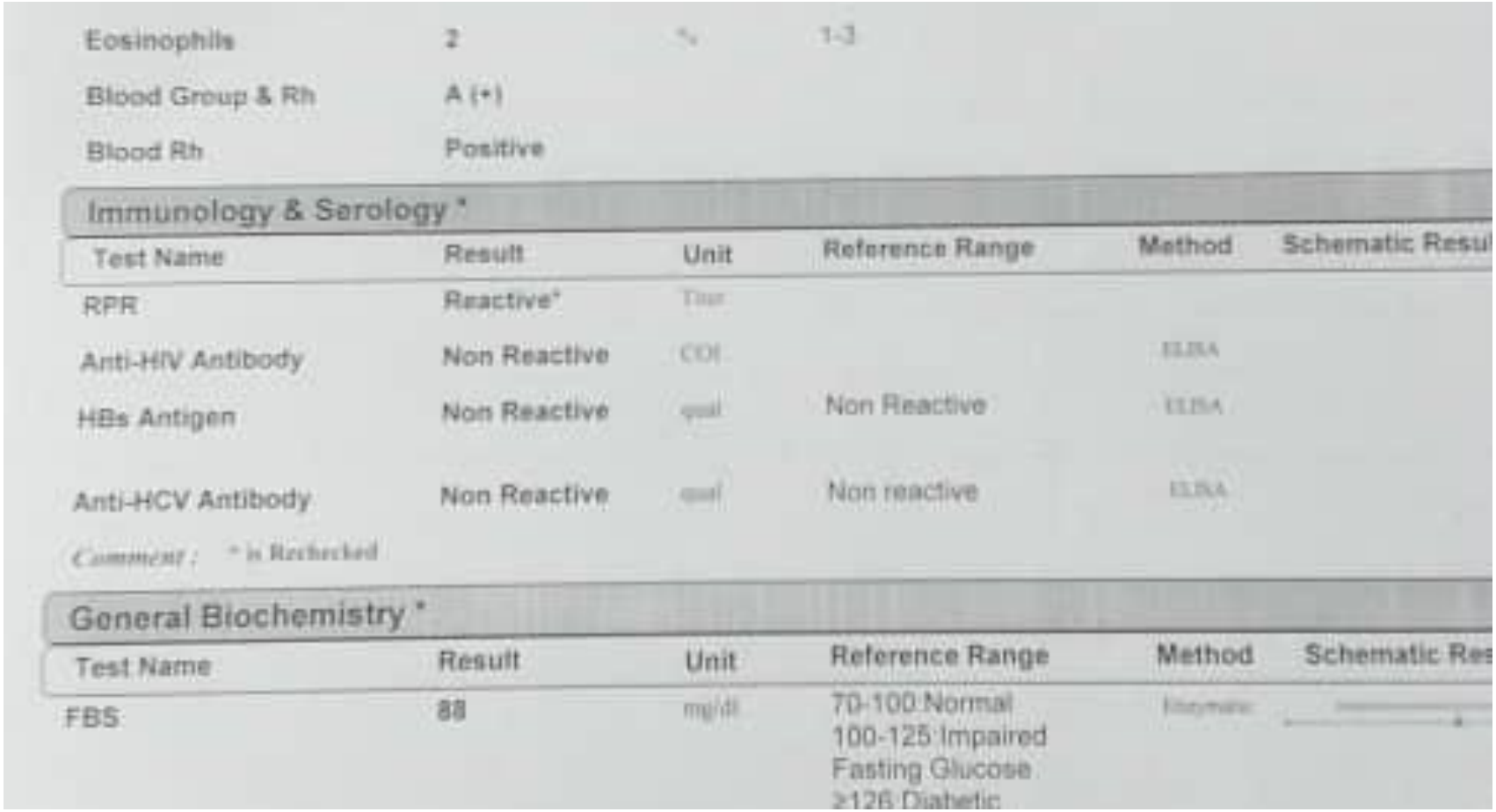
Reaction de Kahn positive + آزمایش کان

دکتر محمد حسن مُرشد

تلفن { مطب ۲۵۵۸۸۴
منزل ۲۵۵۸۸۵

Your Action?!?

1- What is the titer?



Eosinophils	2	%	1-3		
Blood Group & Rh	A (+)				
Blood Rh	Positive				

Immunology & Serology *

Test Name	Result	Unit	Reference Range	Method	Schematic Resul
RPR	Reactive*	Titer			
Anti-HIV Antibody	Non Reactive	COI		ELISA	
HBs Antigen	Non Reactive	qual	Non Reactive	ELISA	
Anti-HCV Antibody	Non Reactive	qual	Non reactive	ELISA	

*Comment : * is Rechecked*

General Biochemistry *

Test Name	Result	Unit	Reference Range	Method	Schematic Res
FBS	88	mg/dl	70-100 Normal 100-125 Impaired Fasting Glucose ≥126 Diabetic	Enzymatic	

2- One of my usual comments below such reports:

NOTE: Some cases of Reactive VDRL/RPR may reveal the presence of Anti-Phospholipid Antibody (and not an STD condition) which merits more workup.

False-Positive serologic Reactions -1

- All of the available serologic tests for syphilis produce occasional reactive results in patients who have no other evidence of syphilitic infection.
- These reactions usually are called ***biologic false-positive*** *(BFP) reactions* and are distinct from positive reactions owing to technical errors.
- Most BFP reactions occur with nontreponemal tests; approximately 1% of normal adults have a BFP reaction by nontreponemal antigen tests.
- These reactions probably are more common in pregnant women than in the general population.
- Reaginic antibody is reactive with at least 200 antigens other than those of *T. pallidum*, and although the specific stimulus for this antibody in syphilis and other diseases is unknown, it may represent antibody to cellular lipoidal antigens of the host that are liberated during various diseases.
- For clinical purposes, BFP reactions may be classified as **acute**, in which the reactivity resolves within 6 months, or **chronic**, in which reactivity is persistent.

From Feigin & Cherry's Txtbk Paed Inf Dis 2019 ed 8

False-Positive serologic Reactions -2

- ***Acute biologic false-positive reactions***.
  - –Most BFP reactions are detected by nontreponemal tests and occur in patients with other acute illnesses, especially pneumonia, hepatitis, and viral exanthematous disease, or after receiving vaccinations. The prognosis for the patient's health is not affected by the finding. The titer of antibody usually is low (<1 : 8), and in most instances the FTA-ABS or TP-PA test is nonreactive.
  - –Approximately two-thirds of patients with BFP reactions have acute reactions, and reactivity subsides within 6 months.
- ***Chronic biologic false-positive reactions.***
  - –Many patients with chronic BFP reactions have or develop systemic disease. Drug addiction, chronic hepatitis, old age, leprosy, and collagen vascular disease, especially systemic lupus erythematosus, are associated highly with chronic BFP reactions.
- A familial predisposition to BFP may exist (in endemic and non-venereal (non-sexual) treponemal infections and in autoimmune states).

False-Positive serologic Reactions -3

- The antibody detected by the VDRL test in chronic BFP reactions predominantly is IgM, whereas it mainly is IgG in syphilis.
- A finding of particular concern (in the western countries, Feigin 2019, ed 8) is that there seems to be a relative increase in acute and chronic BFP reactions in women who are infected with HIV.
- Screening with EIA/CIA treponemal tests has resulted in some individuals having a reactive EIA/CIA test but a nonreactive nontreponemal test. If the individual does not have a history of adequately treated syphilis, a second treponemal test (TP-PA preferred) should be performed, and if it is negative, the positive EIA/CIA is more likely to represent a false-positive test.

About Screening and Confirmation (regarding sensitivity & Specificity) and the Disease Management

- Both **treponemal** and **non-treponemal** tests are only **moderately sensitive** (~70%-85%) for **primary syphilis**.
- **Both types of tests** are **very sensitive** (>95%) for **secondary syphilis**.
- **Treponemal tests** are **very sensitive** for **tertiary and latent syphilis**.

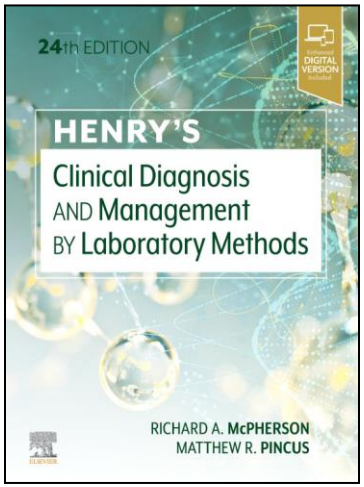
  In contrast, **non-treponemal** antibody titers fall with time and so **non-treponemal tests** are somewhat **less sensitive** for **tertiary or latent syphilis**.

- Both **non-treponemal** and **treponemal** tests can be used to **screen** for syphilis, but positive results should be **confirmed** using a test of the other type (e.g., **confirm non-treponemal** positive test results with a **treponemal** test & vice versa). **Confirmatory testing** is needed because false positive results can occur in both **non-treponemal** and **treponemal** tests.
- Causes of false-positive results in these tests include pregnancy, autoimmune diseases, and infections other than syphilis.

- **Non-treponemal** antibody levels fall with successful **treatment of syphilis**, and so changes in the titers detected in these tests can be used ***to monitor therapy***.
- **Treponemal tests**, usually non-quantitative, remain positive even after **successful therapy**.

Sensitivities of Serologic Tests for Syphilis in Different Stages of Disease

Test	STATE OF SYPHILIS, % SENSITIVITY (RANGE)				% Specificity (Range)
	Primary	Secondary	Latent	Late	
Nontreponemal					
VDRL	78 (74-87)	100	95 (88-100)	71 (37-94)	98 (96-99)
RPR	86 (77-100)	100	98 (95-100)	73	98 (93-99)
Treponemal					
FTA-ABS	84 (70-100)	100	100	96	97 (94-100)
MHA-TP	76 (69-90)	100	97 (97-100)	94	99 (98-100)
TPPA	88 (86-100)	100	100	NA	96 (95-100)
IgG ELISA	100	100	100	NA	100
CLIA	98	100	100	100	99



From Henry's Clin D&M by LM 2022, ed 24, p.1234

	% Sensitivity at Given Stage of Infection				
	Primary	Secondary	Latent	Tertiary	% Specificity
Serology, nontreponemal					
VDRL	78 (74–87)	100	95 (88–100)	71 (37–94)	98 (86–99)
RPR	86 (77–100)	100	98 (95–100)	73	98 (93–99)
USR	80 (72–88)	100	95 (88–100)	—	99
RST	82 (77–86)	100	95 (88–100)	—	97
TRUST	85 (77–86)	100	98 (95–100)	—	99 (98–99)
Serology, treponemal					
FTA-ABS	84 (70–100)	100	100	96	97 (94–100)
FTA-ABS double staining	80 (69–90)	100	100		98 (97–100)
MHA-TP	76 (69–90)	100	97 (97–100)	94	99 (98–100)
PCR (PBMC/serum)	46 (25–64)	86	66 (62–71)	—	100
RT-PCR (whole blood)	28 (10–53 CI)	36 (19–55 CI)	0	—	100
Tissue (skin/mucosa/exudates)					
Dark-field microscopy	84 (71–100)	60 (25–100)	—	—	98 (97–100)
Silver stain histochemistry*	86 (50–100)	40 (0–92)	—	4 (0–11)	—
Direct immunofluorescence	90 (80–100)	70 (68–71)	—	—	—
Immunohistochemistry	100	87 (58–100)	—	36 (11–60)	100†
PCR (tissue)	100	67 (42–100)	—	7 (0–14)	—
PCR (lesional smear)	94 (91–96)	80	—	—	98 (96–100)
RT-PCR (lesional smear)	80 (44–97 CI)	20 (0.5–72 CI)	—	0‡	—

- Serologic tests are the most sensitive ones.
- Treponema pallidum cross-reacts with Borrelia burgdorferi (Lyme disease).

(MHA-TP, microhemaggl. Assay for T. pallidum; RST, regain screen test; TRUST, toluidine red unheated serum test; USR, unheated serum regain test)

Am J Dermatopathol Volume 33, Number 5, July 2011

CSF-VDRL is preferred to RPR in neurosyphilis samples

- The RPR (nontreponemal test) is a serum assay. It is not approved for CSF specimens. The VDRL-CSF is the nontreponemal test approved for detection of neurosyphilis but it has low **sensitivity** (around 60-80%).
- The CSF VDRL has high diagnostic **specificity** in neurosyphilis. A false-positive VDRL titer occur only if the CSF is bloody. However, a negative CSF FTA-ABS may be useful to rule out neurosyphilis.
- It is also said that the presence of **treponemal** antibody in the CSF is not diagnostic for neurosyphilis since it may represent passive diffusion of **treponemal** antibody from the blood into the CSF due to the leakge of damaged meninges.

A short Recall:

- Yaws, bejel (endemic syphilis), and pinta are not sexually transmitted, are endemic to various tropical and Middle Eastern regions, and are caused *respectively* by:

✓ *T. pallidum subsp. pertenue (=T. pallidum pertenue),*

✓ *T. pallidum subsp. Endemicum,* and

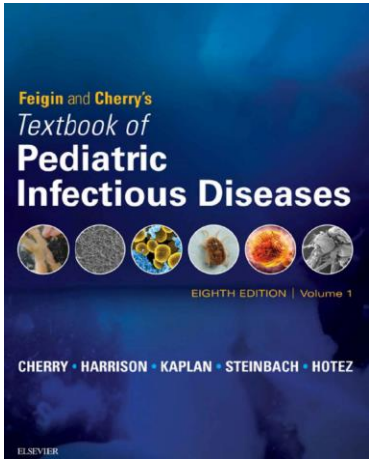
✓ *T. carateum.*

- They are transmitted person to person, neither sexually nor congenitally, but acquired by means of skin or mucous membrane contact. Close contact with infected children or young adults, traumatized areas of skin, lack of clothing, and poor personal hygiene are important factors in the transmission of these diseases.
- Presumptive diagnosis of the endemic treponematoses can sometimes be made on the basis of clinical and epidemiologic data; however, as with venereal syphilis, **serologic tests** and direct microscopic techniques may prove useful.
- Human studies suggest that *T. carateum* induces significant **protection** against homologous reinfection and **cross-immunity** against the treponemes of yaws and syphilis; however, these two subspecies of *T. pallidum* fail to confer cross-protection against *T. carateum.*

Comparison of the Treponematoses

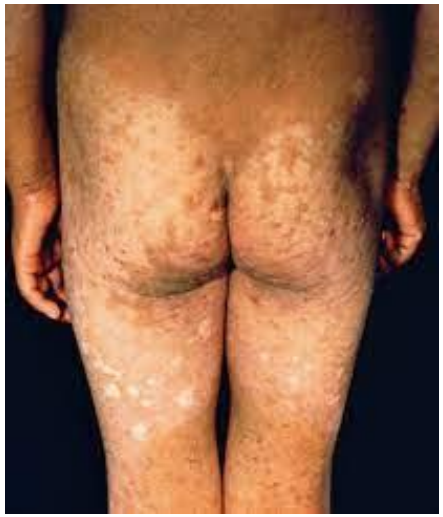
	Syphilis	Yaws	Endemic Syphilis	Pinta
Organism	*T. pallidum* subsp. *pallidum*	*T. pallidum* subsp. *pertenue*	*T. pallidum* subsp. *endemicum*	*T. carateum*
Distribution	Worldwide	Tropics, Africa, Asia, Pacific	Arid areas of Africa, Arabia	Tropical Central, South America
Peak ages affected	All ages	1–12 y	<15 y	15–20 y
Route of entry	Mucous membranes	Skin	Oral mucosa, fomites	Skin
Incubation period	2–10 wk	3–9 wk	3 wk	2–3 wk
Experimental hosts	Rabbit, primate, guinea pig, hamster	Rabbit, hamster	Rabbit, hamster	Primate only
Sexual transmission	Yes	No	No	No
Congenital transmission	Yes	No	No	No
Skin infection	Yes	Yes	Yes	Yes
Bone involvement	5–10%	20–40%	20–40%	0
Neurologic involvement	Yes	No	Rare	No
Cardiologic involvement	Yes	No	Rare	No

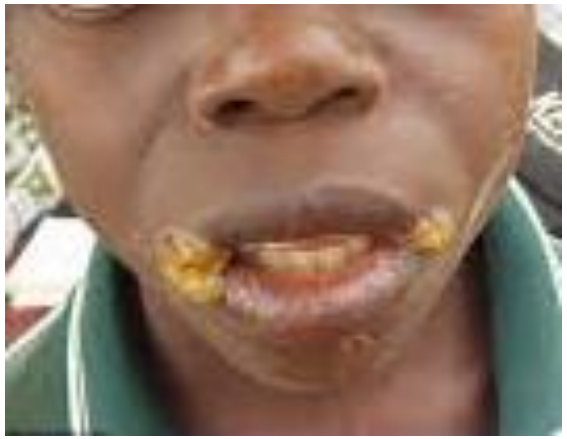
On the basis of experimental studies in inbred hamsters, *T. pallidum subsp. Endemicum* stimulates a high degree of homologous **resistance to reinfection** and **cross-immunity** to *T. pallidum subsp. pallidum* and *T. pallidum subsp. Pertenue.*

