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# Serologic Testing for Syphilis

## Comparison of the Traditional and Reverse Screening Algorithms

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# Disclosures

None

# Objectives

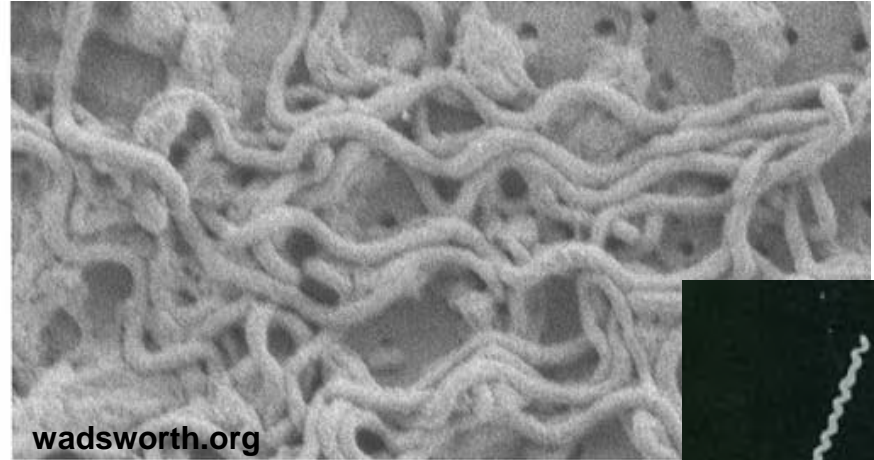
- Describe the treponemal and non-treponemal assays for syphilis screening
- Discuss the advantages and limitations of both the traditional and reverse syphilis screening algorithms
- Result interpretation from the reverse syphilis screening algorithm

# Outline

- Syphilis Infection
  - Causative Agent
  - Clinical Manifestations
- Laboratory Tests for Diagnosis of Syphilis
  - Non-treponemal Tests
  - Treponemal Tests
- Traditional Algorithm for Syphilis Screening
- Reverse Algorithm for Syphilis Screening
- Interpretation and Follow-up

# *Treponema pallidum* – The Agent of Syphilis

- Spirochete
- Obligate human parasite
- Transmission
  - Sexual
  - Trans-placental
  - Percutaneous following contact with infectious lesions
  - Blood Transfusion
    - No reported cases of transmission since 1964



# Syphilis – The “Great Imitator”

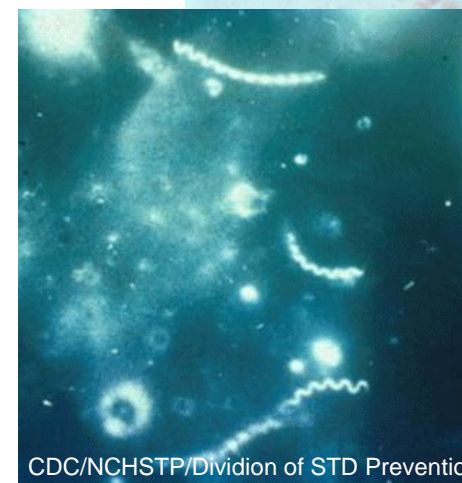
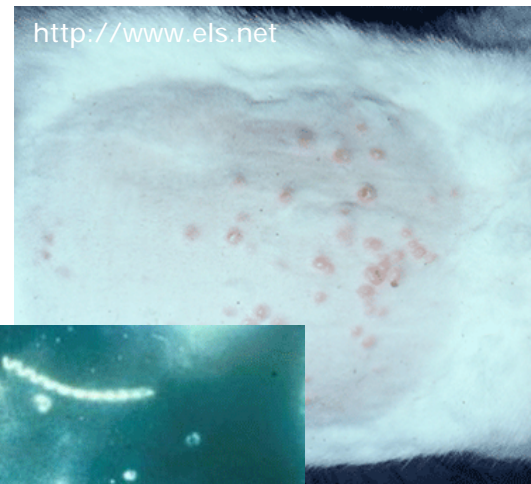
- Infectious Dose: ~57 organisms<sup>1</sup>
- Incubation Period – 21 days (median)
- 3 clinical stages of syphilis
  - **Primary:**
    - Painless sore (chancre) at inoculation site
  - **Secondary:**
    - Rash, Fever, Lymphadenopathy, Malaise
  - **Tertiary/Latent:**
    - CNS invasion, organ damage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



# Laboratory Diagnosis of Syphilis

## The Uncommon Methods

- Rabbit Infectivity Test (RIT)
  - High Sensitivity and Specificity
  - Long turn-around-time
  - Limited to research settings
- Dark Field Microscopy
  - Useful only during primary infection
  - Technician expertise required
- Immunostaining
  - Direct fluorescent antibody or silver stain
- Polymerase Chain Reaction (PCR)
  - Not commercial available



