Session III
Syphilis

1. Syphilis Testing Overview – Megan Crumpler
2. Syphilis Automation – Mayur Shukla
3. Syphilis Case Studies and Discussion – Eric Tang

Syphilis Testing Overview

HIV, HCV and Syphilis Diagnostic Testing Workshop

ID Lab Con 2023

March 12, 2023

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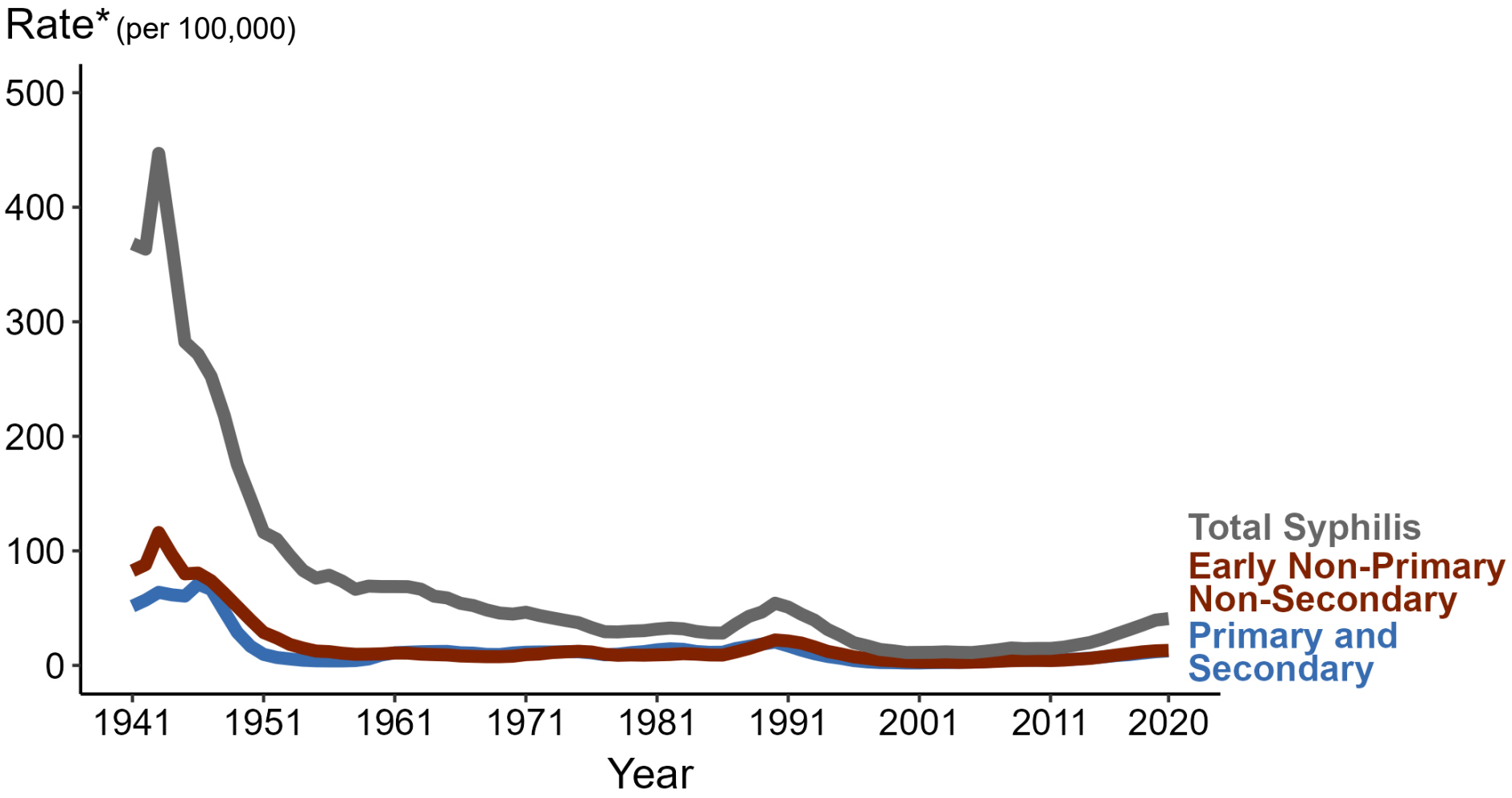
Outline

1. General Syphilis Overview and Statistics
2. Review Stages of Syphilis
3. Review Syphilis Testing Algorithms
4. Overview of Syphilis Testing Procedures
5. Syphilis Reporting

Syphilis Overview

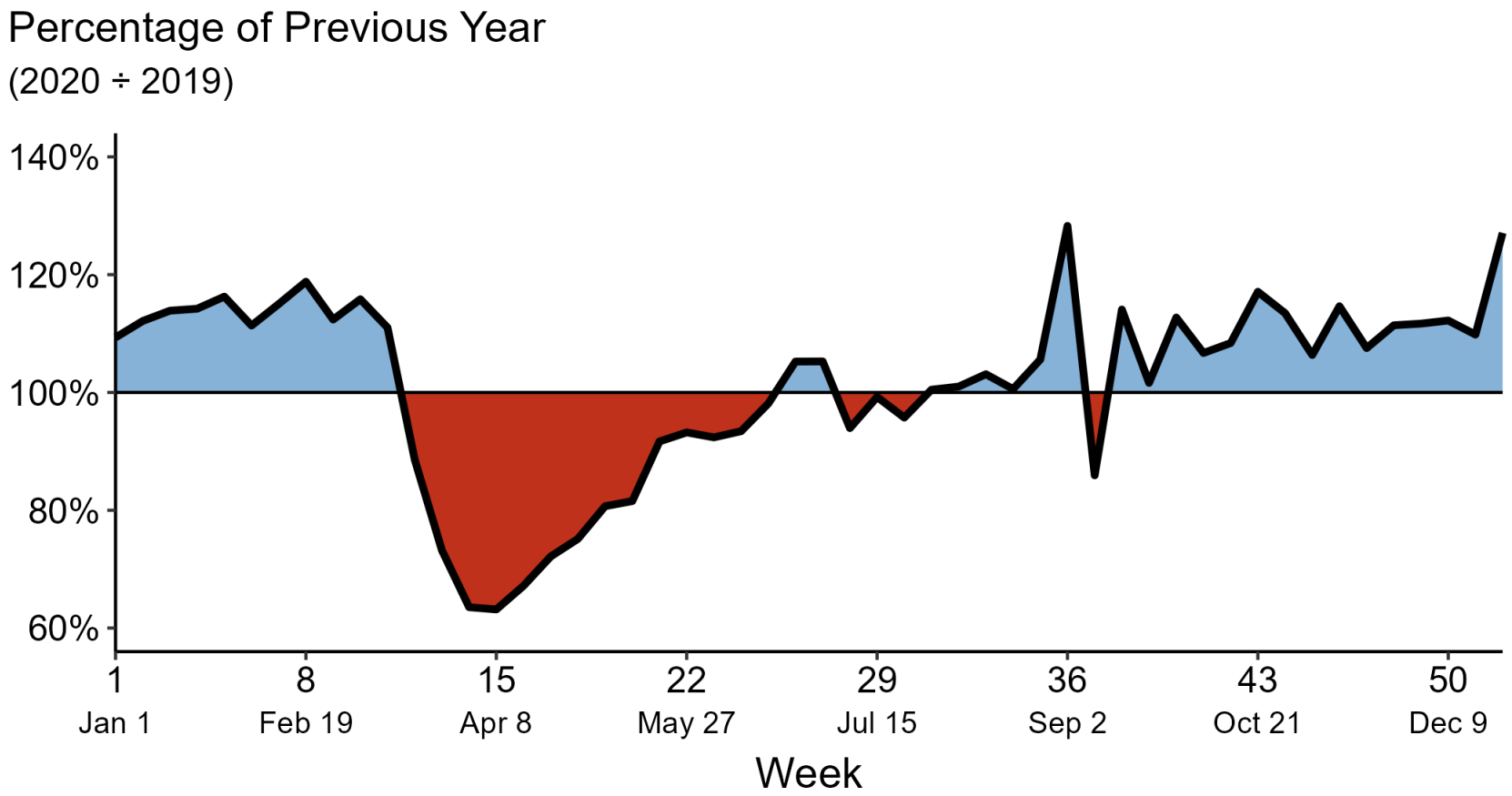
- Sexually transmitted infection caused by the spirochete *Treponema pallidum*
- Spread by:
  - direct contact with a syphilis sore during vaginal, anal, or oral sex
  - Infected mother to fetus
- Causes a systemic infection and might lead to serious sequalae in multiple organ systems, including the CNS
- Vertical transmission can cause congenital syphilis
  - May results in spontaneous abortions, miscarriages, or stillbirths
  - infants with congenital syphilis can present with clinical signs of infection at birth or months to years after birth
- Treated with single or three weekly IM injections of benzathine penicillin G, depending on stage of infection