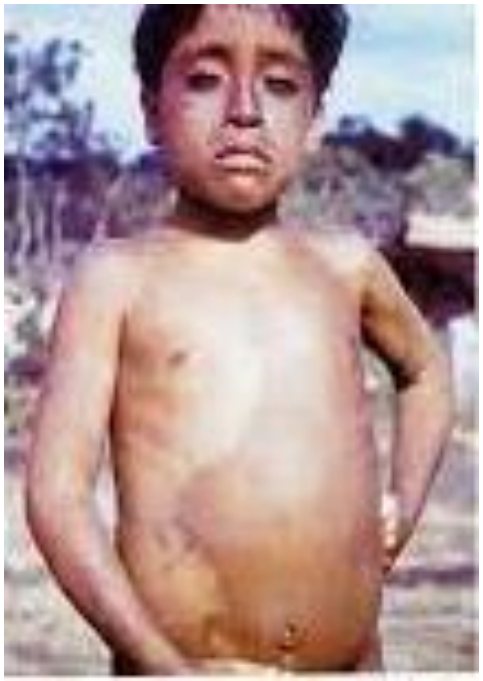


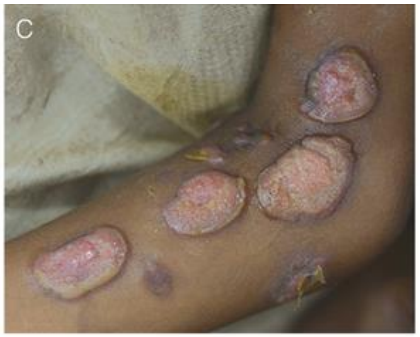
2019 ed 8
on page 1285

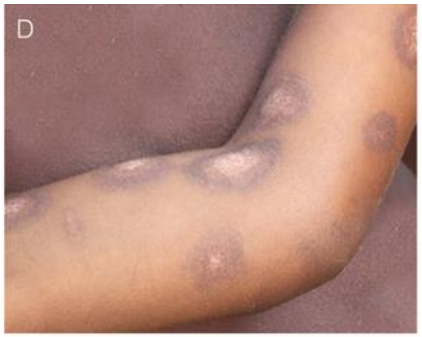
Pinta, Bejel, Yaws

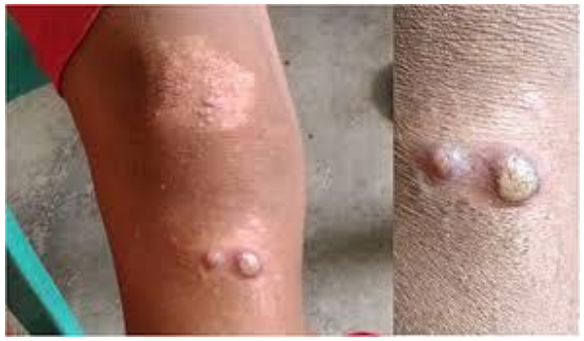


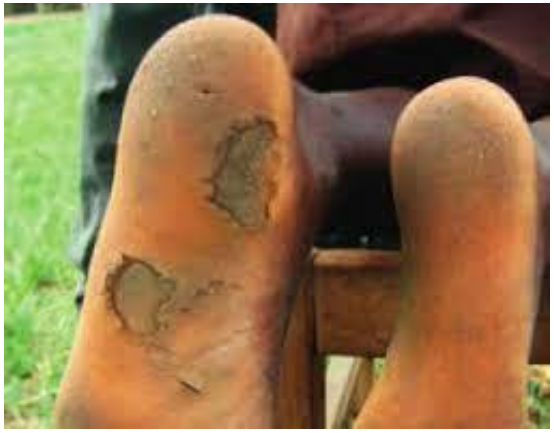


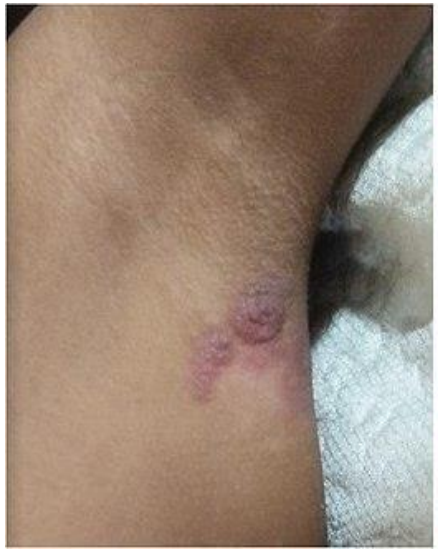
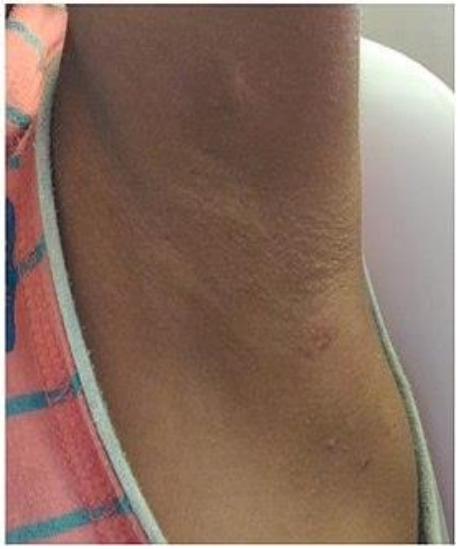
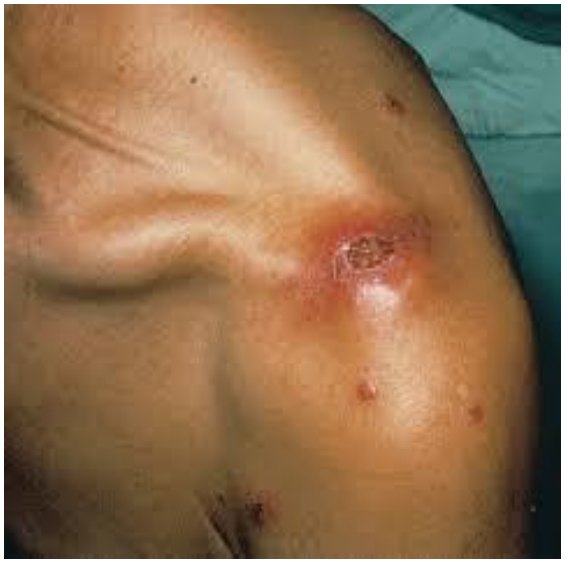
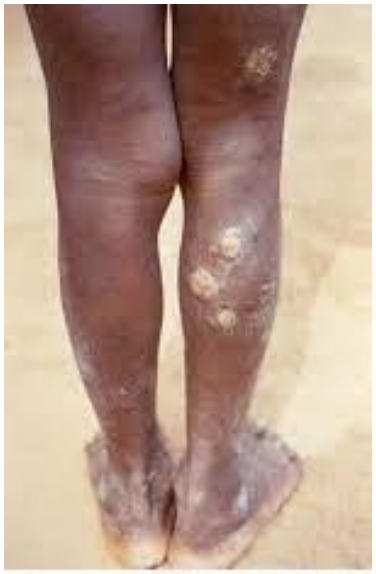
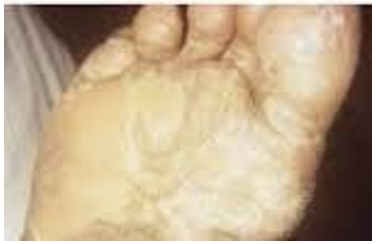
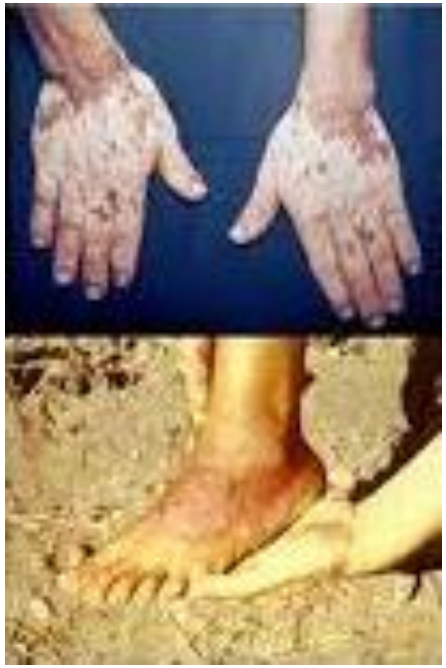
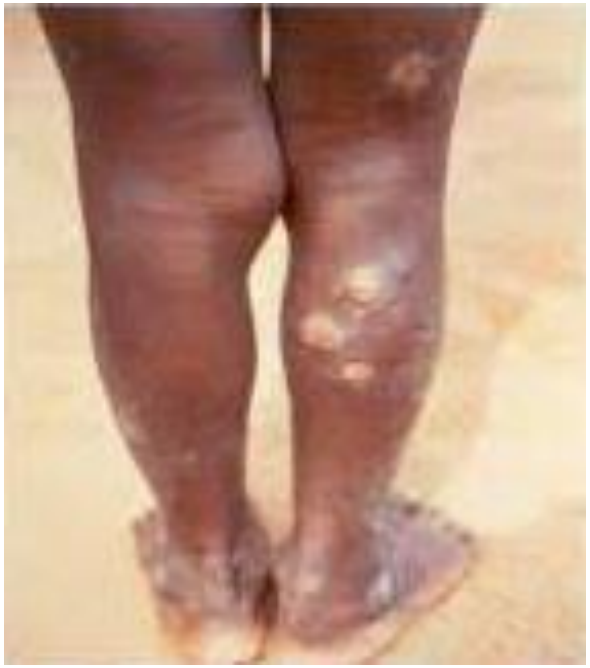
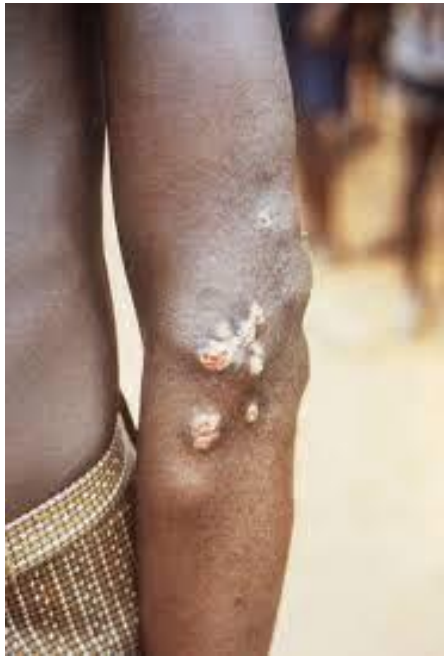










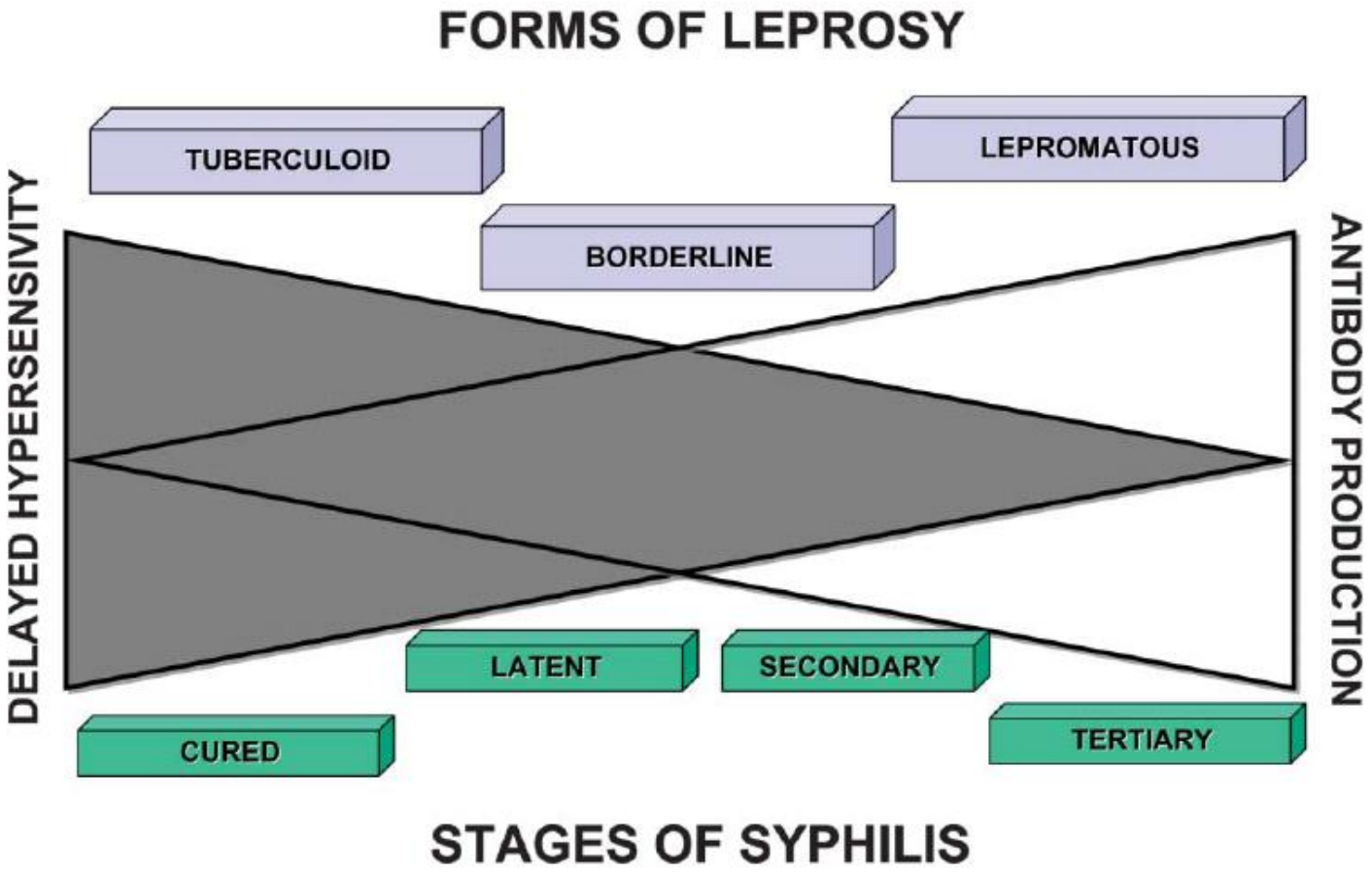










افتراق ترپونماتوز سیفیلیسی از ترپونماتوز غیرآمیزشی

1. نحوه انتقال
2. سن پیدایش آلودگی
3. ظاهر بالینی ضایعات
4. نواحی مبتلا روی بدن یا در داخل
5. آزمایش منفی برای قارچ یا کشت باکتری
6. توزیع جغرافیایی در کشور

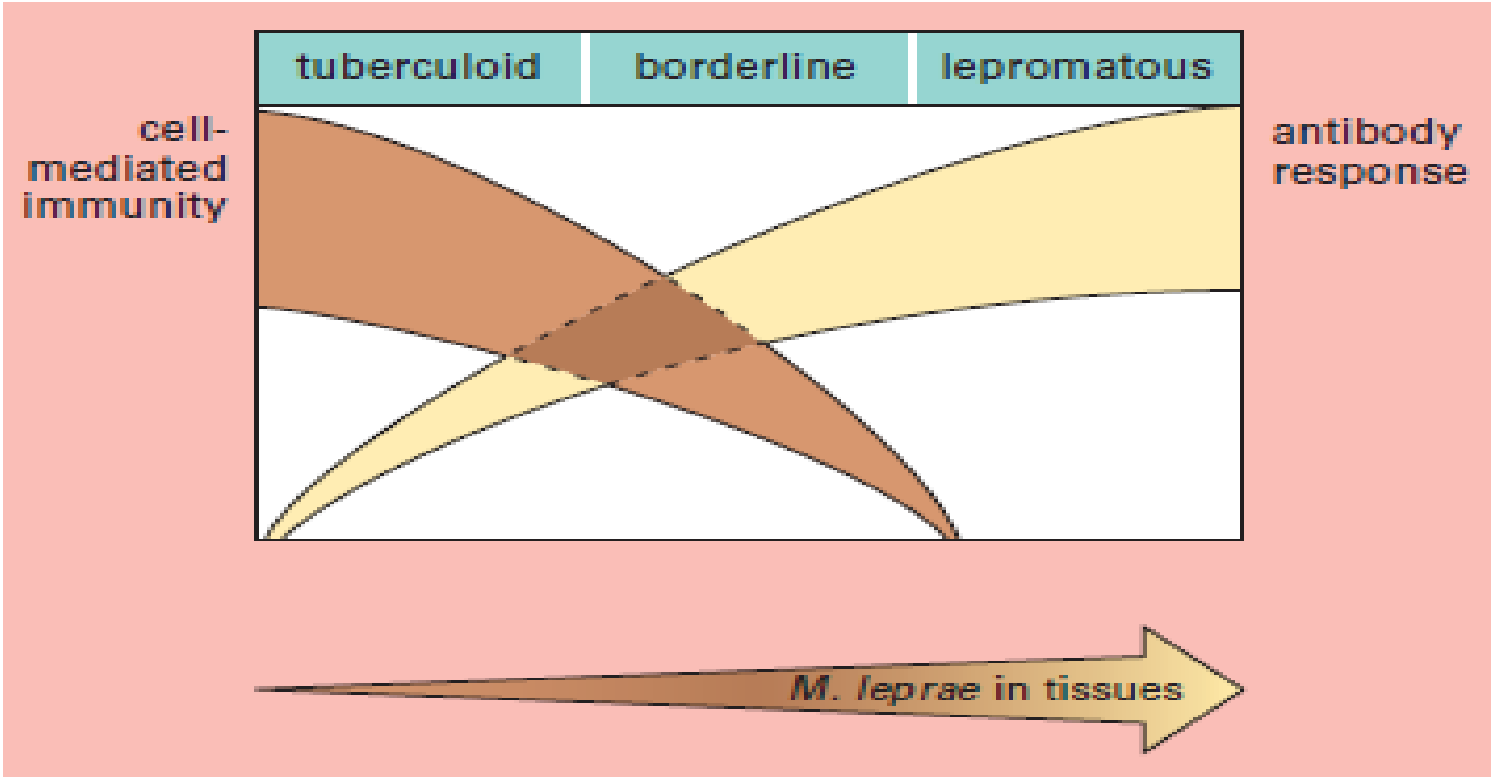
A comparison of the stages of syphilis and leprosy

and their relationship to the balance of Humoral and Cellular Imm.