# Laboratory Diagnosis of Syphilis

## The Common Methods

- Serology
  - Mainstay for syphilis testing
  - Two classes of serologic tests
    - Non-treponemal
    - Treponemal



# Serologic Tests for Syphilis:

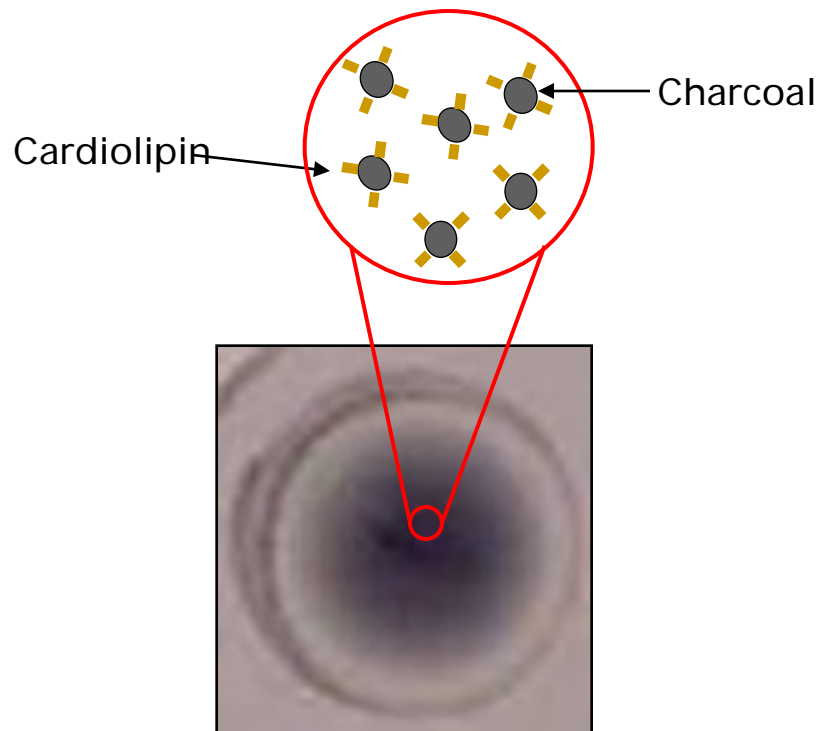
## Non-Treponemal Assays

- Principle:
  - *T. pallidum* infection leads to the production of reagin
    - **Reagin** – Antibodies to substances released from cells damaged by *T. pallidum*
    - Reagin reacts with cardiolipin
      - **Cardiolipin** – a phospholipid component of certain eukaryotic and prokaryotic membranes
- Examples of non-treponemal tests:
  - Rapid Plasma Reagin (RPR)
  - Venereal Disease Research Laboratory (VDRL)