Syphilis — Rates of Reported Cases by Stage of Infection, United States, 1941–2020



Content source: Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention

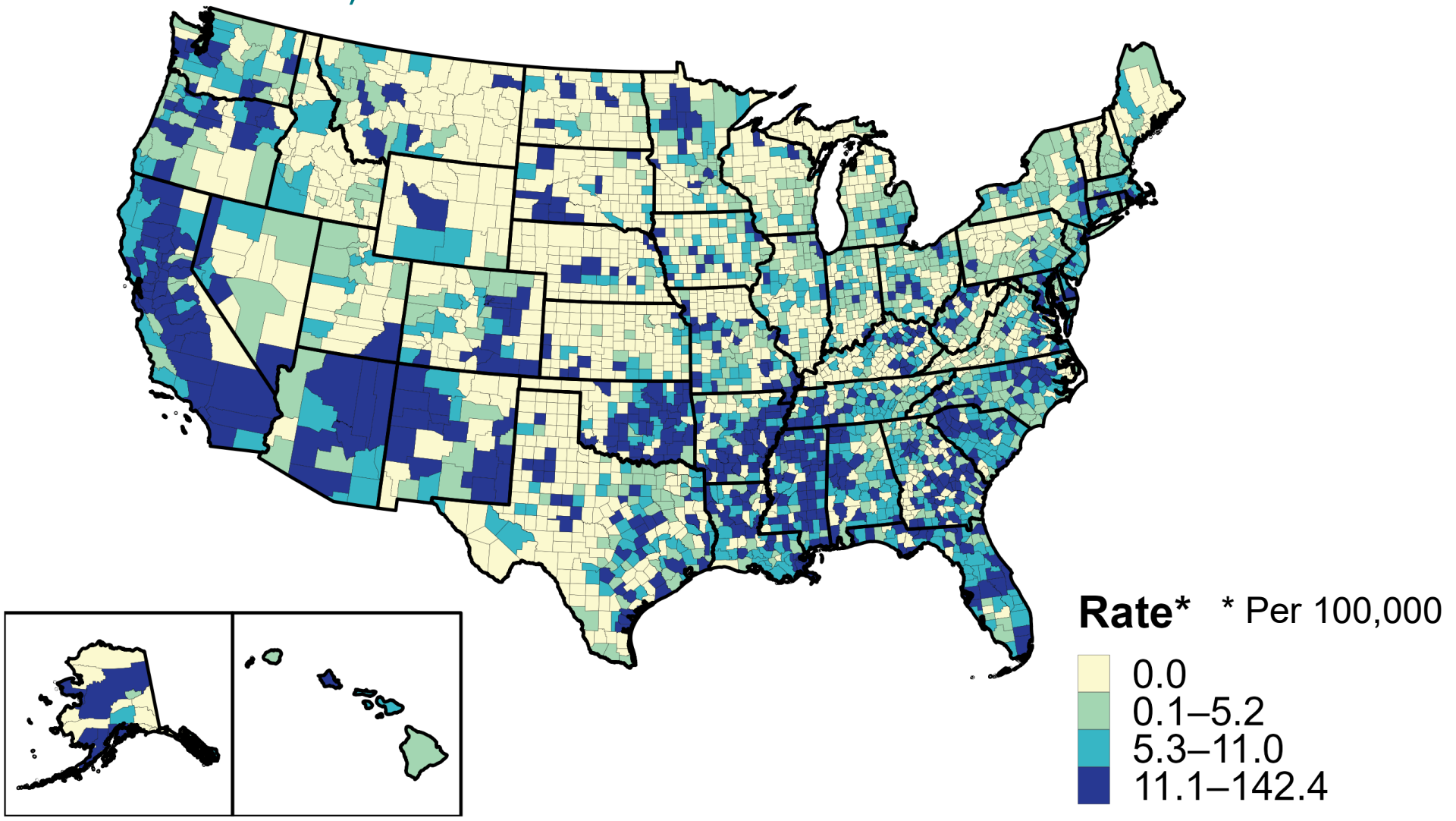
Syphilis (All Stages) — Reported 2020 Cases as a Percentage of 2019 by *MMWR* Week, United States



NOTE: The *MMWR* week is the week of the epidemiologic year for which the case is assigned by the reporting local or state health department.