- Non-Trep. Tests frequently yield F.P. results in Leprosy **(Jawetz, ed 20)**
- High levels of DTH are associated with cure; weak DTH is associated with progressive disease.
- Progression of syphilis to the tertiary stage is most likely more related to depressed T-cell immunity, with or without high levels of antibody production.

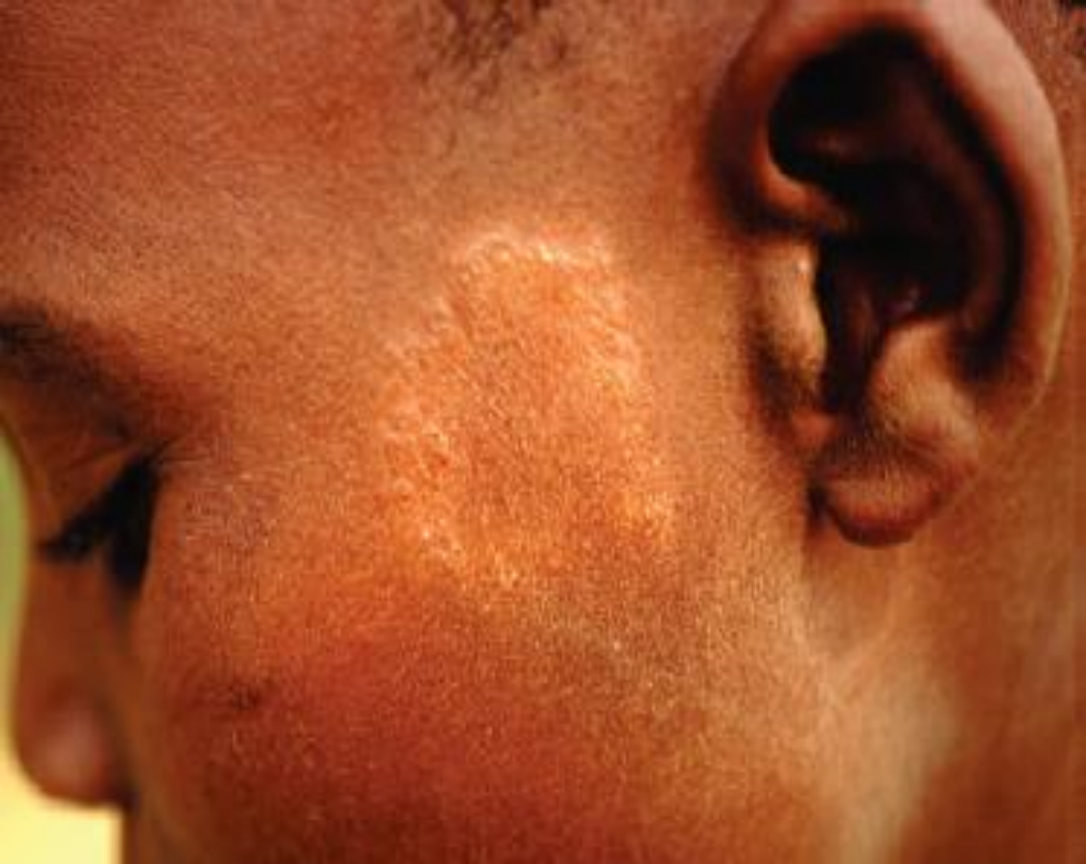
The immunological spectrum of leprosy

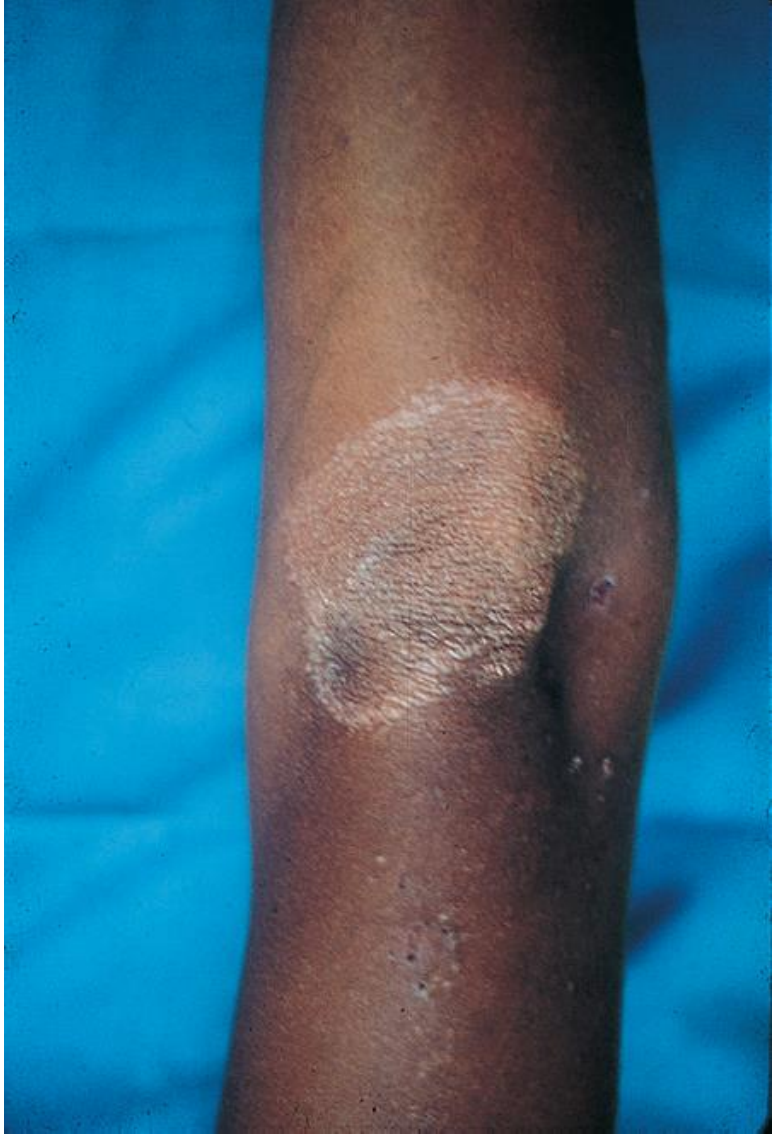


- The clinical spectrum of leprosy ranges from **tuberculoid** disease with few lesions and bacteria to **lepromatous** leprosy, with multiple lesions and uncontrolled bacterial proliferation.
- This range reflects host immunity as measured by specific CMI and antibody responses to *M. leprae*, and the tissue expression of cytokines.

Comparative table for Bipolarized Leprosy

Tuberculoid L.	Lepromatous L.	معیار مقایسه
intact CMI, low Ab, Skin Th1 lymphocytosis, Normal serum Ig. levels	Weak CMI, Hi Ab, Foamy Mϕ, Low IFNγ, Low IL2, Hypergammaglobulinemia	پاسخ ایمنی
Scanty- Paucibacillary L	Many- Multibacillary H	تعداد باسیل **Infectivity**
Limiting & Benign	Progressive & Destructive	سیر بیماری
Few erythematous macules or hypopigmented plaques	Many erythematous macules, papules, or nodules (=Leproma)	نوع ضایعات در پوست
Sudden onset, Asymmetric, Complete sensory loss, Visible nerve enlargement	Slow, Symmetric, Patchy sensory loss, Usually no nerve enlargement	**Nerve Involvement**
Pos	Neg	**Lepromin Skin Test**
Y	N	**Granuloma**
mouse footpad	nine-banded armadillo	**Lab Cultivation**

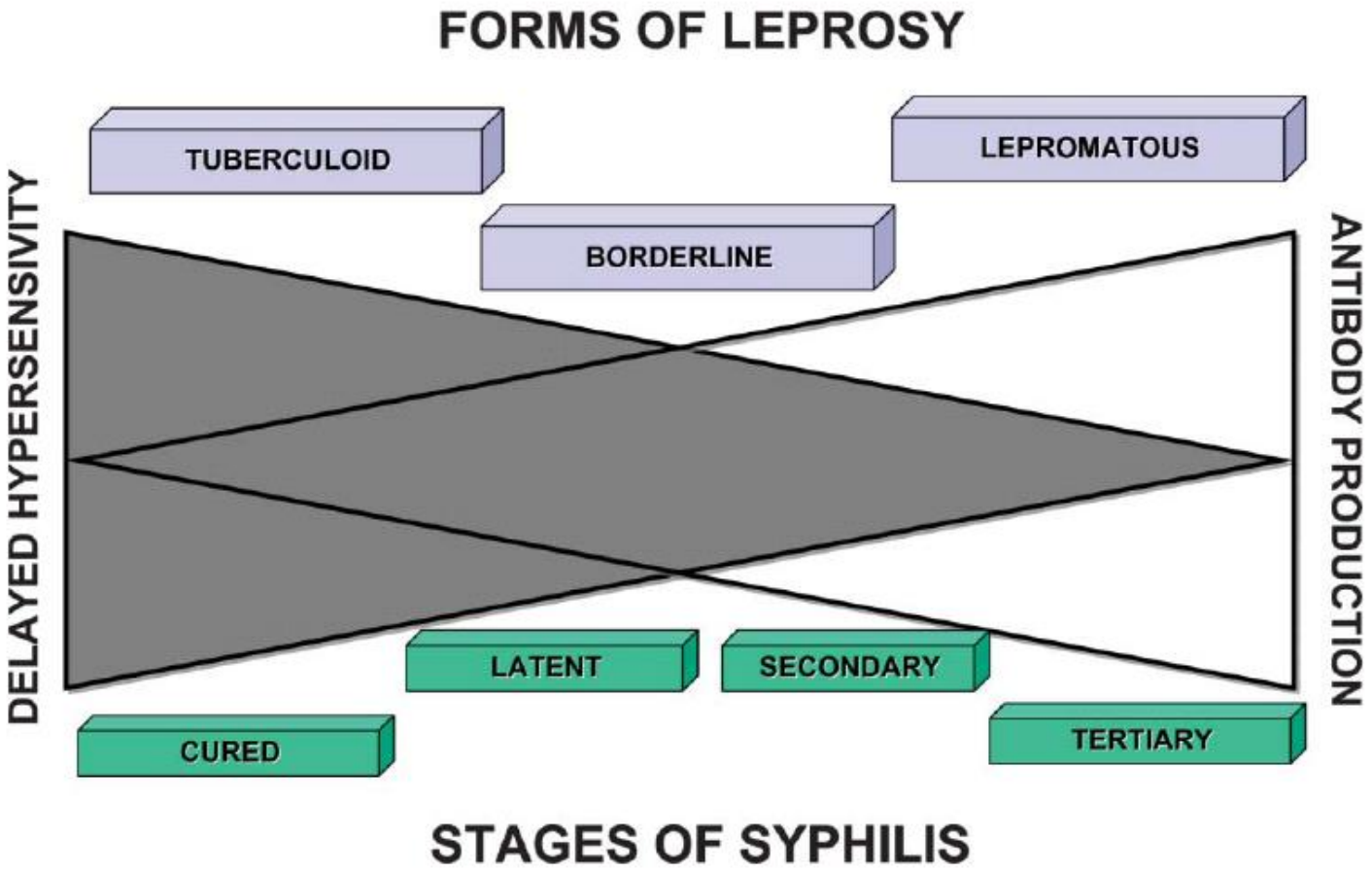




Early tuberculoid lesions in leprosy are usually characterized by **anesthetic macules with hypopigmentation**.

A comparison of the stages of syphilis and leprosy

and their relationship to the balance of Humoral and Cellular Imm.



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Jarisch–Herxheimer reaction

- Within 24 hours after antibiotic treatment of the spirochetal infections (syphilis, Lyme disease, leptospirosis, and relapsing fever), patients experience:
  - ✓ shaking chills, a rise in temperature, malaise, tachycardia,
  - ✓ intensification of skin (any existing skin lesions become more prominent), a flush due to vasodilation,
  - ✓ and leukocytosis,
  - ✓ with symptoms resolving a few hours later over a period of 6-12 hours.
- JHR can also include uterine contractions in pregnancy, worsening liver and renal function, acute respiratory distress syndrome, myocardial injury, hypotension, meningitis, alterations in consciousness, seizures, and strokes. JHR is not seen with subsequent treatment.
- JHR is caused by non-endotoxin pyrogen and spirochetal lipoproteins and is then mediated by the pro-inflammatory cytokines (TNF, IL6, and IL8).
- The reaction should not ascribe to a sensitivity to the antibiotic.
- Syphilis may be suspected by the appearance of this reaction when treating another infection!
- Candidiasis (treated with systemic fluconazole) has also been reported as cause.
- The frequency of JHR is:
  - ➢ Seropositive primary syphilis (95%)
  - ➢ Secondary syphilis (95%)
  - ➢ Lyme disease (7-30%)
  - Seronegative primary syphilis (55%)
  - Latent and late syphilis (0%)
  - Leptospirosis (9%)

آزمون ایمونوسرولوژی بیماریهای اسپیروکتال
برای انجمن متخصصین آزمایشگاه
(مبحث دوم- دوشنبه 10 آبان 1400)

1	سوال: پدیده منطقه‌ای (یا فنومن دوزون!) در کدامیک از آزمایش‌های زیر رخ می‌دهد؟	
	الف	FTA-Abs
	ب	TPI
	ج	VDRL
	د	نلسون – مایر
منبع:	Harrison’s PIM, 2019, ed 20	

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Let's have another review in a Lab perspective

- All non-treponemal tests have **similar performance** characteristics. They detect both IgM and IgG.
- All non-treponemal tests in current use are **flocculation tests**.
- Interpretation of flocculation tests is **subjective** and, therefore, depends on staff experience.
- The non-treponemal tests currently licensed and available in I.R. Iran are the RPR and the VDRL.
- The VDRL is now conserved primarily for testing **CSF** mainly in referral hospitals (such as Imam Khomeini Hosp. Complex, Tehran).
- Non-treponemal tests are usually used in a traditional algorithm as **qualitative** assays or as **quantitative** assays to assess the **response to treatment** or to estimate the probability of the presence of a **Biologic False Reaction**. Screening tests are performed using undiluted serum.
- Results are reported as non-reactive or reactive.
- Reactive specimens are re-tested in a **two-fold dilution** series for quantitative results. Titres usually decline significantly after successful therapy. Treated patients should be followed for up to 24 months; this prolonged follow-up is necessary to ensure titres decline to the expected extent.
- A **prozone** phenomenon occurs in high-titre specimens, which appear as non-reactive unless the samples are diluted.
- False-positive reactions are categorized as either **acute** (occurring for <6 months) or **chronic**.
- The recognized causes of **acute false-positive reactions** include other febrile illnesses, immunizations and pregnancy. Patients with acute false-positive reactions should be re-tested in three to six months.
- **Chronic false-positive reactions** are associated with hepatitis C infection, connective tissue diseases, intravenous drug use, malignancy, older age, malaria, Chagas disease, tuberculosis and leprosy.