# Serologic Tests for Syphilis:

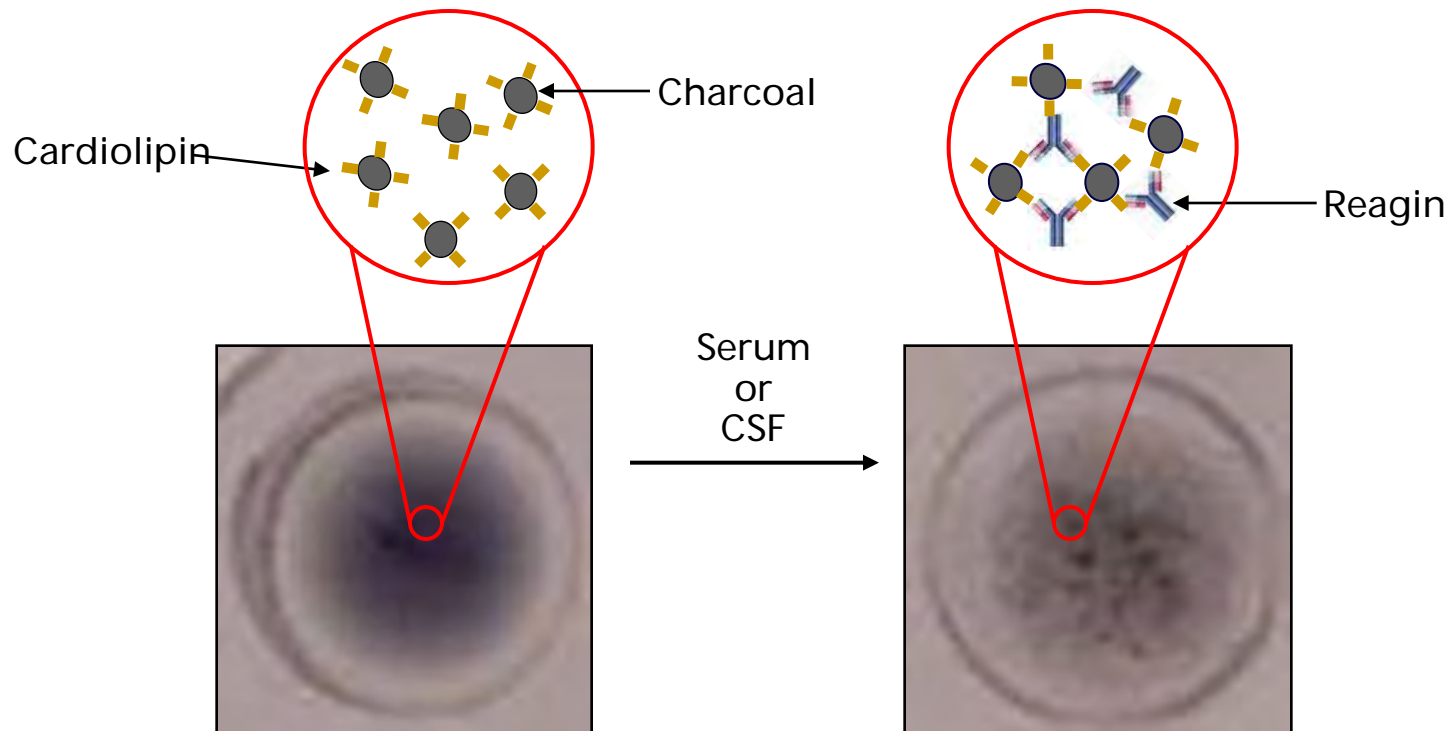
## Non-Treponemal Assays

- RPR and VDRL are agglutination assays



# Serologic Tests for Syphilis: Non-Treponemal Assays

- RPR and VDRL are agglutination assays



# Non-Treponemal Tests:

## Advantages

- Rapid turnaround time – Minutes
- Inexpensive
- No specialized instrumentation required
- Usually revert to negative following therapy
  - Can be used to monitor response to therapy

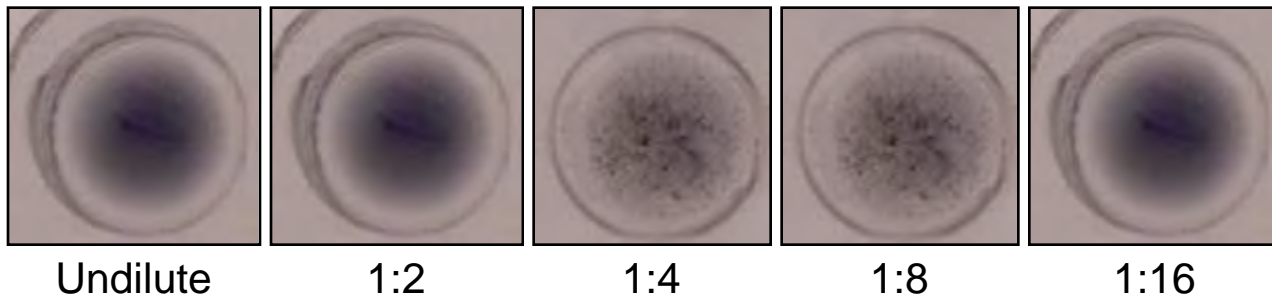
# Non-Treponemal Tests: Limitations

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

# Non-Treponemal Tests:

## Limitations, continued

- Possibility for prozone effect
  - High levels of antibody may inhibit the agglutination reaction
  - To identify prozone, labs must serially dilute samples



# Serologic Tests for Syphilis:

## Treponemal Assays

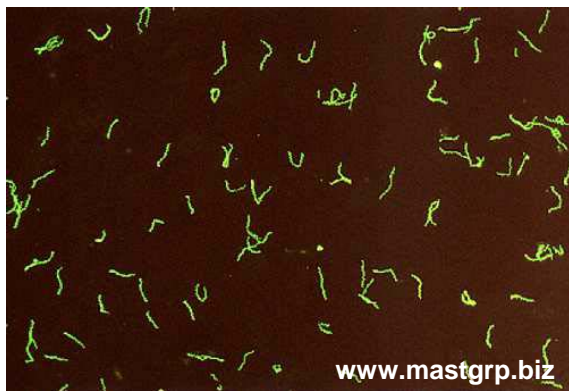
- Principle:
  - Infection leads to production of specific antibodies directed against *T. pallidum*
- Treponemal tests detect IgG or total IgM/IgG antibodies directed against *T. pallidum*