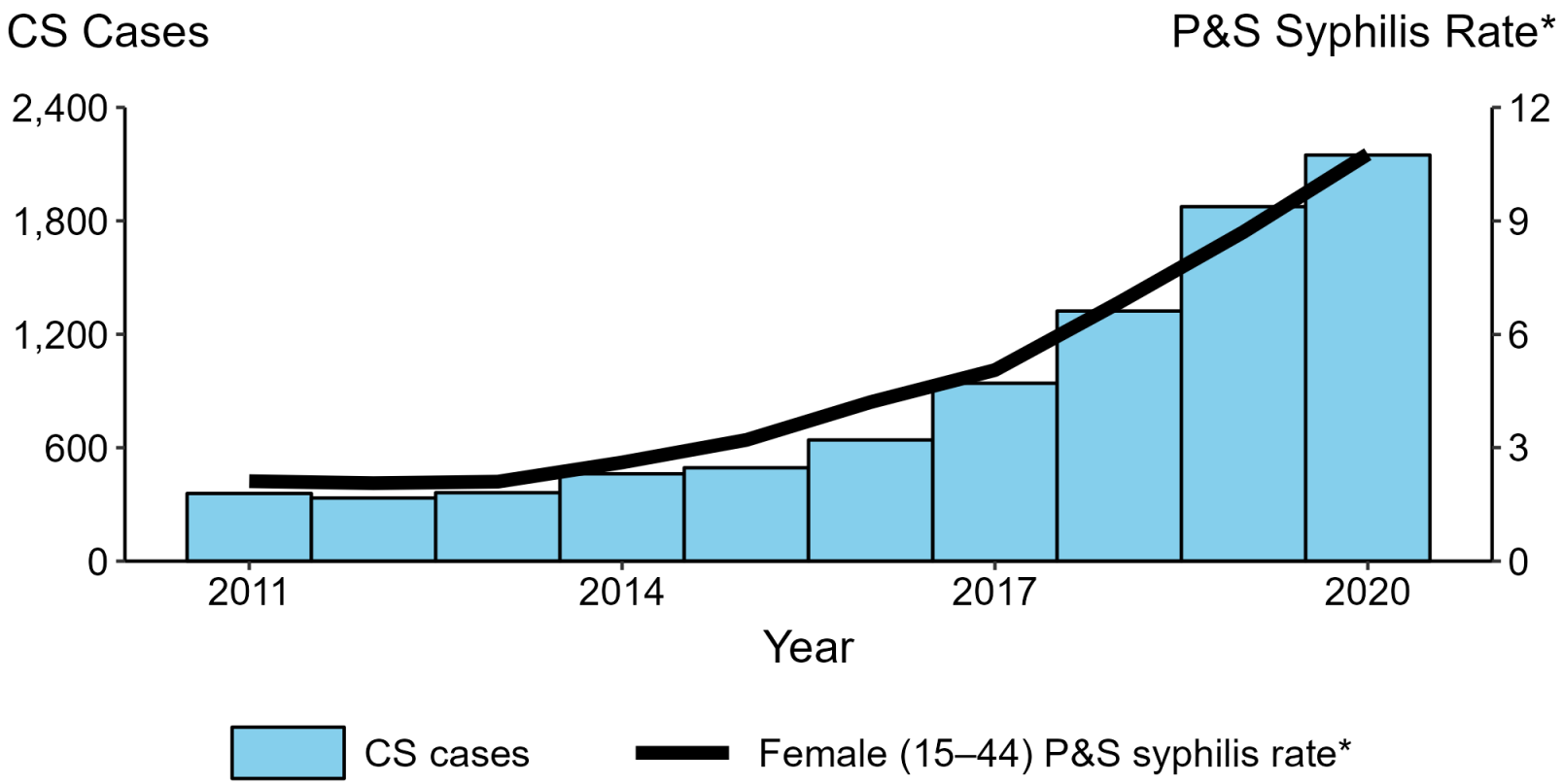
Adapted from Pagaoa et al, *Sexually Transmitted Diseases*, 2021

Primary and Secondary Syphilis — Rates of Reported Cases by County, United States, 2020



Content source: Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention

Congenital Syphilis — Reported Cases by Year of Birth and Rates of Reported Cases of Primary and Secondary Syphilis Among Women Aged 15–44 Years, United States, 2011–2020



* Per 100,000

ACRONYMS: CS = Congenital syphilis; P&S = Primary and secondary syphilis

Content source: Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention

Stages of Syphilis

Table 1. Stages, Time Course, and Manifestations of Syphilis

		Manifestations	
Stage	*Time*	*Common*	*Uncommon*
Primary	10 to 90 days	Chancre	Local lymphadenopathy
Secondary	1 to 3 months	Arthralgia, condylomata lata, fatigue, generalized lymphadenopathy, headache, maculopapular/papulosquamous exanthema, myalgia, pharyngitis	Annular syphilis, iritis, pustular syphilis, pyrexia, syphilitic alopecia, ulceronodular syphilis
Early latent	After primary or secondary stages, 1 year or less of no symptoms	None	None
Late latent	More than 1 year of no symptoms	None	None
Tertiary	Months to years	Late neurosyphilis*	Cardiovascular syphilis, gummatous syphilis

*—Neurosyphilis may occur at any stage of infection.

Information from reference 5.

Mattei, P.L., et.al. 2012 Syphilis: A Reemerging Infection

Syphilis Diagnostic Tests

Syphilis Darkfield

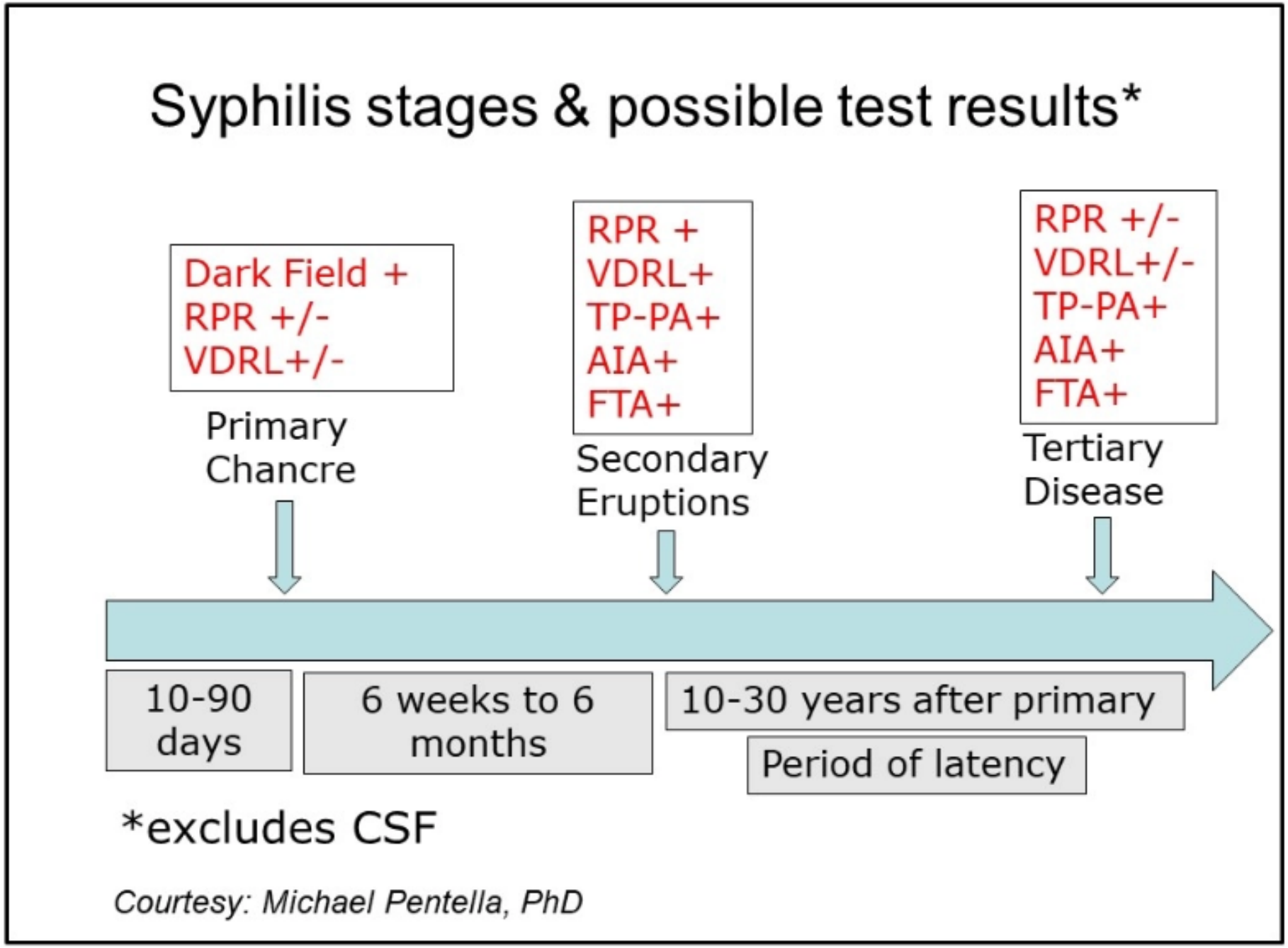
- Definitive method for diagnosing syphilis
- Examine exudate from lesion with darkfield microscope
- Rarely performed
  - Slide must be ready within 30 minutes of collection
  - Training and experience necessary
  - Cannot be used for oral specimens

Common Syphilis Serology Tests

- Non-treponemal tests
  - VDRL (Venereal Disease Research Laboratory)
  - RPR (Rapid Plasma Reagin)
- Treponemal tests
  - TP-PA (Treponema Pallidum Particle Agglutination)
  - FTA-abs (Fluorescent Treponemal Antibody - Absorbed)
  - Immunoassay

Coming soon!