Borreliosis

- *Borrelia spp. are divided into two major groups:*

–*the **Lyme** borreliosis* group that are transmitted exclusively by hard-bodied ticks, and

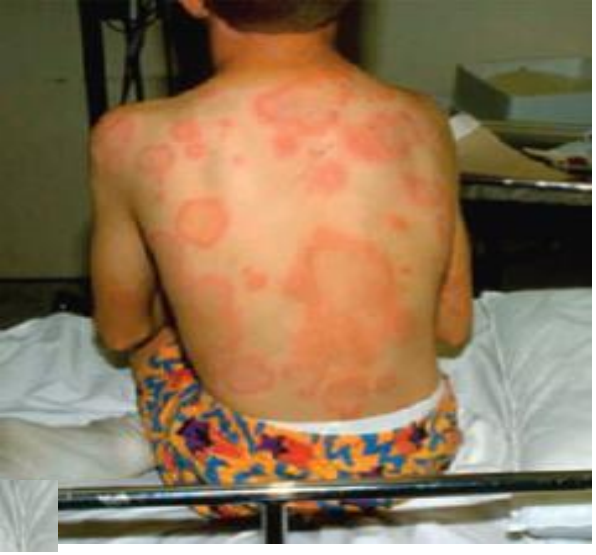
–the **relapsing fever** group that are primarily transmitted by soft-bodied ticks but also by hard-bodied ticks and lice.

- ***Borrelia recurrentis*** is the common sp. in **Iran**. The most common tick-borne disease in North America and Europe, Lyme disease is caused chiefly by ***Borrelia burgdorferi*** (senso lato or senso stricto!)***, Borrelia garinii****, and* ***Borrelia afzelii****.*
- Epidemic, louse-borne relapsing fever has a worldwide distribution but Endemic, tick-borne relapsing fever has a limited geographic distribution and is caused by several *Borrelia species.*
- Diagnosis of relapsing fever is most often established through the demonstration of spirochetes in **peripheral blood smears** stained by a Giemsa technique.

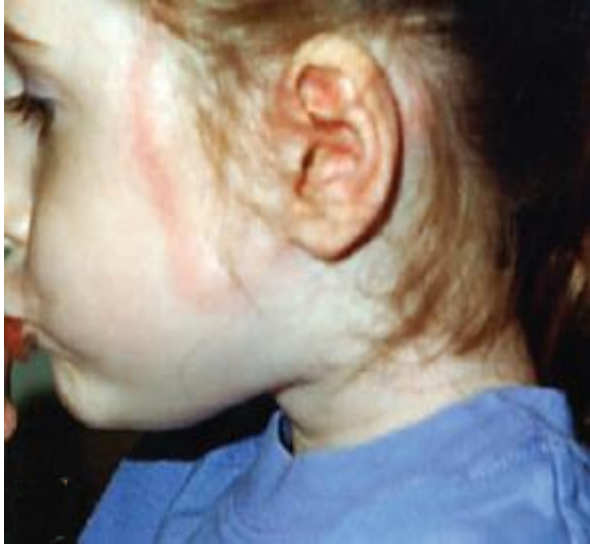
Immune Response against Borrelias

- *Borrelia are* initially phagocytized in the bloodstream by neutrophils, macrophages, and dendritic cells that cause the release of cytokines and other immune factors, resulting in symptoms.
- Antibodies, especially IgM plays a central role in bacterial eradication, and most *Borrelia are cleared from the bloodstream after 2 to 3 days. Surviving Borrelia are able to change* ***outer membrane proteins*** *so that the original* IgM antibodies are no longer effective. A new **antigenic variant** emerges and causes recurrence of fever and other symptoms. The same process repeats itself for several cycles in untreated patients.
- Because there is some antibody cross-reactivity, subsequent spirochetemia and clinical manifestations usually are milder.
- *Borrelia* ***antigenic variation*** is a result of new outer surface VMPs that are encoded by alternative *vmp genes found on linear plasmids. There is a copy of silent and* expressed genes on the same or different plasmids within each borrelial microorganism.
- **Antigenic switching** occurs when a silent *vmp gene* begins to encode for protein while the existing *vmp gene is shut down.*
- The stimulus for antigenic switching is unknown. Recent data suggest that the antigenic switching that occurs when relapsing *Borrelia move* from mouse to tick is induced by the temperature change from the warm-blooded host to the cooler tick vector.

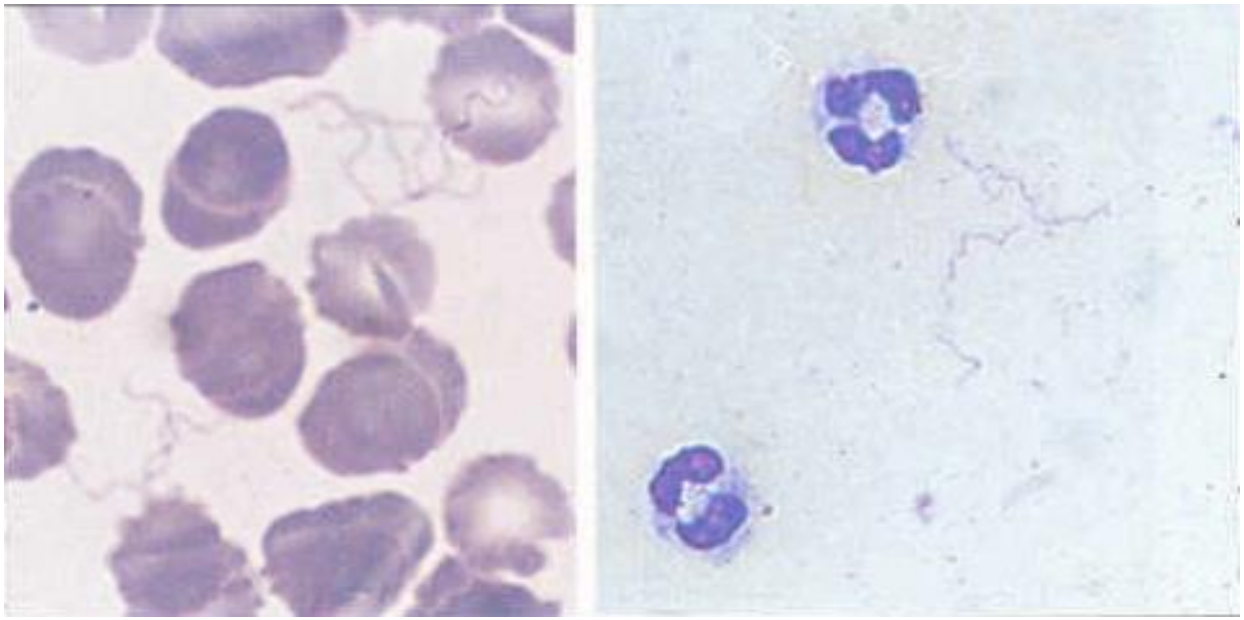
- Relapsing fever ***Borrelia*** are not confined to the bloodstream but also infect the nervous system, eyes, lung, liver, kidney, heart, and spleen. They may persist in these tissues.
- There is evidence in animal models that the same serotype antigenic variation that is seen in the blood also occurs in brain tissue, where ***Borrelia*** have been shown to persist for up to 3 years.
- Tissue damage in fatal human cases has been noted in the central nervous system (meningitis, degenerative lesions, hemorrhage, and perivascular infiltrates), heart (myocarditis), lung (pneumonia), gastrointestinal tract (hemorrhage), liver (enlargement, hepatic necrosis), and spleen (splenic abscess) probably due to exacerbated **immune response**.
- Relapsing fever *Borrelia* can cross the placenta or be transmitted during birth, causing abortion, premature birth, or severe perinatal infection.



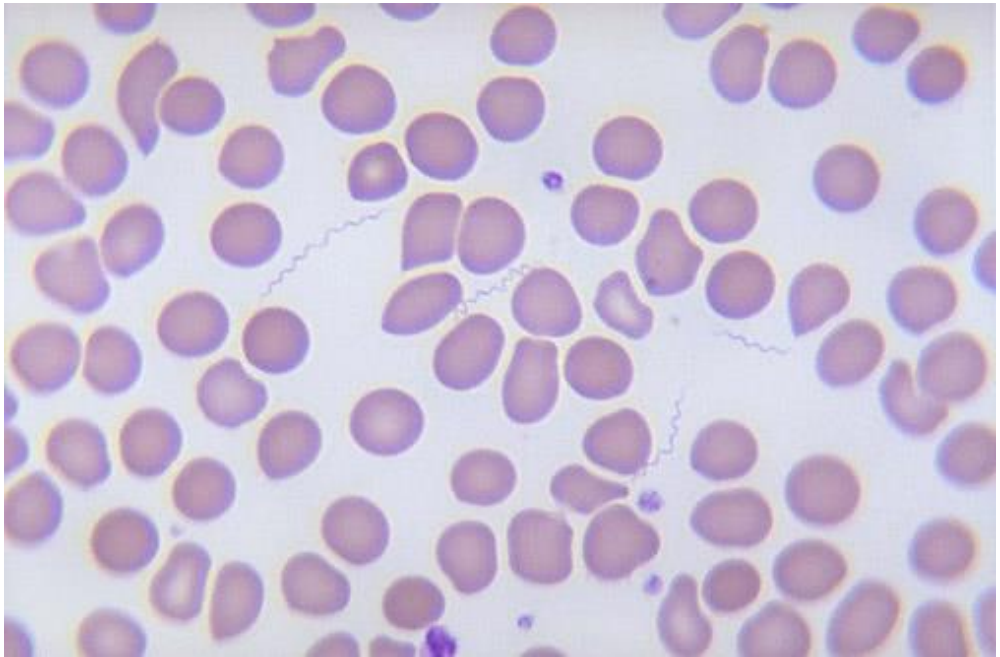
Lyme Disease



- Lyme disease, not yet reported from Iran is a common arthropod-borne illness caused by the spirochete, ***Borrelia burgdorferi***, which can be localized or disseminated with a tendency to cause persistent chronic arthritis.
- It is caused by several subspecies of the spirochete ***Borrelia burgdorferi***, which is transmitted from rodents to people by *Ixodes* deer ticks.
- Lyme disease is endemic in the US, Europe, and Japan.
- In endemic areas, *B. burgdorferi* infects up to 50% of ticks, which may also be infected with ***Ehrlichia*** and ***Babesia***.
- Serology is the main method of diagnosis, but PCR can be done on infected tissue.



PBS (thin & thick) of a patient with Relapsing fever
Giemsa stained (100x objective)



Lyme Clinical Picture

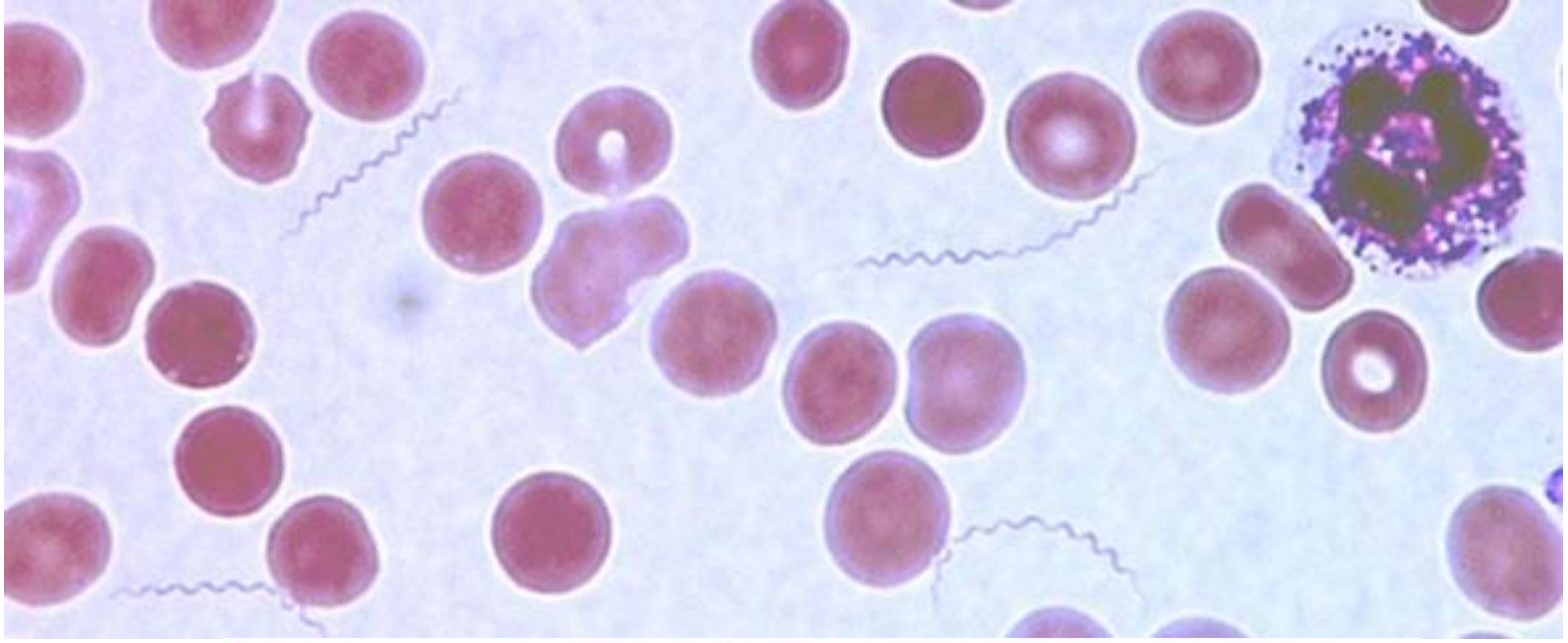
- Lyme disease involves multiple organ systems and is divided into **three stages**.

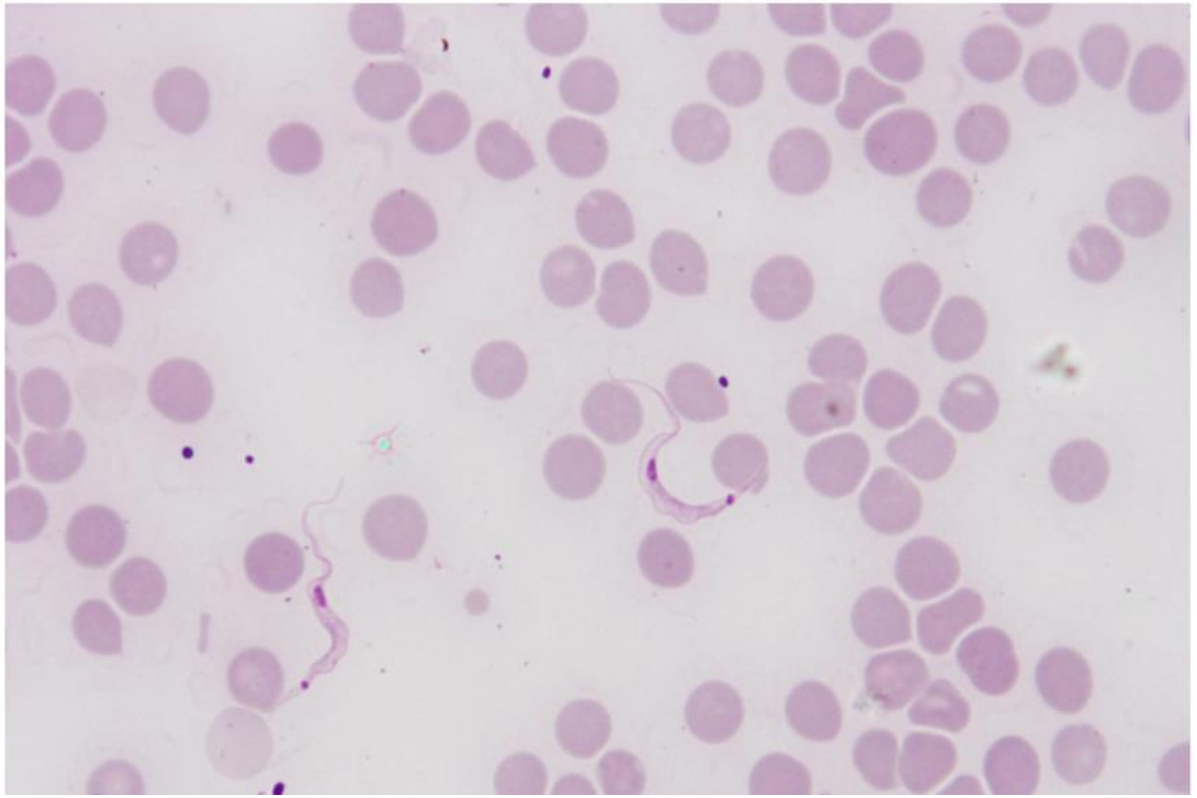
- In **stage 1** (**localized infection**) spirochetes multiply and spread in the dermis at the site of a tick bite, causing an expanding circular area of redness, often with a pale center. This lesion, called **erythema migrans**, may be accompanied by fever and lymphadenopathy. The rash spontaneously disappears in 4 to 12 weeks.

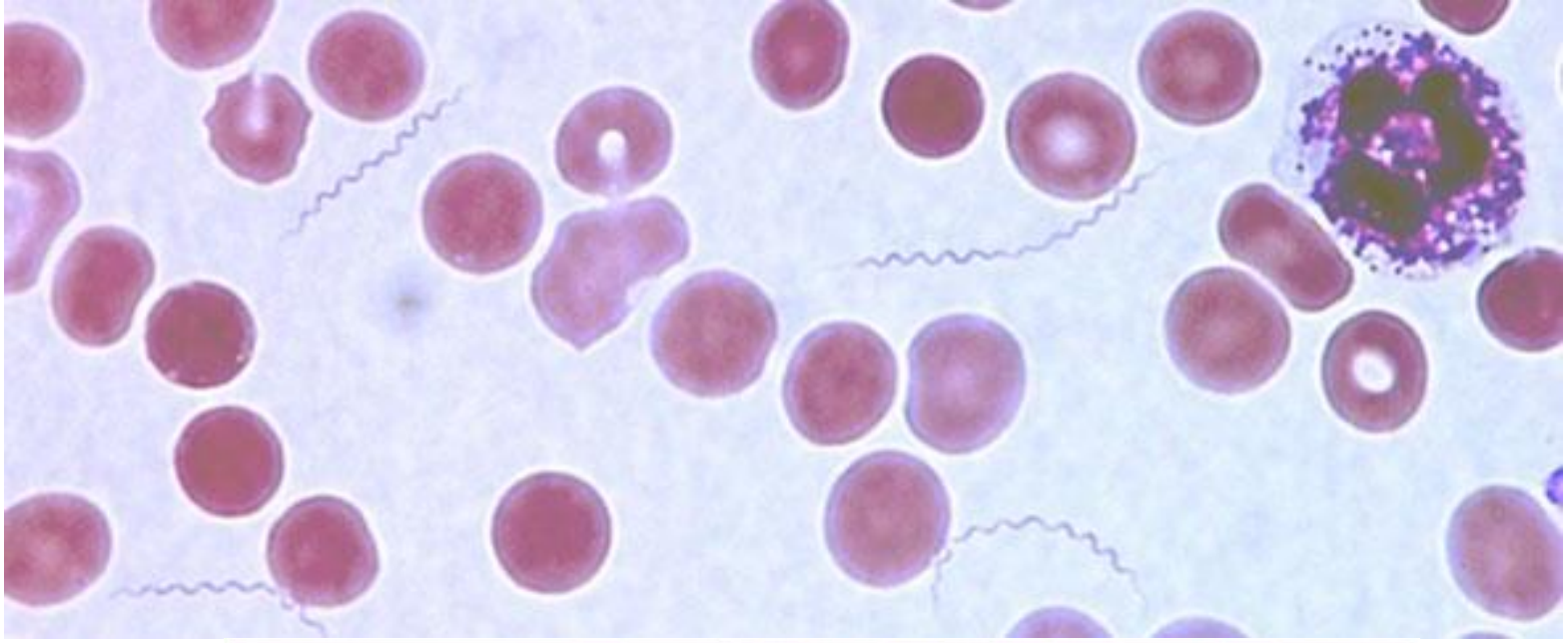
- In **stage 2** (**disseminated infection**) spirochetes spread hematogenously throughout the body and cause secondary skin lesions, lymphadenopathy, migratory joint and muscle pain, cardiac arrhythmias, and meningitis often associated with cranial nerve involvement.

- **Stage 3** (**persistent infection**) manifests many months after the tick bite.

  B. burgdorferi usually causes a **chronic arthritis** sometimes with severe damage to large joints. Less often, patients will have polyneuropathy and encephalitis that vary from mild to debilitating.

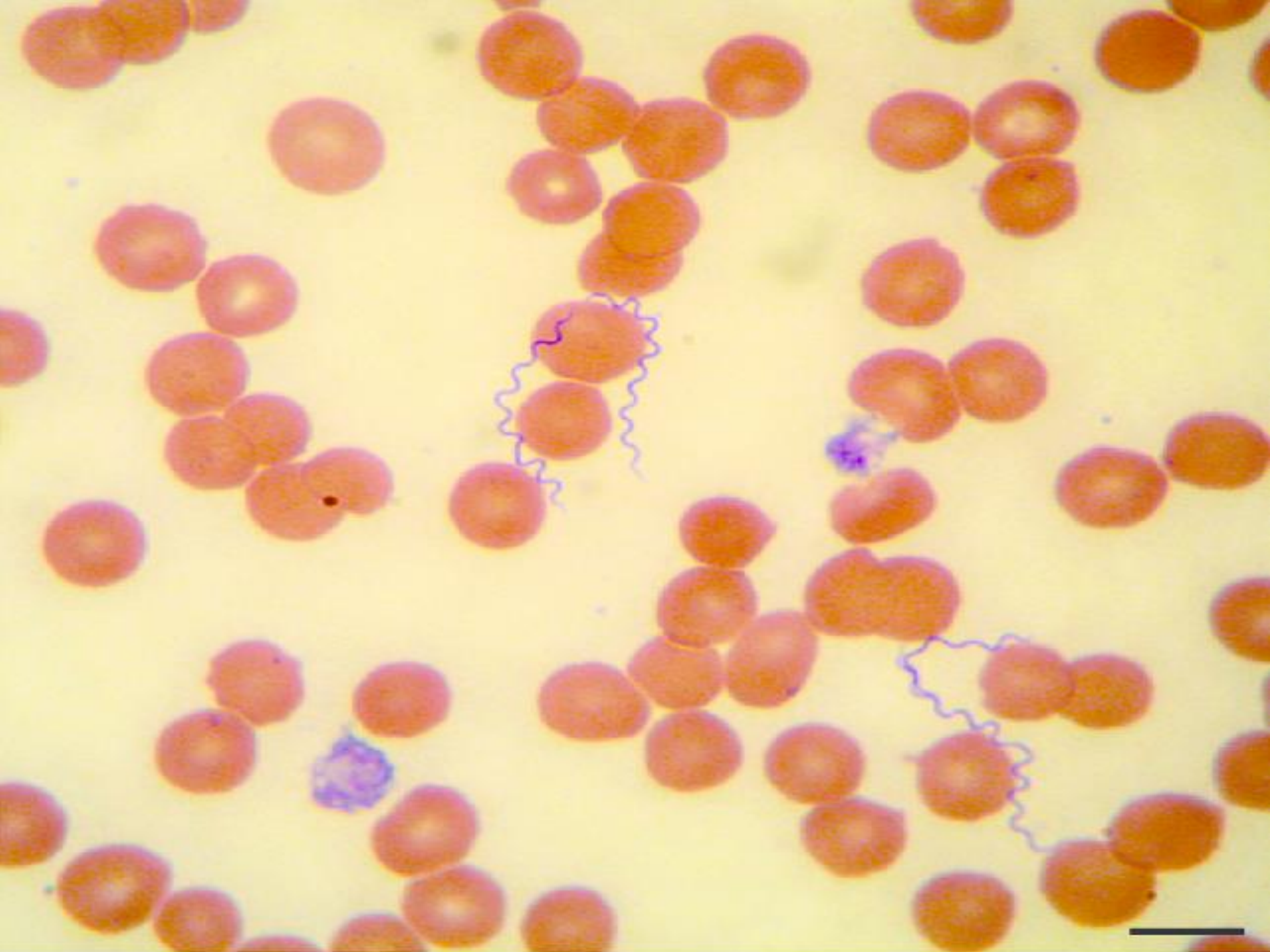






تریپانوزوم!

انواع انسانی آن را در ایران نداریم ولی درسهایش را زیاد میخوانیم!



Emerging and Re-emerging Infectious Diseases

Emerging infectious diseases:

- Arisen or increased in the human population in the past 2 decades, such as:
  - ✓ COVID-19, MERS and Zika nowadays,
  - ✓ AIDS, H. pylori & Ebola in the past!

Re-emerging pathogens:

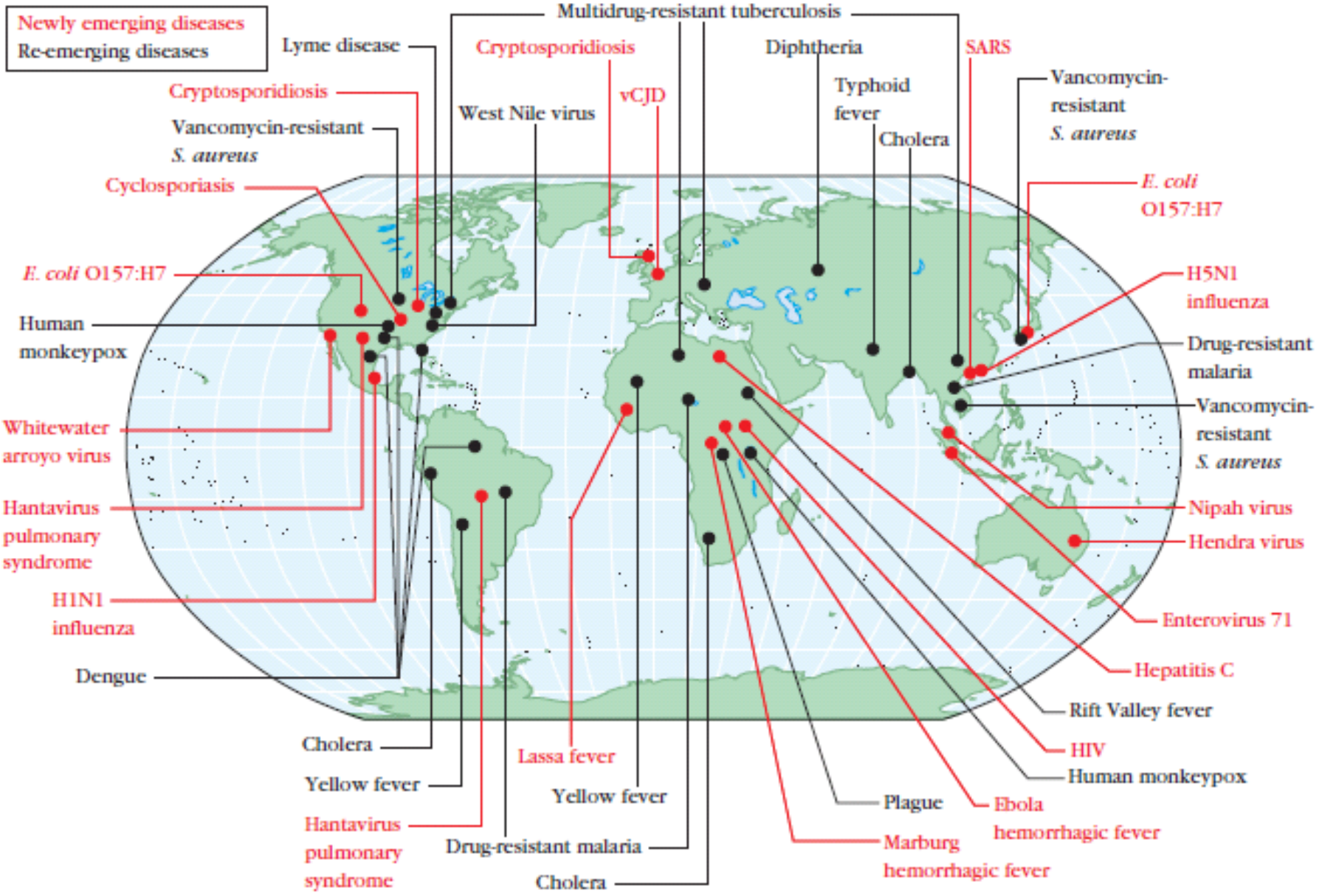
- Those that were formerly rare, viewed as largely under control or exhibiting reduced infection rates such as:
  - ✓ Diphtheria and Measles in recent years in neighbor countries
  - ✓ Influenza and CCHF inside Iran

پیدایش متناوب عفونتهای بازپدید یا Re-emerging میتواند انعکاسی از شیوه های موفق گریز پاتوژنها از چنگال سیستم ایمنی باشد

Emerging pathogens in the end of the last century

Year	Pathogen	Disease
1973	Rotavirus	Major cause of infantile diarrhea globally
1974	Hepatitis C	Non-A, non-B hepatitis commonly transmitted via transfusions
1976	*Cryptosporidium parvum*	Acute chronic diarrhea
1977	Ebola virus	Ebola haemorrhagic fever
	Legionella pneumophilia	Legionnaires' disease
	Hantavirus	Haemorrhagic fever with renal syndrome
	Campylobacter jejuni	Enteric diseases distributed globally
1980	Human T-lymphotrophic virus I (HTLV-1)	T-cell lymphoma
1981	Toxin-producing strains of *Staphylococcus aureus*	Toxic shock syndrome
1982	*Escherichia coli* 0157:H7	Haemorrhagic colitis
	HTLV-II	Hairy cell leukemia
	Borrelia burgdorferi	Lyme disease
1983	HIV	AIDS
	Helicobacter pylori	Peptic ulcers
1988	Hepatitis E	Enteric non-A, non-B hepatitis
1990	Guanarito virus	Venezuelan haemorrhagic fever
1991	*Encephalitozzon hellem*	Conjunctivitis, disseminated disease
1992	*Vibrio cholerae* 0139	New strain of epidemic cholera
	Bartonella henselae	Cat scratch disease
1994	Sabia virus	Brazilian haemorrhagic fever
1995	Human herpes virus-8	Associated with Kaposi sarcoma in AIDS patients
1996	TSE causing agent	New variant of Creutzfeldt-Jakob disease (mad cow disease)
1997	Influenza A subtype H5N1	Avian influenza
1999	Influenza A subtype H9N2	New strain of human influenza
	Nipah virus	Encephalitis
	West Nile virus	Encephalitis

Examples of Emerging & Re-emerging Diseases showing point of origin



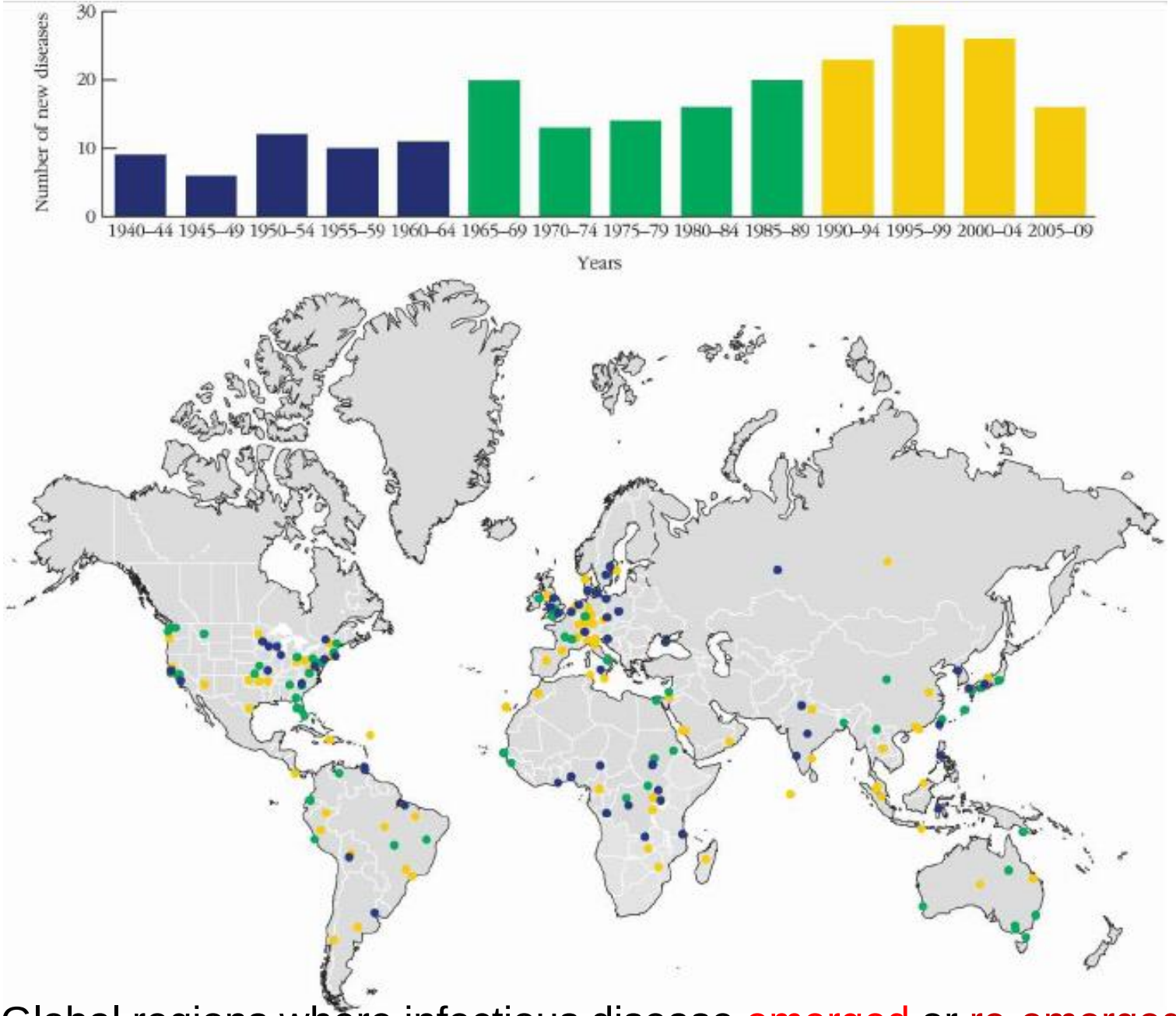
Emerging and Re-emerging Infectious Diseases