- *Laboratory Recommendations for Syphilis Testing in the United States*
- CDC, Division of STD Prevention
- In 2017 APHL assisted with the literature review for development of evidence-based recommendations for syphilis testing in the US
- Will be posted for public comment period soon



https://labtestsonline.org/tests/syphilis-tests

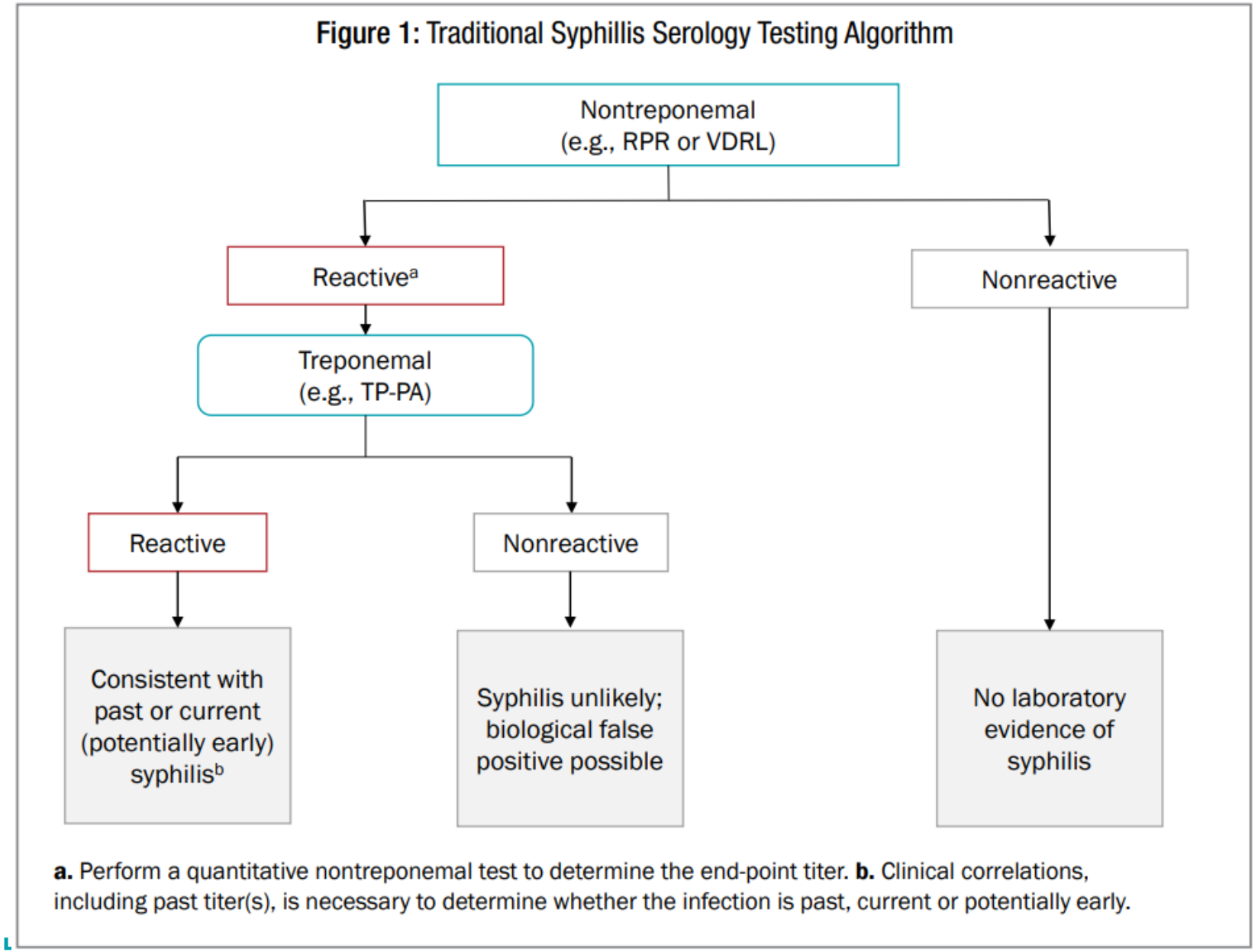
Table 3. Sensitivity and Specificity of Commonly Used Syphilis Tests*[25,29–32]

Syphilis screening test	Mixed	Sensitivity by stage of untreated syphilis, % (range)†				Specificity % (range)‡
		Primary	Secondary	Latent	Tertiary	
Nontreponemal						
VDRL‡		78 (74-87)	100	96 (88-100)	71 (37-94)	98 (96-99)
RPR‡		86 (77-99)	100	98 (95-100)	73	98 (93-99)
TRUST‡		85 (77-86)	100	98 (95-100)		99 (98-99)
USR‡		80 (72-88)	100	95 (88-100)		99
Treponemal						
FTA-ABS‡		84 (70-100)	100	100	96	97 (84-100)
TPPA‡		88 (86-100)	100	97 (97-100)	94	96 (95-100)
EIA: Trep-Chek Trep-Sure	 95.9§ 96.9§	(77-100)	(85-100)	(64-100)	NA	(99-100) 98.5§ 95.4§
CIA: LIAISON‖	 99.2	98	100	100	100	99 99.9
MFI: BioPlex 2200 Syphilis IgG	96.9§					98.0§
Syphilis Health Check	95.6¶, 98.5**					90.5¶, 97.4**

* This is not a comprehensive list of tests available in the United States.
† Sensitivity and specificity of tests also depends on the disease prevalence in the population tested, and may vary considerably by manufacturer or the standard used as a comparison.
‡ Unknown reference standard.
§ When compared with FTA-ABS test results.
‖ When compared with results from western blotting.
¶ When compared with nontreponemal test results.
** When compared with treponemal test results.