Emerging infectious diseases:

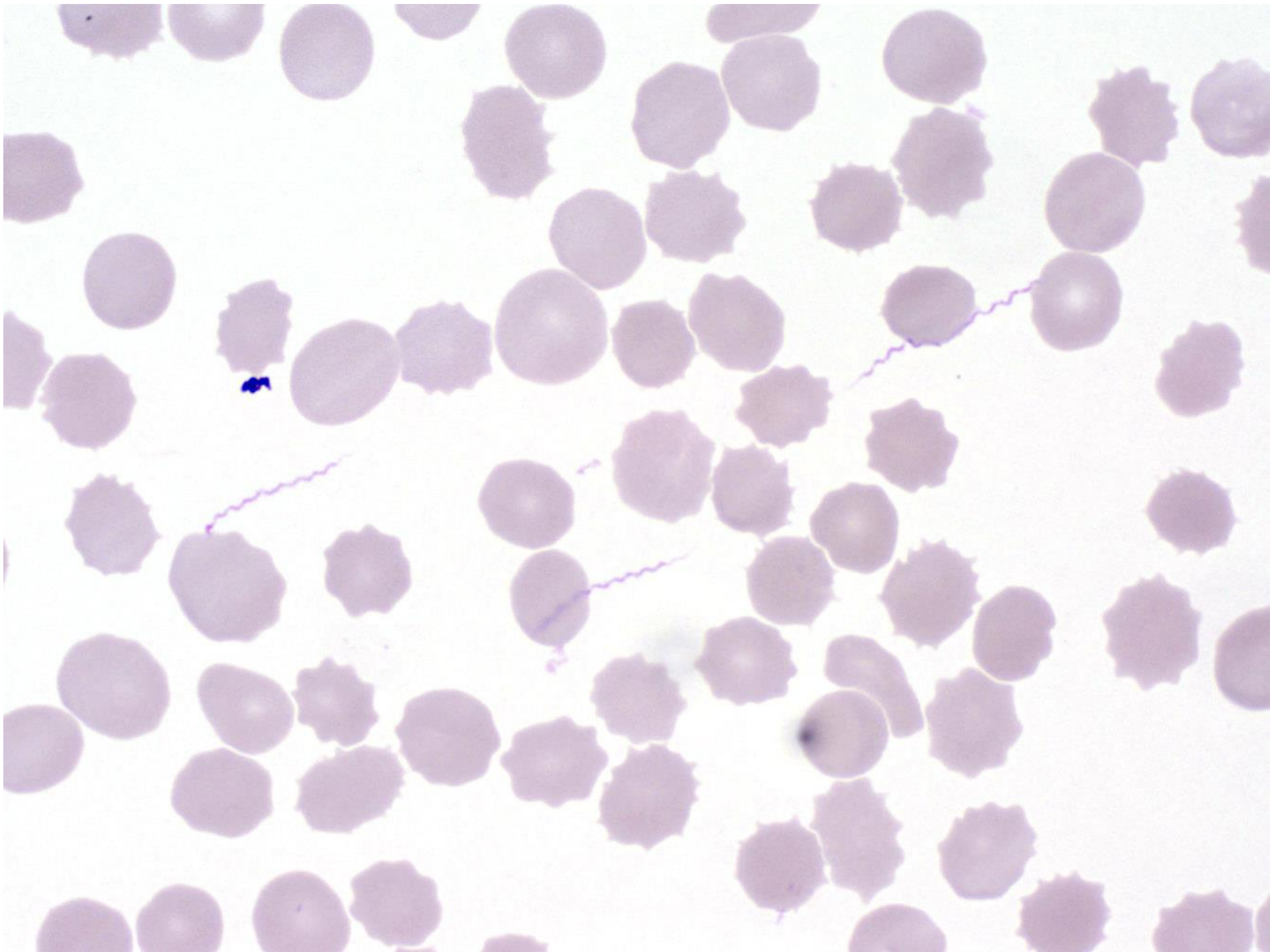
- Arisen or increased in the human population in the past 2 decades, such as:
  - ✓ COVID-19, MERS and Zika nowadays,
  - ✓ AIDS, H. pylori & Ebola in the past!
- Emerging infectious disease outbreaks may seem to come from nowhere!

Re-emerging pathogens:

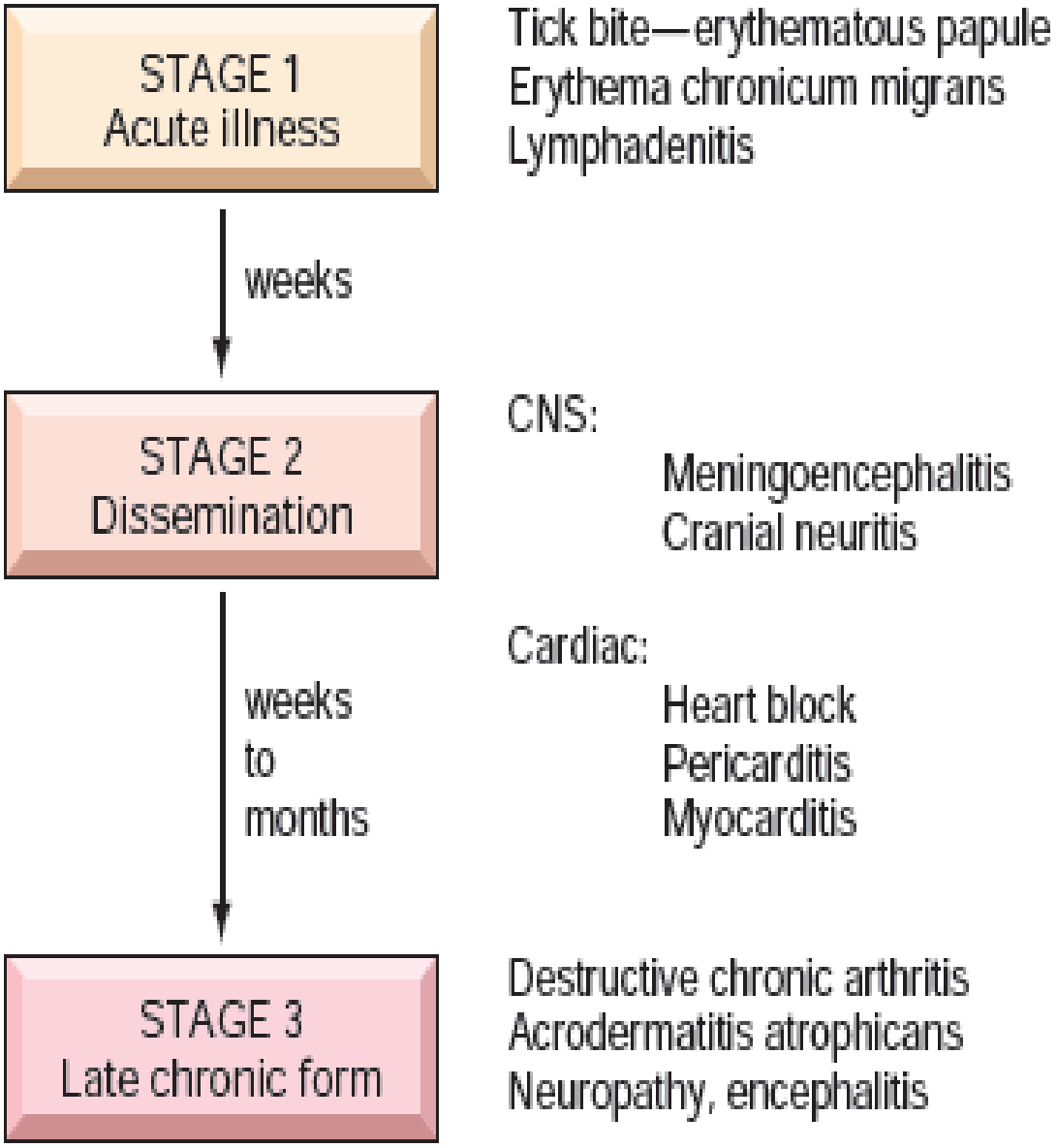
- Those that were formerly rare, viewed as largely under control or exhibiting reduced infection rates, but that have recently begun to resurge due to:
  - ✓ development of drug resistance (e.g., tuberculosis),
  - ✓ acquisition of new virulence factors (e.g., MRSA),
  - ✓ environmental changes that impact spreading (e.g., H1N1)
  - ✓ biological changes that increase transmission rates (e.g., diphtheria)
  - ✓ socio-behavioral changes that enhance geographic range (e.g., CCHF)



Global regions where infectious disease emerged or re-emerged in the past years, highlighting the expanding geography of this issue



Clinical stages of **Lyme disease**



Lyme Pathogenesis & Immunity

- ***B. burgdorferi*** does not produce LPS or exotoxins that damage the host.
- Much of the **pathology** associated with ***B. burgdorferi*** is thought to be secondary to the **immune response** against the bacteria and the **inflammation** that accompanies it.
- The initial immune response is stimulated by binding of bacterial lipoproteins to **TLR2** on **macrophages.** In response, these cells release **pro-inflammatory cytokines (IL-6 and TNF)** and generate bactericidal **reactive nitrogen intermediates**, reducing but usually not eliminating the infection.
- The **inflammatory lesions** are likely triggered by **T cells** and **cytokines**.
- Borrelia-specific **antibodies**, made 2 to 4 weeks after infection, direct **complement-mediated phagocytosis** and killing of the bacteria; however, ***B. burgdorferi*** escapes the antibody response through **antigenic variation**.
- Similar to ***Borrelia hermsii,*** a cause of endemic relapsing fever, ***B. burgdorferi*** has a plasmid with a single promoter sequence and multiple coding sequences for an antigenic surface protein, VlsE, each of which can shuttle into position next to the promoter and be expressed. Thus, as the antibody response to one VlsE protein is mounted, bacteria expressing an alternate VlsE protein can escape immune recognition.
- Chronic manifestations of **Lyme disease**, such as the late arthritis, are probably caused by the **immune response** against persistent bacteria.

Lyme Pathogenesis & Immunity, cont.

- ***Borrelia*** spirochetes are deposited in the skin during tick feeding. The organism does **not** produce toxins, so disease is primarily a result of the **immunologic host response**.
- Major antigens of the bacteria include the outer-surface lipoproteins (Osp) A, B and C, as well as the flagellar protein. Outer surface proteins are important for survival both in the tick and in mammals.
- **Innate immune activation** begins with spirochete binding to PRRs such as **toll-like receptors** on dendritic cells and macrophages in the dermis. This initiates signal transduction pathways that result in production of chemokines and cytokines by macrophages and dendritic cells. Spirochetes are ingested by **macrophages and neutrophils** and degraded within phagocytic vacuoles. Degradation products bind to intercellular pattern recognition receptors that further amplify the inflammatory response.
- **Adaptive immune responses** also help to clear spirochetes. Borrelia-specific **T cells** are activated. These aid in macrophage killing and help **B cells** differentiate into antibody-producing plasma cells.
- Patients with **Lyme arthritis** typically have higher Borrelia-specific **antibody titers** than do patients with other manifestations of Lyme disease. Despite a vigorous immune response, the spirochetes are not immediately killed, partly owing to **immune evasion** strategies, such as **downregulation of expression** of outer surface proteins and **antigenic variation** from the variable major protein-like sequence expression locus. ***B. burgdorferi*** produces specific proteins that inhibit **complement** activity.
- Several lines of evidence suggest that persistent symptoms do **not** result from active infection. Synovial tissue from patients with Lyme arthritis typically shows hypertrophy, vascular proliferation, and a marked mononuclear cell infiltrate, but **joint cultures** in patients with recurrent arthritis are negative.
- Some adult patients with Lyme arthritis, particularly those with HLA-DRB1 alleles, will develop a chronic, antibiotic refractory, **autoimmune arthritis**.

Leptospirosis

- One of the most common zoonotic diseases throughout the world, leptospirosis is caused by various serovars of different species within the genus *Leptospira* (such as ***L. interrogans***).
- Although leptospirosis is usually subclinical or mild and flulike, a small proportion of patients develop severe symptoms related to gastrointestinal/hepatic disease, meningitis, renal failure, and/or myocarditis (also referred to as *Weil's disease).*
- Although *Leptospira spp. can be cultivated in vitro, diagnosis is* usually made by demonstrating **seroconversion**.
- Demonstration of spirochetes along the colonic epithelial cell borders using routine, **immunohistochemical,** or in situ hybridization microscopy from biopsy material is more commonly performed than cultivation or genomic amplification assays.

Leptospiral Antigenic Structure

- Leptospires have a double-membrane structure, making it unique and similar to both gram-positive and gram-negative bacteria, attracting also different **immune responses** against them.
- Describing the membrane from inside out, leptospires have an inner cytoplasmic membrane, a peptidoglycan cell well, periplasm, outer membrane with outer-membrane proteins (OMPs), phospholipids, and lipopolysaccharide (LPS).
- Endothelial cell activation and **immune activation** were associated with disease severity in leptospirosis patients.
- Pathogenic ***Leptospira*** may inhibit the activation of the **complement** system through the secretion of proteases that cleave and inactivate key complement proteins.
- The ability of spirochetes to interact with the host fibrinolytic system may contribute to the degradation of the extracellular matrix components, **immune evasion**, and tissue penetrations and invasion.

Leptospiral Immunity & Immune Evasion

- Leptospiral immunoglobulin-like proteins (Lig), surface proteins found in pathogenic leptospires, are believed to be involved in cell adhesion and are among those that have been studied as potential vaccine candidates.
- Lig-bound plasmin was shown to cleave fibrinogen and the complement proteins **C3b and C5**, which may allow for invasion and complement immune system evasion by *Leptosira.*
- Surface-exposed proteins that might have potential roles in adhesion and pathogenesis include OmpL36, OmpL37, OmpL47, and OmpL54.
- OmpL1 displayed significant adhesin activity binding to glycosaminoglycans and monolayers of human cells in vitro and may be a promising component for a **subunit vaccine**.
- The outer surface proteins LigA, LigB, and LigC contain immunoglobulin-like domains found in virulence factors such as intimin and invasin.
- Leptospiral endostatin-like proteins (Len) have been identified that have been shown to be involved in the organism's ability to **evade the complement system**.
- The humoral response is the main mechanism of resistance against leptospirosis. IgG and IgM are detected in patients who have recovered from infection.
- Antibodies are produced against leptospiral LPS. LPS **vaccines** are effective, but immunity is limited to homologous serovars.
- PMN leukocyte is not an efficient defense factor for pathogenic leptospires in non-immune hosts.
- The virulence of leptospires appears to be related to their ability to resist killing both by serum and by neutrophils.