Screening for Syphilis in Nonpregnant Adolescents and Adults: June 2016 - https://www.ncbi.nlm.nih.gov/books/NBK368467/pdf/Bookshelf_NBK368467.pdf

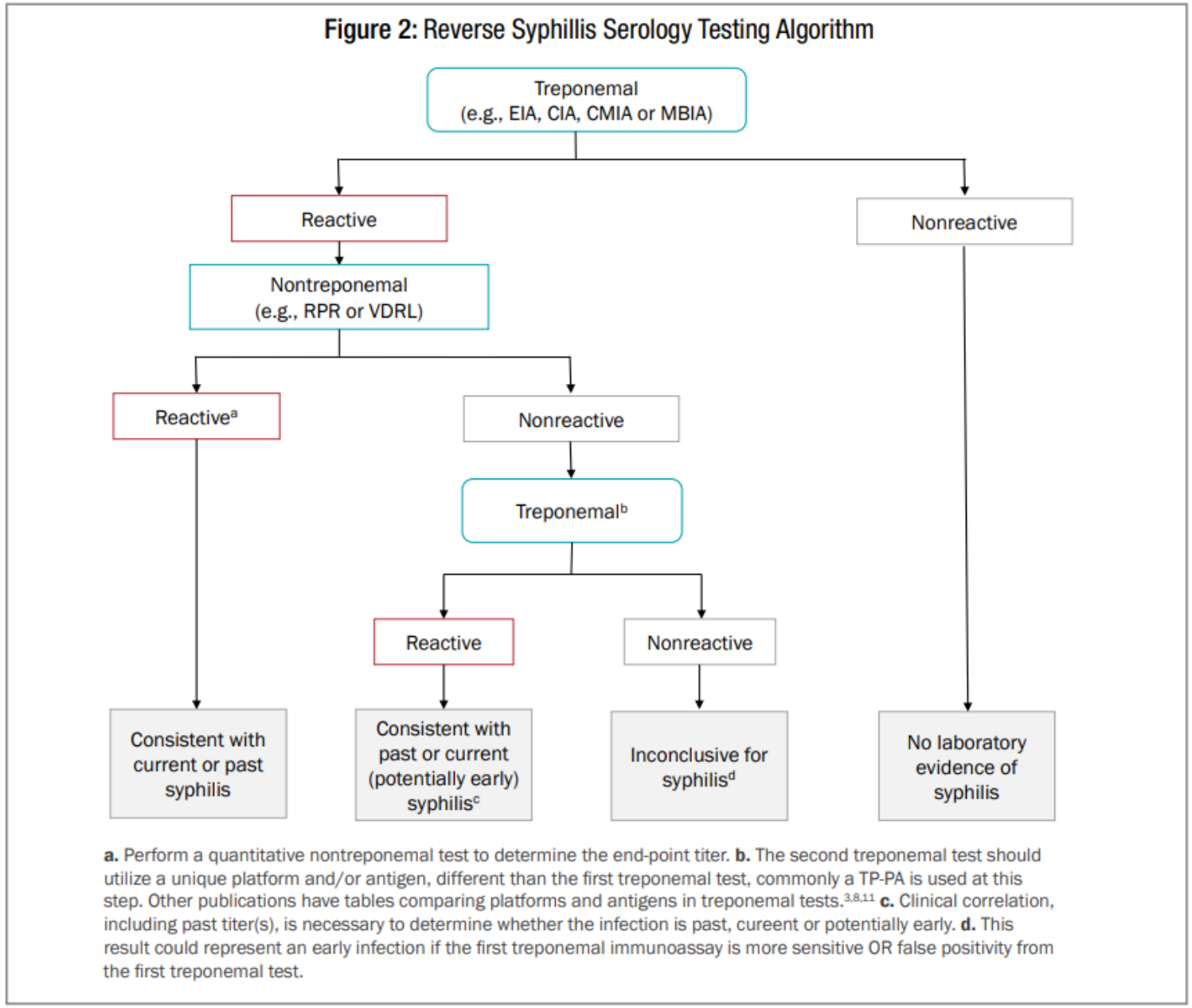
Traditional Syphilis Testing Algorithm



RPR/VRDL

TP-PA/FTA-ABS

Reverse Syphilis Testing Algorithm



Immunoassay

RPR/VRDL

TP-PA/FTA-ABS

Nontreponemal Syphilis Assays

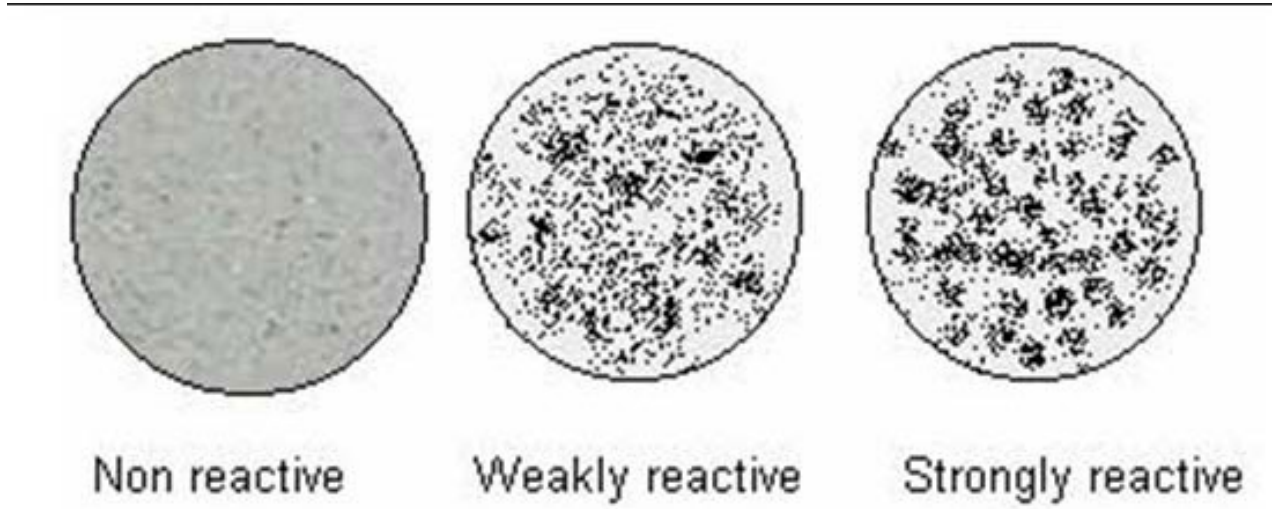
- Detect “reagin”, a non-specific antibody-like substance present in serum or plasma in persons infected with syphilis
- Both qualitative and quantitative
- Used for:
  - Screening
  - Evaluation of patients with symptoms or possible re-infection
    - 4 fold rise in titer indicates reinfection
  - Follow-up assessment after treatment
    - 4-fold drop in titer

Venereal Disease Research Laboratory (VDRL)

- Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin.
  - Antigen must be made up fresh daily and is very dependent on proper technique.
- Acceptable specimens: serum or CSF
  - Only FDA-approved test available for CSF specimens to test for neurosyphilis
  - Serum must be heated to 56 C for 30 minutes to remove anti-complementary activity which may cause false positive

VDRL

- Read microscopically at 100x and grade reaction if positive.
- Perform titer on positive samples, report out titer.



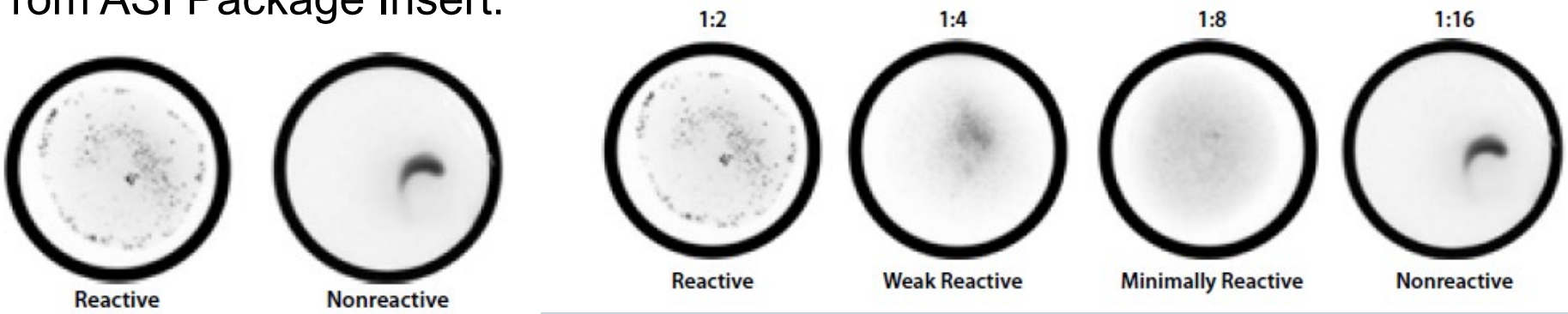
Rapid Plasma Reagin (RPR) Test

- RPR antigen suspension is a carbon particle cardiolipin antigen which detects "reagin“
  - The reagin binds to the test antigen, is bound to charcoal, causing macroscopic flocculation.
- Antigen is more stable that VDRL and does not need to be made daily
- Acceptable specimens: serum and plasma
- Read with naked eye under a high intensity incandescent lamp or strong daylight.
- Qualitative and quantitative:
  - Used to monitor patient titer in response to treatment
- Instruments for automation are now available

Rapid Plasma Reagin (RPR) Test

- Reading and reporting:
  - Reactive - showing characteristic clumping ranging from slight but definite (minimum-to-moderate) to marked and intense.
    - Reactive minimal-to-moderate (showing slight, but definite clumping) is always reported as Reactive
  - Nonreactive - Showing no clumping. Note: There are only two possible final reports with the Card Test: Reactive or Nonreactive, regardless of the degree of reactivity.

From ASI Package Insert:



Nontreponemal Test Limitations

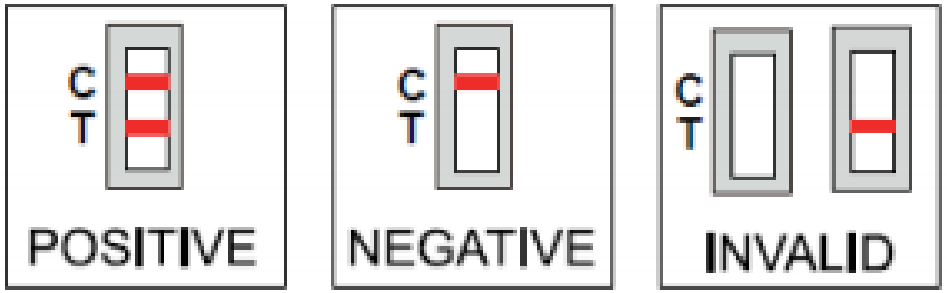
- False negative due to prozone
  - occurs in 1% to 2% of patients with secondary syphilis
  - may exhibit a nonreactive pattern that is slightly granular or “rough”
  - Should be repeated using an alternative procedure and/or titer for quantification.
- Biological False Positives
  - infectious mononucleosis, leprosy and malaria, lupus erythematosus, vaccinia and virus pneumonia.
  - pregnancy, narcotic addiction and autoimmune diseases
  - Pinta, yaws, bejel and other treponemal diseases
  - Typically below a titer of 1:8
- Lipemic or hemolyzed specimens: as a general rule, should be able to read newsprint through the serum.

Treponemal Syphilis Assays

- Measure antibody (IgM and/or IgG) directed against *T. pallidum* antigens
- Qualitative
- Reactivity can persist over lifetime
- More expensive and labor intensive
- Can not quantitate - not useful for following response to treatment
- Traditionally used for confirmation

CLIA-Waived Rapid Testing

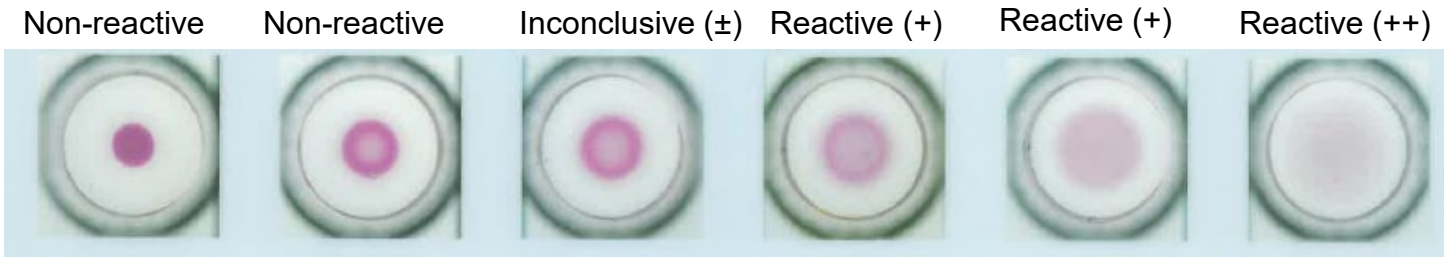
- Two FDA-cleared, CLIA-waived for the detection of *T. pallidum* antibodies:
  - Syphilis Health Check (Trinity Biotech)



  - Dual Path Platform (DPP) HIV-Syphilis assay (Chembio Diagnostics, Inc)
    - Requires DPP Micro Reader optical analyzer
- Could be utilized as the treponemal test in a syphilis testing algorithm to expedite results

FUJIREBIO SERODIA TP-PA

- Qualitative gelatin particle agglutination assay intended to be used for the detection of *Treponema pallidum* antibodies
- Acceptable specimens: Serum and EDTA, Sodium citrate or heparin plasma
- Visually observe the pattern of agglutination in each well.
  - A plate viewer may be used with indirect lighting.
- Ensure each of the Unsensitized Particle wells is non-reactive and interpret the agglutination pattern of the Sensitized Particles (next slide)