Immunodiagnosis in **Leptospirosis**

- A confirmed case of leptospirosis fulfills one of the following criteria:

(1) Specimens that are **culture positive** for leptospires or

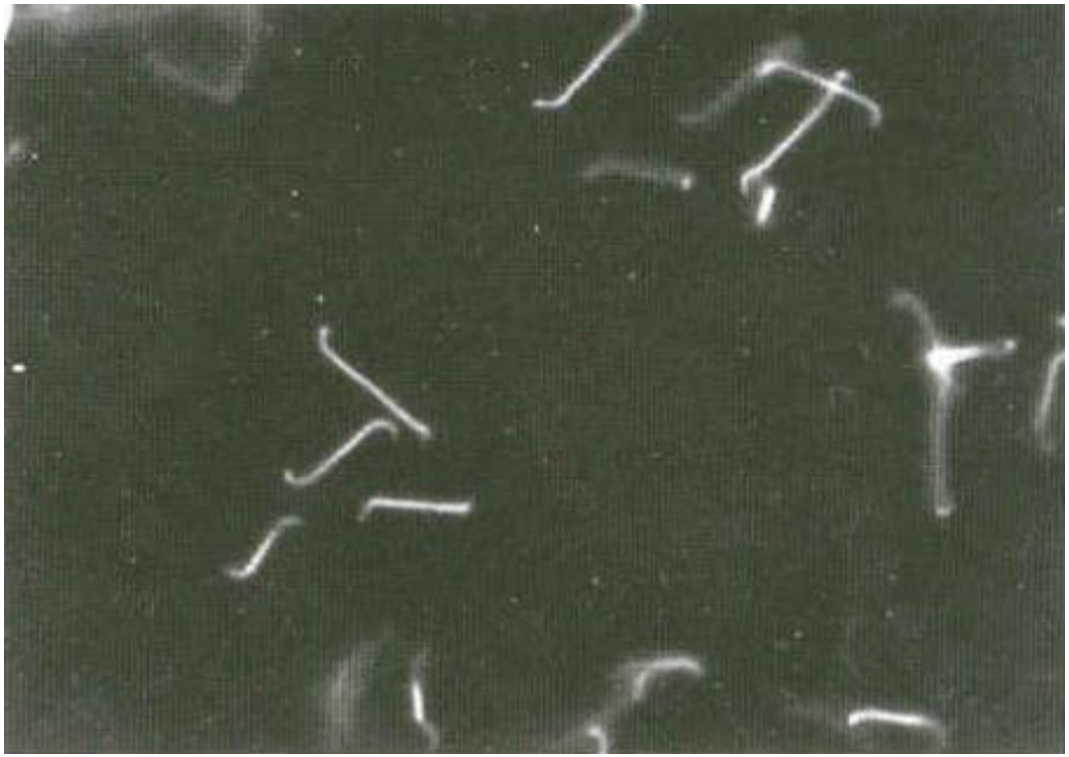
(2) clinical symptoms compatible with leptospirosis and **serological** evidence (ELISA, Strip, etc).

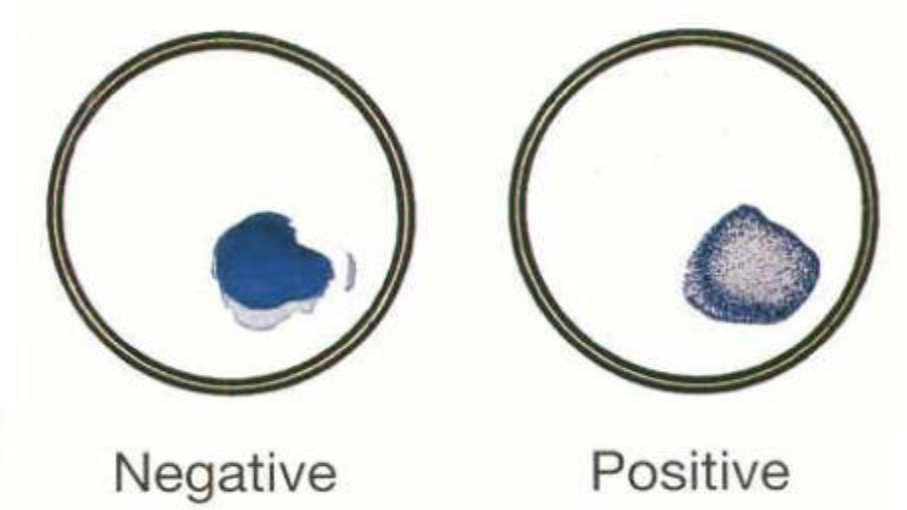
- **Positive Serology** (*like in other infections*) is either:
  - As an obvious **seroconversion** or
  - as a remarkable (usually fourfold [2-tube] or greater) **rising titer**

  in the serologic tests (usually MAT or Micro Agglutination Test) between:
  - the 1st (e.g. the acute) and
  - the 2nd (e.g. the convalescent) serum specimens

  obtained 2 or more weeks apart and studied at the **same laboratory**.
- **PCR** has been shown to be effective for detecting pathogenic Leptospira but may not be routinely available. Leptospires can be **recovered** from blood or CSF during the septicemic stage. Urine serves as the main source from which leptospires can be **isolated** during the immune and convalescent phases of leptospirosis since prolonged urinary shedding after infection is possible in humans.
- Presumptive leptospirosis is defined as the presence of clinical symptoms that are compatible with leptospirosis and a serologic (MAT) titer of 1 : 100 or greater, a positive slide MAT reaction on a single serum specimen obtained after the onset of symptoms, or a stable MAT titer of 1 : 100 or greater in two or more serum specimens obtained after the onset of symptoms.

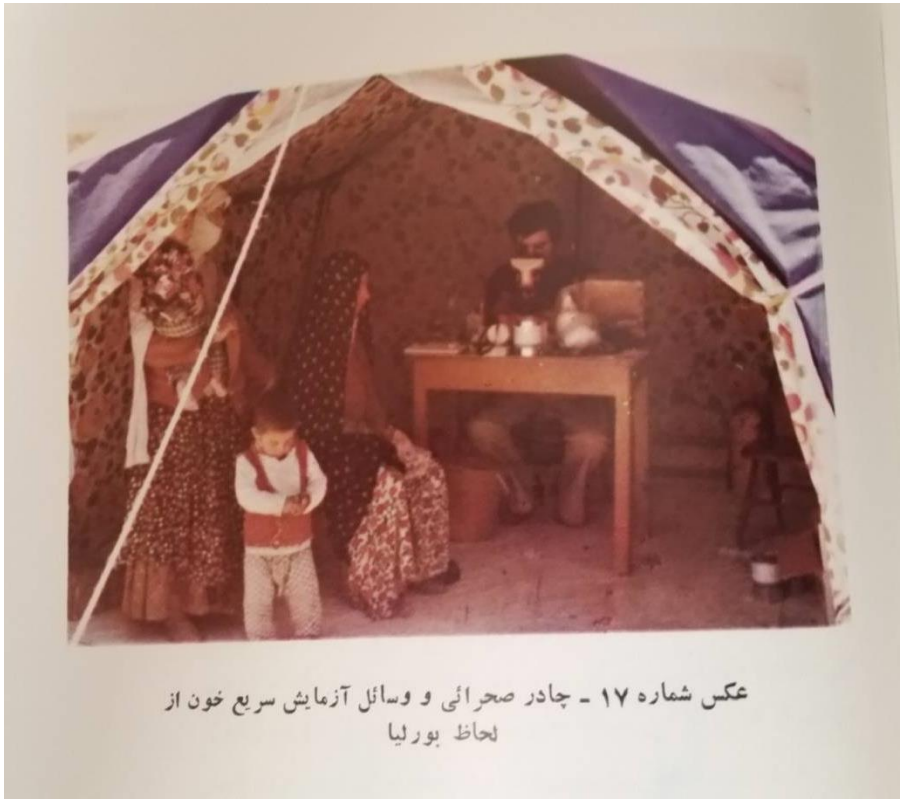
Leptospira in U/A specimen by DFM



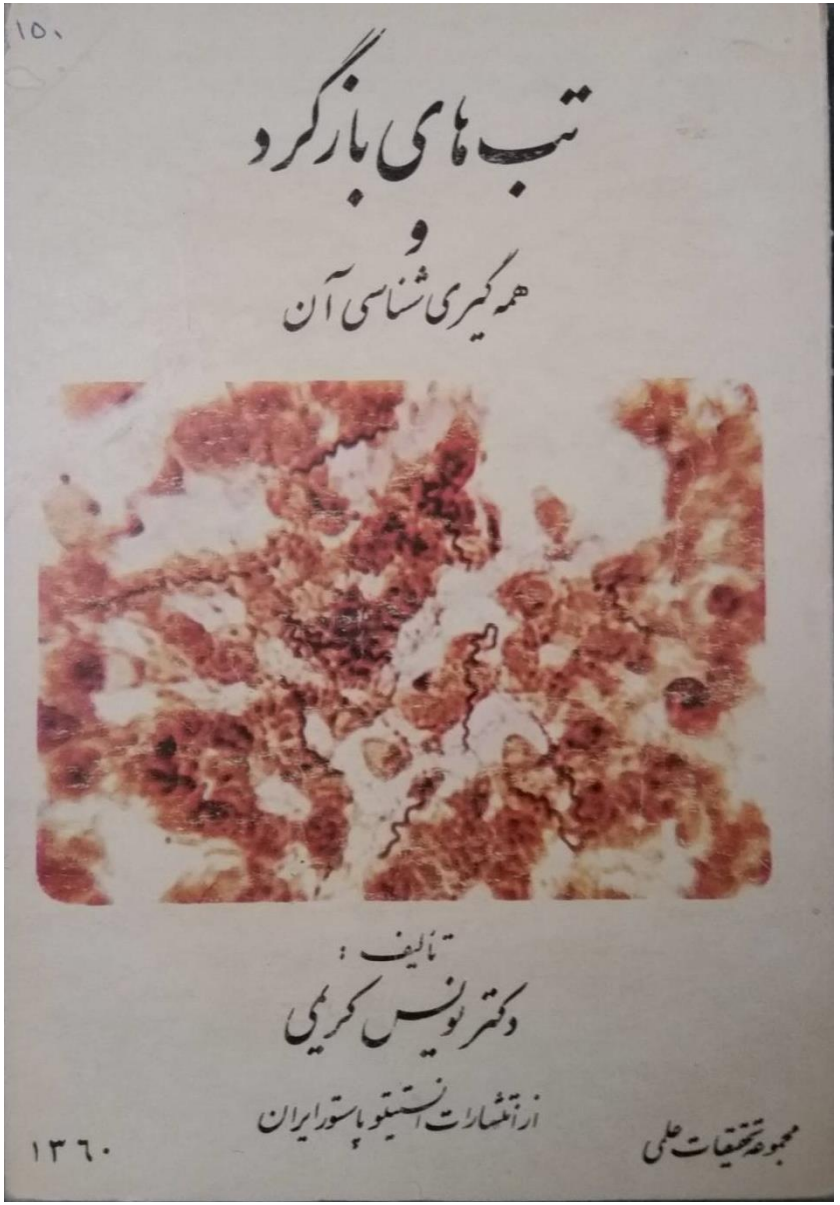


LeptoTek latex agglutination for specific Leptospira Ab

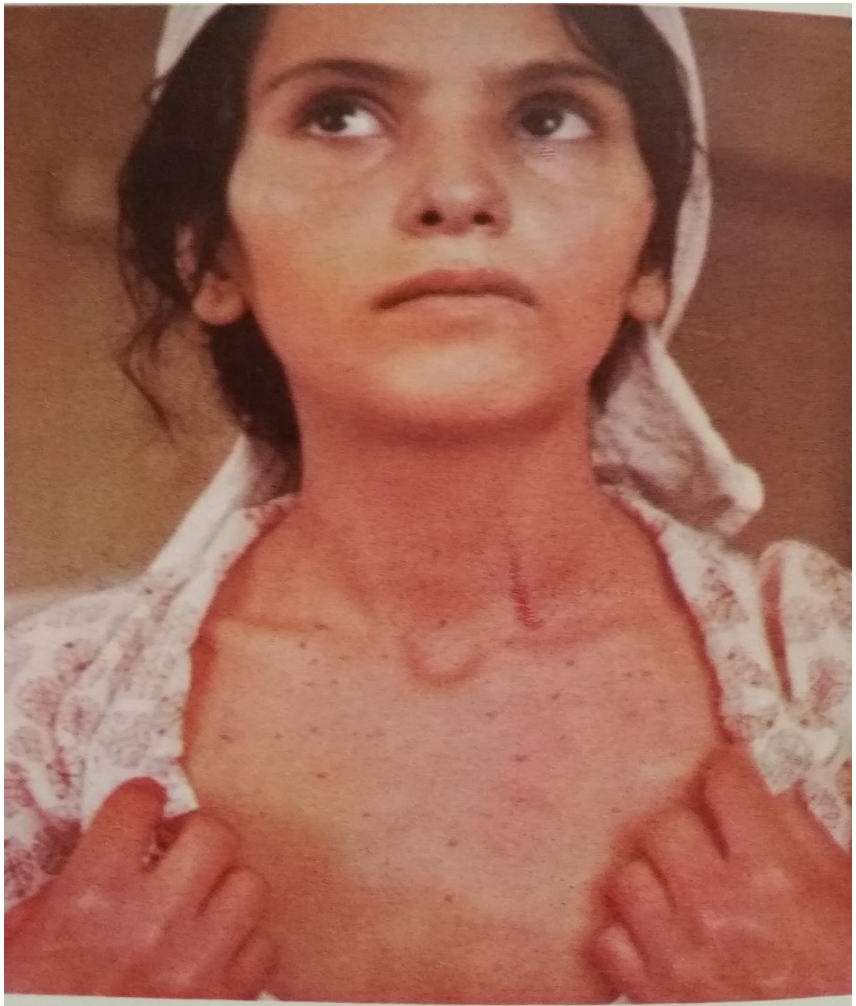
A Recommended Reading



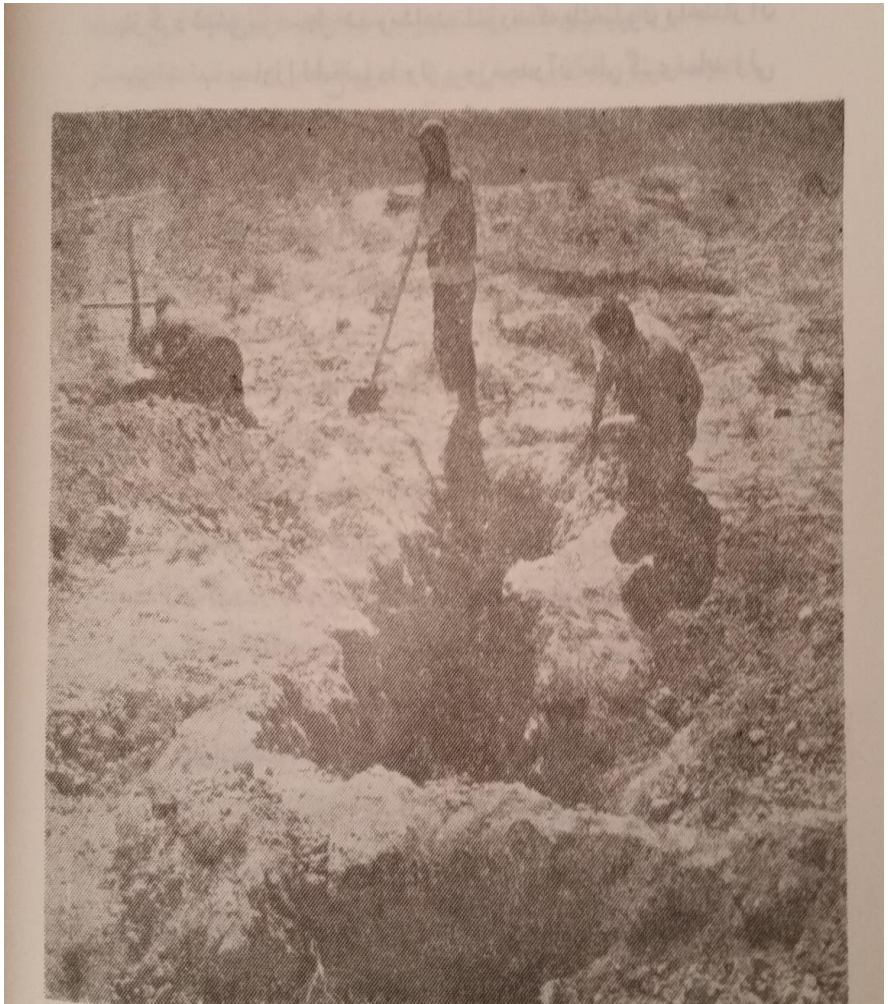
عکس شماره ۱۷ - چادر صحرائی و وسائل آزمایش سریع خون از
لحاظ بورلیا