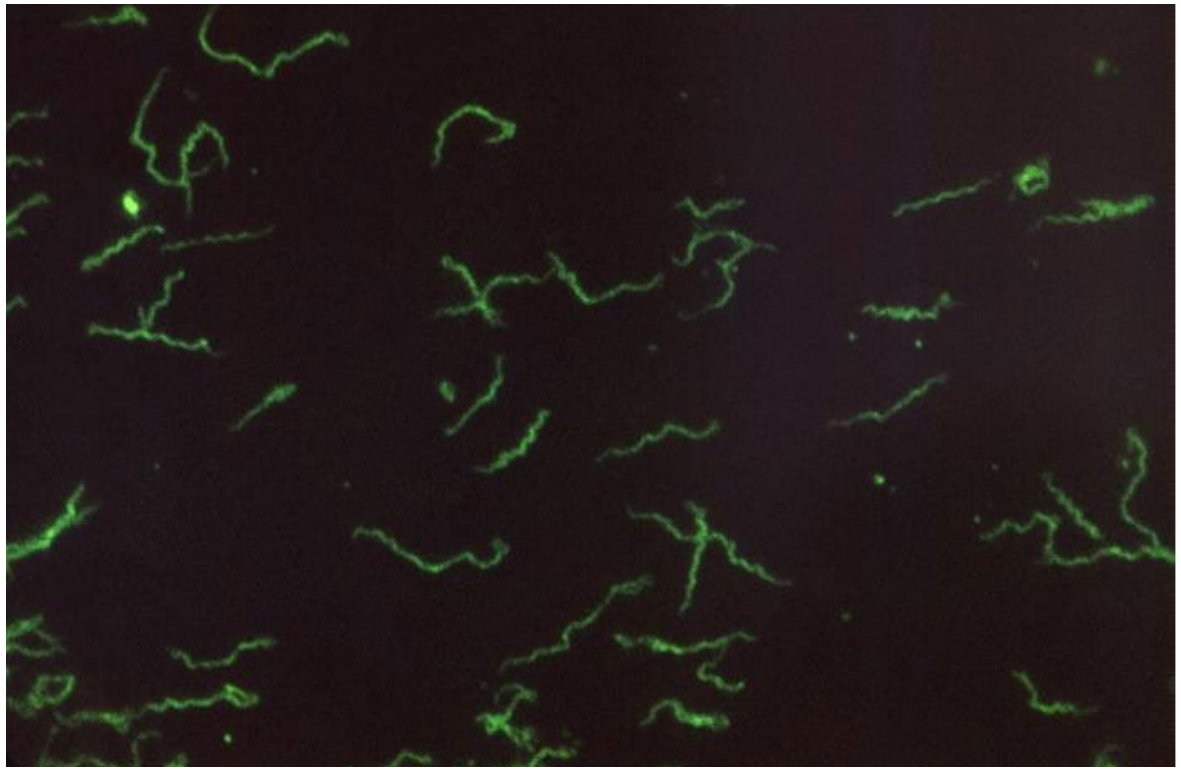
FUJIREBIO SERODIA TP-PA

Table 4

Settling Patterns of Particles	Reading	Interpretation
Particles are concentrated in the shape of a button at the center of the well with a smooth round outer margin.	(-)	Non-Reactive
Particles are concentrated in the shape of a compact-ring with a **very small** "hole" in the center and a smooth round outer margin..	(-)	Non-Reactive
Particles are concentrated in the shape of a compact ring with a "hole" in the center and a smooth round outer margin.	(±)	Inconclusive
Defined large ring with a rough multiform outer margin and peripheral agglutination.	(+)	Reactive
Agglutinated particles spread out covering the bottom of the Well uniformly, edges sometimes folded.	(++)	

FTA-ABS

- Alternative to TP-PA as confirmatory assay
- Serum is layered on slide that is coated with *T. pallidum* and FITC-labeled antihuman IG is added
- Requires experienced microscopist
- Subjective interpretation



Public Health Image Library - CDC/ Russell

Syphilis Immunoassays

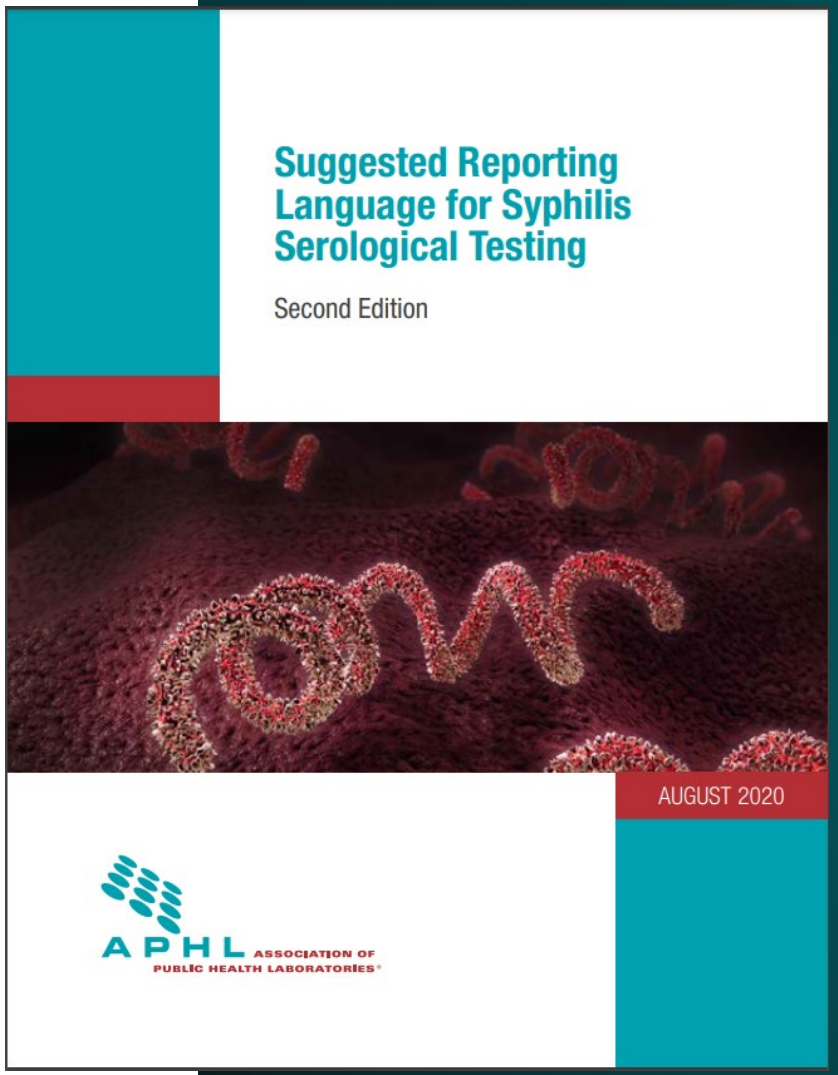
- Qualitative assays that detect anti-treponemal IgM and/or IgG antibodies
- Most are on automated platforms
- Examples:
  - Bio-Rad Syphilis IgG on the EVOLIS
  - Abbott Syphilis TP on the Architect
  - Elecsys Syphilis on the Cobas (IgM and IgG)
- Commonly used as the 1st test in the reverse algorithm

Reporting Language for Syphilis Serological Testing

Use this slide to transition between topics

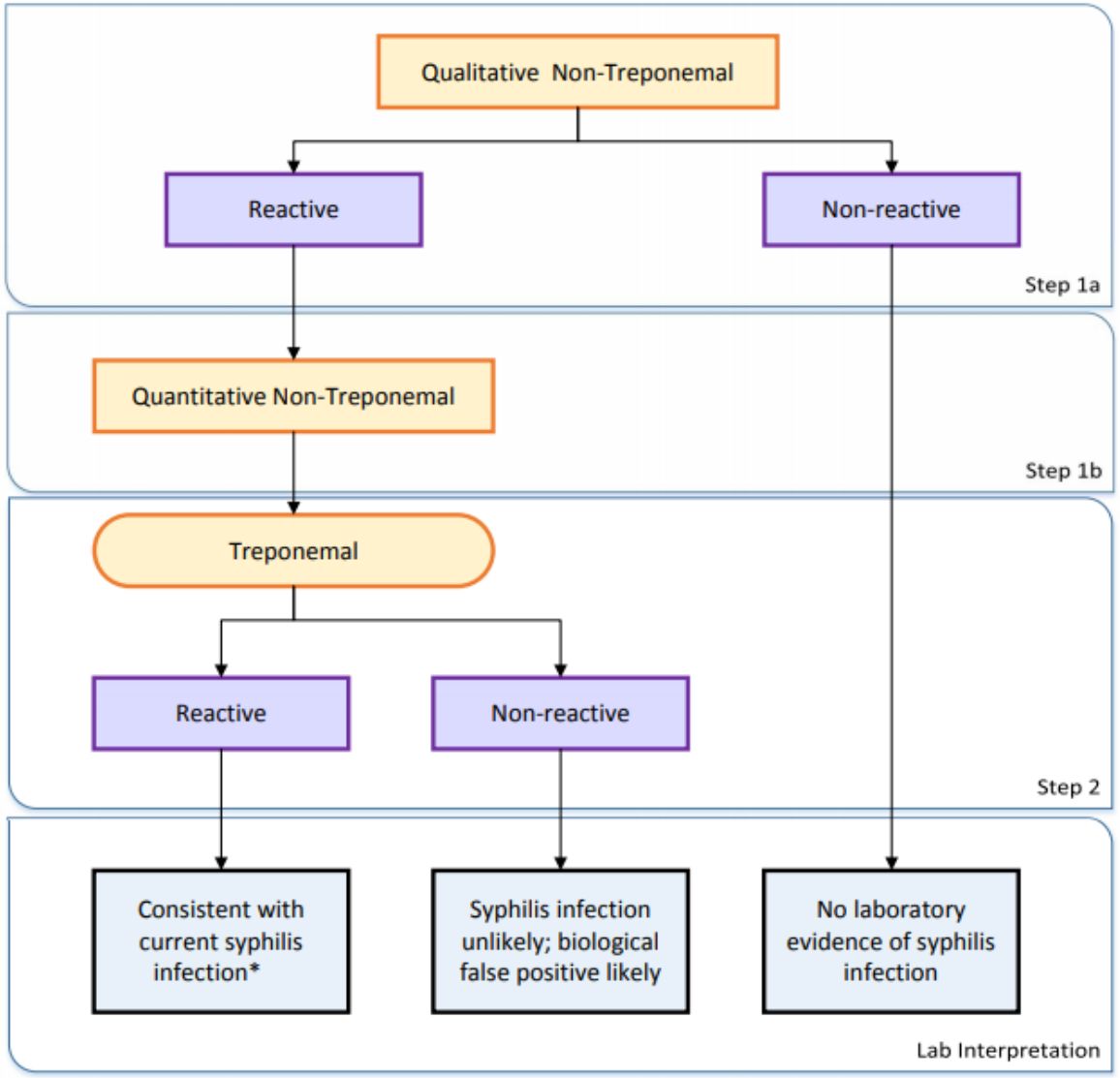
Syphilis Reporting

- With multiple tests being reported for the same specimen, it is important to review all results before reporting.
- Look for discrepancies.
  - For example:
    - RPR Reactive, TPPA Negative
    - RPR Nonreactive, TPPA positive
- If possible, refer to previous result
  - Use this to identify discrepancies
    - TP-PA previous positive, now negative
    - RPR titer increase from previous result when patient has been treated



https://www.aphl.org/aboutAPHL/publications/Documents/ID-2020Aug-Syphilis-Reporting-Language.pdf

Traditional Syphilis Testing Algorithm

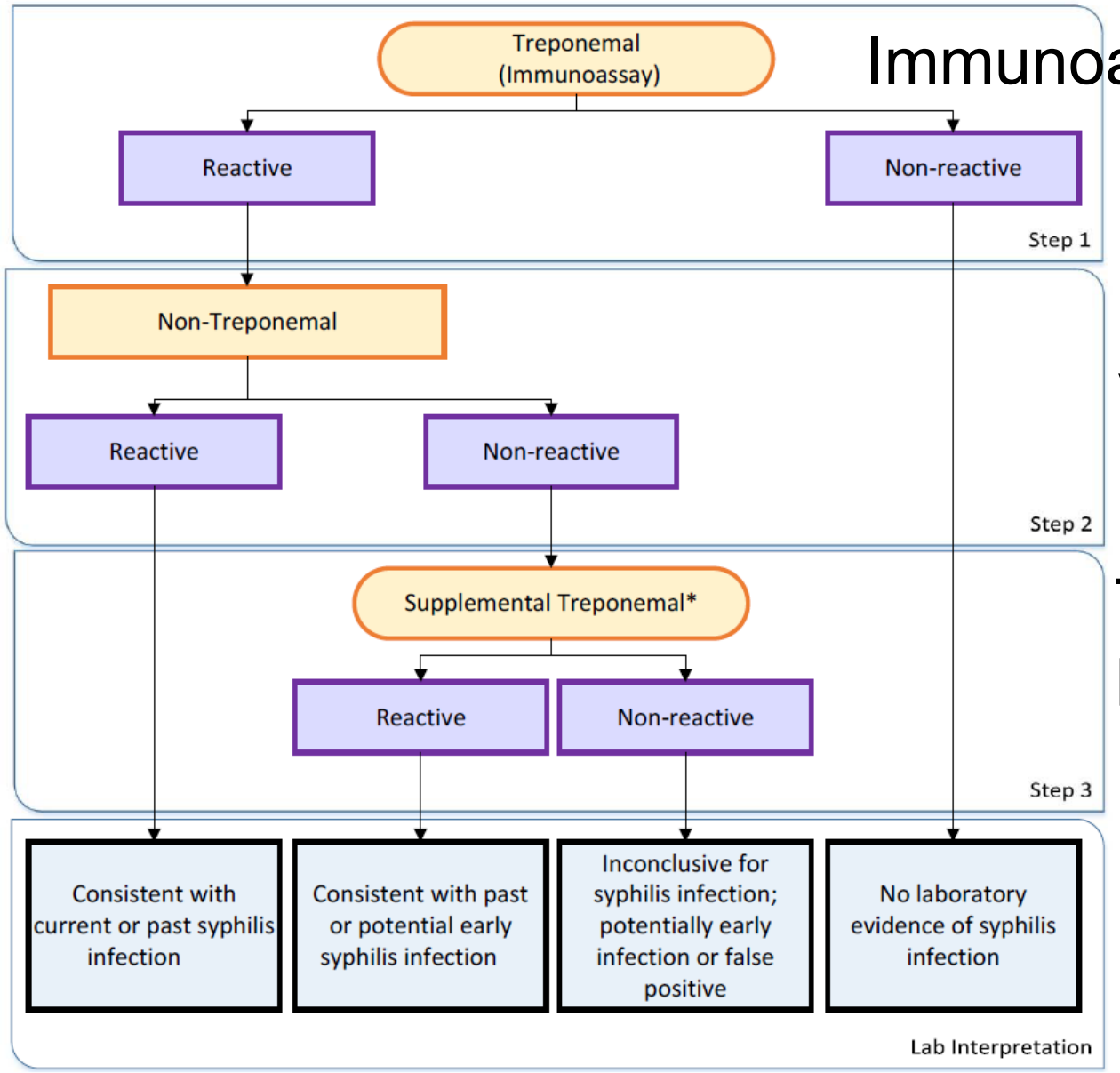


RPR/
VRDL

TP-PA/
FTA-ABS

*If titer <1:4 consider these values associated with possible serofast condition.[22] Serofast is used to refer to those persons with early syphilis with non-treponemal titers that neither increase nor decrease 4-fold after treatment.[2,3]

Reverse Syphilis Testing Algorithm



Immunoassay

RPR/
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Questions and Discussion