از کتاب تب های بازگرد – انستیتو پاستور

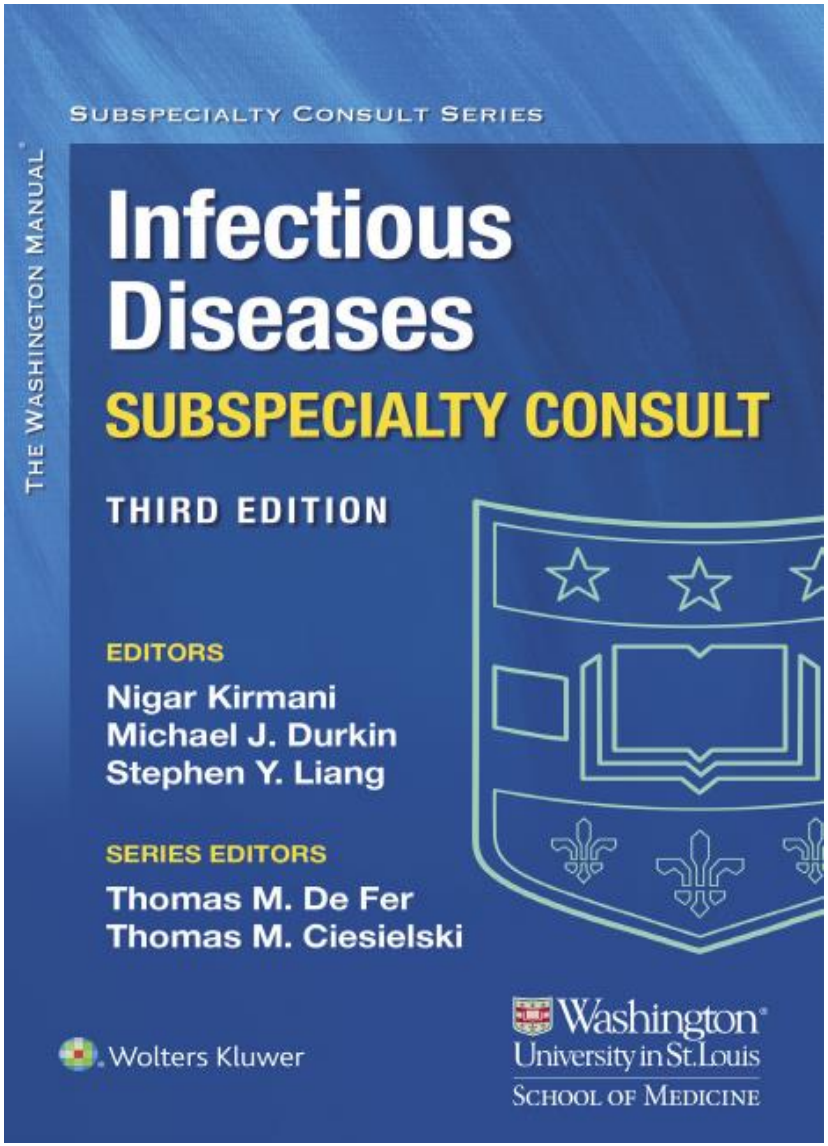


عکس شماره ۱۳ ـ بلوژات جلدی در تب‌های بازگرد



عکس شماره ۱۶ـ منظره عمومی لانه‌گورکن ازلحاظ عمق ووسعت

For Further Study



Antiphospholipid Syndrome

DEFINITIONS

- Antiphospholipid syndrome (APS) is an autoantibody-mediated acquired thrombophilia characterized by **recurrent arterial or venous thrombosis** and/or **pregnancy morbidity**. It affects primarily females.
- APS may occur alone (primary) or in association with other autoimmune diseases, mainly systemic lupus erythematosus (SLE) (secondary).
- Catastrophic APS (CAPS) is a life-threatening rapidly progressive thromboembolic disease involving simultaneously three or more organs.
- The major autoantibodies detected in patients' sera are directed against **phospholipids** and/or phospholipid (PL)-binding plasma proteins such as prothrombin and β2 glycoprotein I (β2GPI).
- PLs are components of the cytoplasmic membrane of all living cells. The antibodies are directed against negatively charged PLs including among others **cardiolipin**, phosphocholine, and phosphatidylserine.
- Another group of antibodies termed *lupus anticoagulant* (LA) prolongs clotting times ***in vitro***, which are not corrected by adding normal plasma.
- Patients with APS often possess antibodies recognizing *Treponema pallidum* PL/cholesterol complexes, detected by Venereal Disease Research Laboratory (VDRL) tests and characterized as biologic false-positive serologic tests for syphilis (BFP-STS).

Classification and Nomenclature of Antiphospholipids Ab's

NAME	ASSAY FOR THEIR DETECTION	COMMENTS
Antibodies against cardiolipin (aCL)	Enzyme-linked immunosorbent assay (ELISA) using as antigen cardiolipin (CL), a negatively charged phospholipid	aCL from patients with APS recognize β2GPI existing in the human serum as well as in bovine serum, which is used to block the nonspecific binding sites on the ELISA plate. CL simply stabilizes β2GPI at high concentration on the polystyrene surface.
Antibodies against β2GPI (anti-β2GPI)	ELISA using as antigen affinity purified or recombinant β2GPI in the absence of PL	Antibodies recognize β2GPI bound in the absence of CL to an oxidized polystyrene surface, where oxygen atoms in the moieties C–O or C=O were introduced by γ-irradiation.
Lupus anticoagulant (LA)	Activated partial thromboplastin time (aPTT) Kaolin clotting time (KCT) Dilute Russel viper venom test (DRVVT)	Antibodies recognize β2GPI or prothrombin (PT) and elongate aPTT, implying that they interfere with the generation of thrombin by prothrombin. Prolongation of the clotting times is an in vitro phenomenon, and LA induces thromboses in vivo.

Antiphospholipid Syndrome -2

EPIDEMIOLOGY

- The incidence of APS is estimated to be around 5 cases per 100,000 persons per year in the US.

- Anti-PL antibodies occur in 1–5% of the general population. Their prevalence increases with age; however, it is questionable whether they are able to induce thrombotic events in elderly individuals. Moreover, one-third of patients with SLE and other autoimmune diseases possess these antibodies, with only 5–10% of them developing APS.

PATHOGENESIS

- The initiating event for the induction of antibodies to PL-binding proteins seems to be **infections, oxidative stress, and major physical stresses such as surgery or trauma**. All these factors appear to induce increased apoptosis of the vessel endothelial cells and subsequent exposure of PLs.
- The latter, bound with serum proteins such as β2GPI or prothrombin, lead to **neoantigen formation**, which in turn triggers the induction of anti-PLs.
- The binding of anti-PLs to the disrupted endothelial cells leads to initiation of intravascular coagulation and thrombus formation.
- **Complement activation** has been also proposed as a mechanism of APS-related fetal injury.

Clinical Features of Antiphospholipid Syndrome

Venous Thrombosis and Related Consequences	
Deep vein thrombosis	39
Livedo reticularis	24
Pulmonary embolism	14
Superficial thrombophlebitis	12
Thrombosis in various other sites	11
Arterial Thrombosis and Related Consequences	
Stroke	20
Cardiac valve thickening/dysfunction and/or Libman-Sacks vegetations	14
Transient ischemic attack	11
Myocardial ischemia (infarction or angina) and coronary bypass graft thrombosis	10
Leg ulcers and/or digital gangrene	9
Arterial thrombosis in the extremities	7
Retinal artery thrombosis/amaurosis fugax	7
Ischemia of visceral organs or avascular necrosis of bone	6
Multi-infarct dementia	3

Neurologic Manifestations of Uncertain Etiology	
Migraine	20
Epilepsy	7
Chorea	1
Cerebellar ataxia	1
Transverse myelopathy	0.5
Renal Manifestations Due to Various Reasons (Renal Artery/Renal Vein/Glomerular Thrombosis, Fibrous Intima Hyperplasia)	**3**
Musculoskeletal Manifestations	
Arthralgias	39
Arthritis	27
Obstetric Manifestations (Referred to the Number of Pregnancies)	
Preeclampsia	10
Eclampsia	4
Fetal Manifestations (Referred to the Number of Pregnancies)	
Early fetal loss (<10 weeks)	35
Late fetal loss (≥10 weeks)	17
Premature birth among the live births	11
Hematologic Manifestations	
Thrombocytopenia	30
Autoimmune hemolytic anemia	10

Antiphospholipid Syndrome -3

CLINICAL MANIFESTATIONS AND LABORATORY FINDINGS

- Clinical manifestations represent the consequences of venous or arterial **thrombosis** and/or **pregnancy morbidity**.
- Venous thrombosis, superficial or deep, occurs primarily in the lower extremities often leading to pulmonary emboli. Thrombosis of the pulmonary arteries leads to pulmonary hypertension and thrombosis of the inferior vena cava to Budd-Chiari syndrome.

- Cerebral venous thrombosis, presents with signs and symptoms of intracranial hypertension and retinal vein thrombosis.
- Arterial thrombosis affects more commonly the arteries of the brain and is manifested as **migraines, cognitive dysfunction**, transient ischemic attacks, **stroke**, and retinal artery occlusion.
- Arterial thrombosis of the extremities presents with **ischemic leg ulcers**, digital gangrene, avascular bone necrosis, while thrombosis of other arteries leads to myocardial infarction, renal artery stenosis, glomerular lesions, and infarcts of spleen, pancreas, and adrenals.

- **Coombs-positive hemolytic anemia** and **thrombocytopenia** are laboratory findings associated with APS.

Antiphospholipid Syndrome -4

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of APS should be seriously considered in cases of **thrombosis**, cerebral vascular accidents in **individuals <55 years** of age, or **pregnancy morbidity** in the presence of **livedo reticularis** or **thrombocytopenia**.

In these cases, aPL antibodies should be measured. The presence of at least one clinical and one laboratory criterion is compatible with the diagnosis, in the absence of other thrombophilia causes.

Clinical criteria include:

(1) vascular thrombosis defined as one or more clinical episodes of arterial, venous, or small vessel thrombosis in any tissue or organ; and

(2) pregnancy morbidity, defined as (a) one or more unexplained deaths of a morphologically normal fetus at or beyond the tenth week of gestation; (b) one or more premature births of a morphologically normal neonate before the thirty-fourth week of gestation because of eclampsia, severe preeclampsia, or placental insufficiency; or (c) three or more unexplained consecutive spontaneous abortions before the tenth week of gestation.

Laboratory criteria include:

(1) LA, (2) anticardiolipin (aCL), and/or (3) anti-β2GPI antibodies, at intermediate or high titers on two occasions, 12 weeks apart.

Antiphospholipid Syndrome -4

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS (cont.)

Differential diagnosis is based on the exclusion of other inherited or acquired causes of thrombophilia, Coombs-positive hemolytic anemia, and thrombocytopenia.

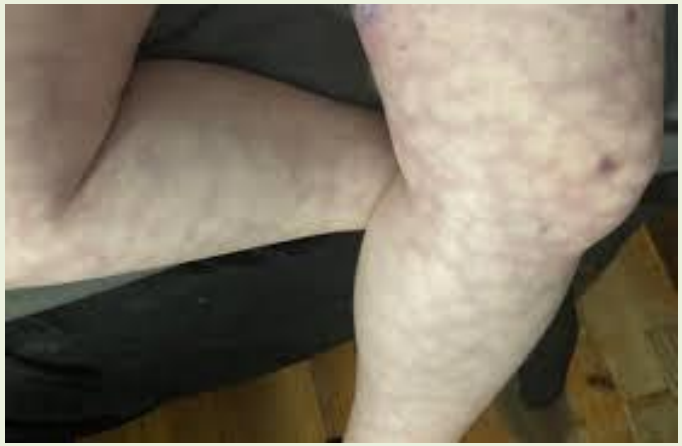
Livedo reticularis with or without a painful ulceration on the lower extremities may be also a manifestation of disorders affecting

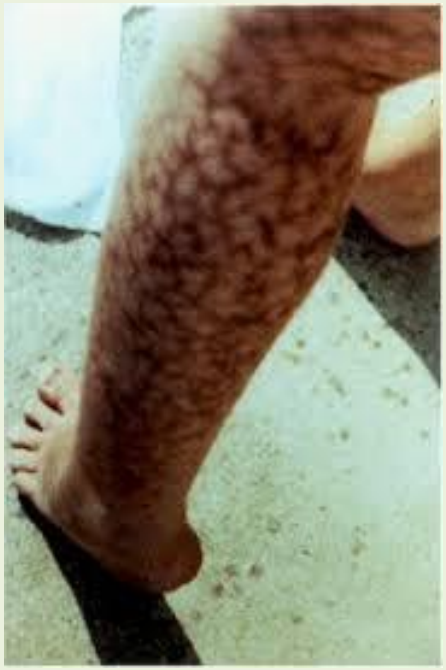
(1) the vascular wall, such as atherosclerosis, polyarteritis nodosa, SLE, cryoglobulinemia, and lymphomas; or

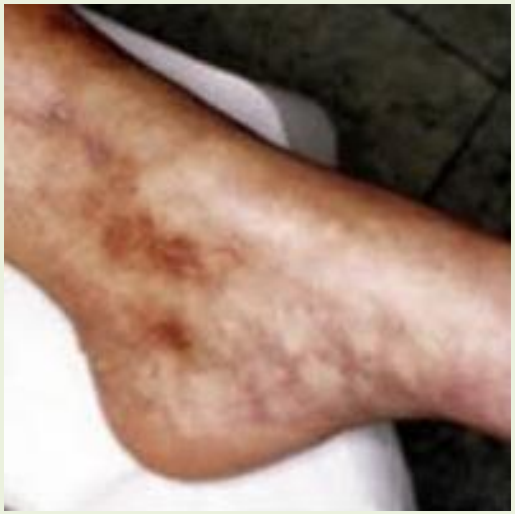
(2) the vascular lumen, such as myeloproliferative disorders, hypercholesterolemia, or other causes of thrombophilia.

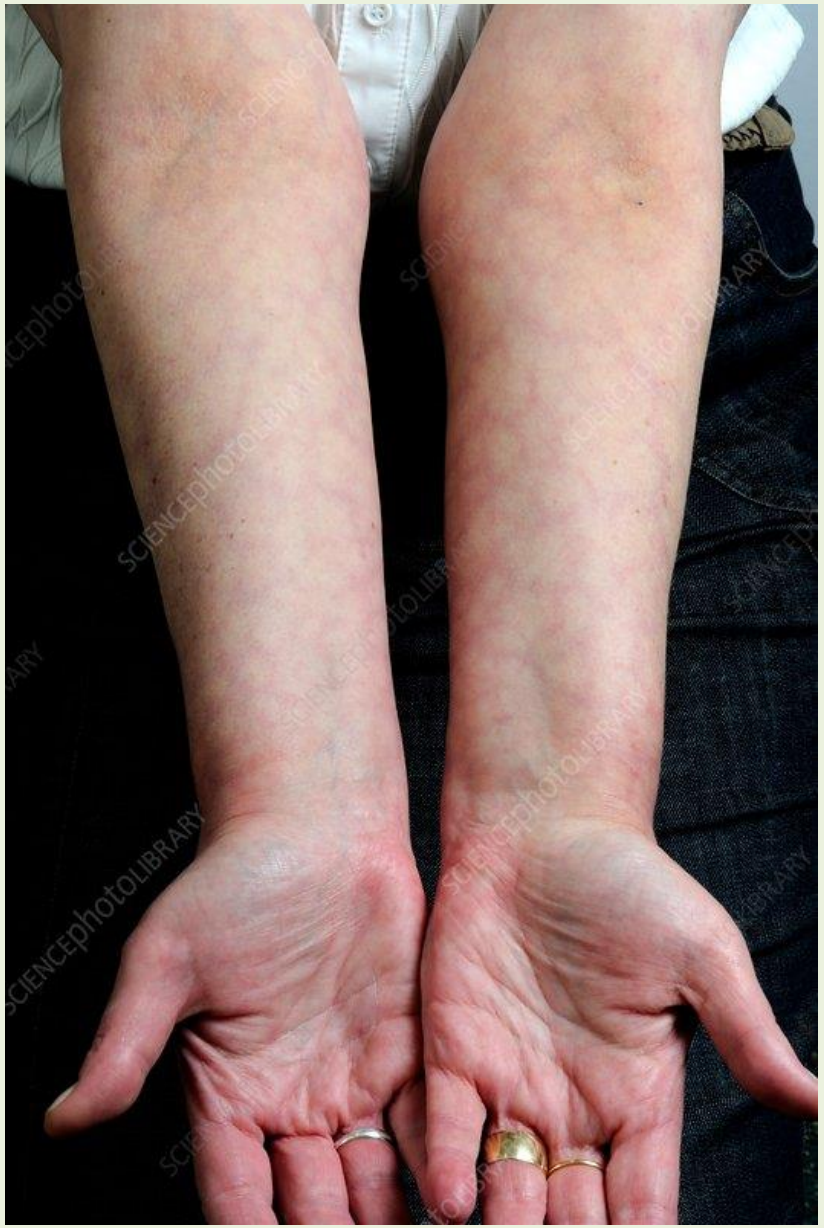
Antiphospholipid Syndrome -5

Livedo Reticularis









از توجه شما متشکرم