# Serologic Tests for Syphilis:

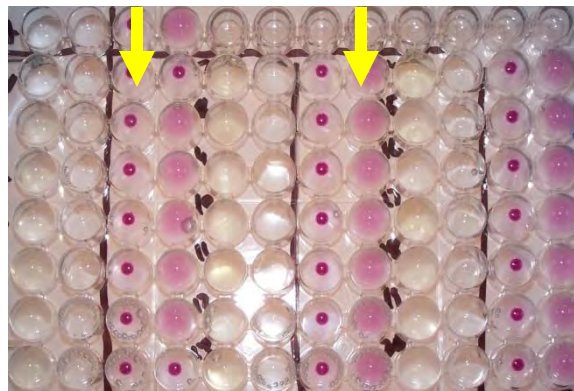
## Treponemal Assays

- Microhemagglutination assay (MHA)
- Fluorescent treponemal antibody (FTA-ABS)
- *Treponema pallidum* particle agglutination (TP-PA)
- Enzyme Immunoassay (EIA)
- Multiplex Flow Immunoassay (MFI)

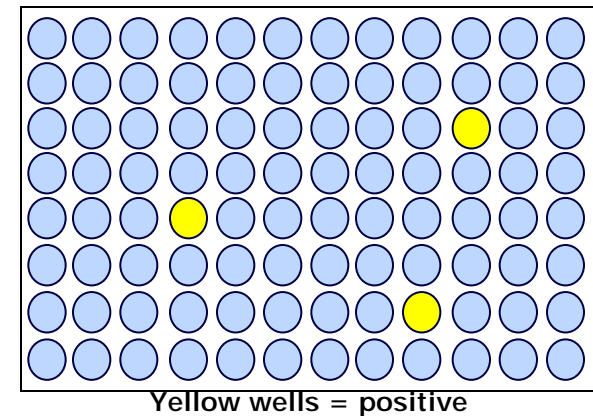
FTA-ABS



TP-PA

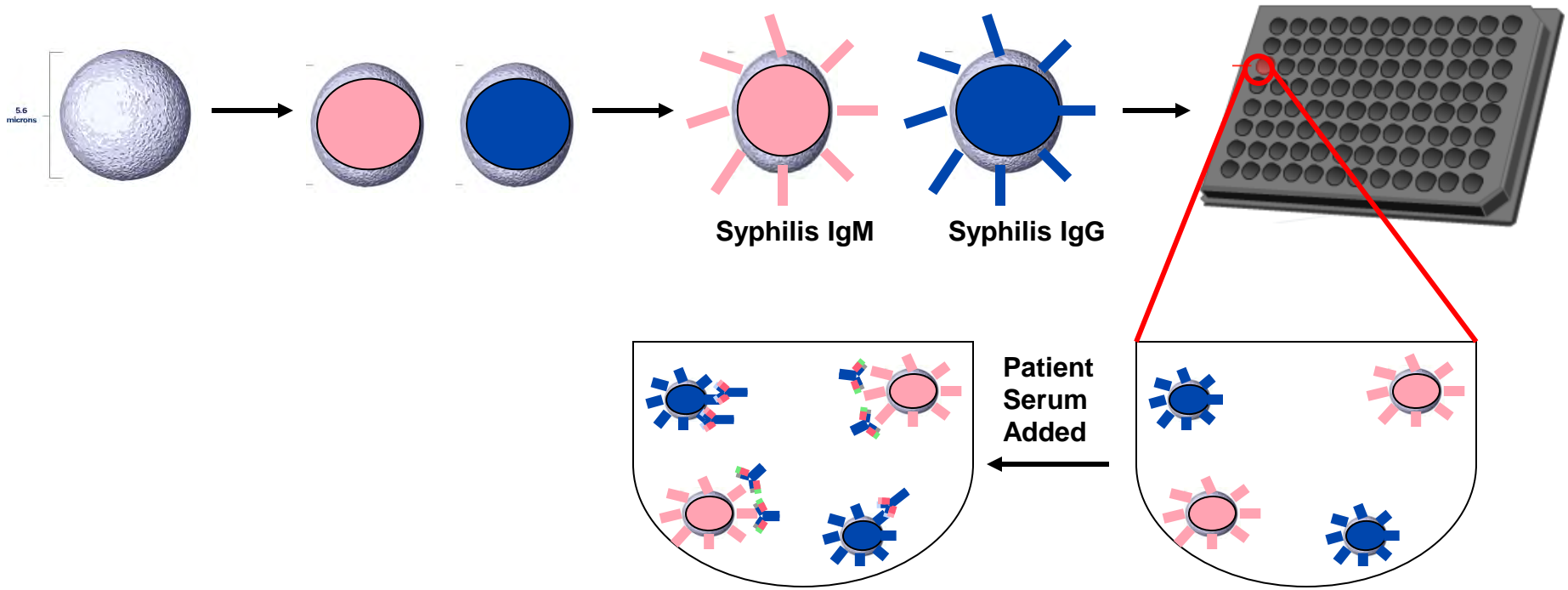


Conventional EIA

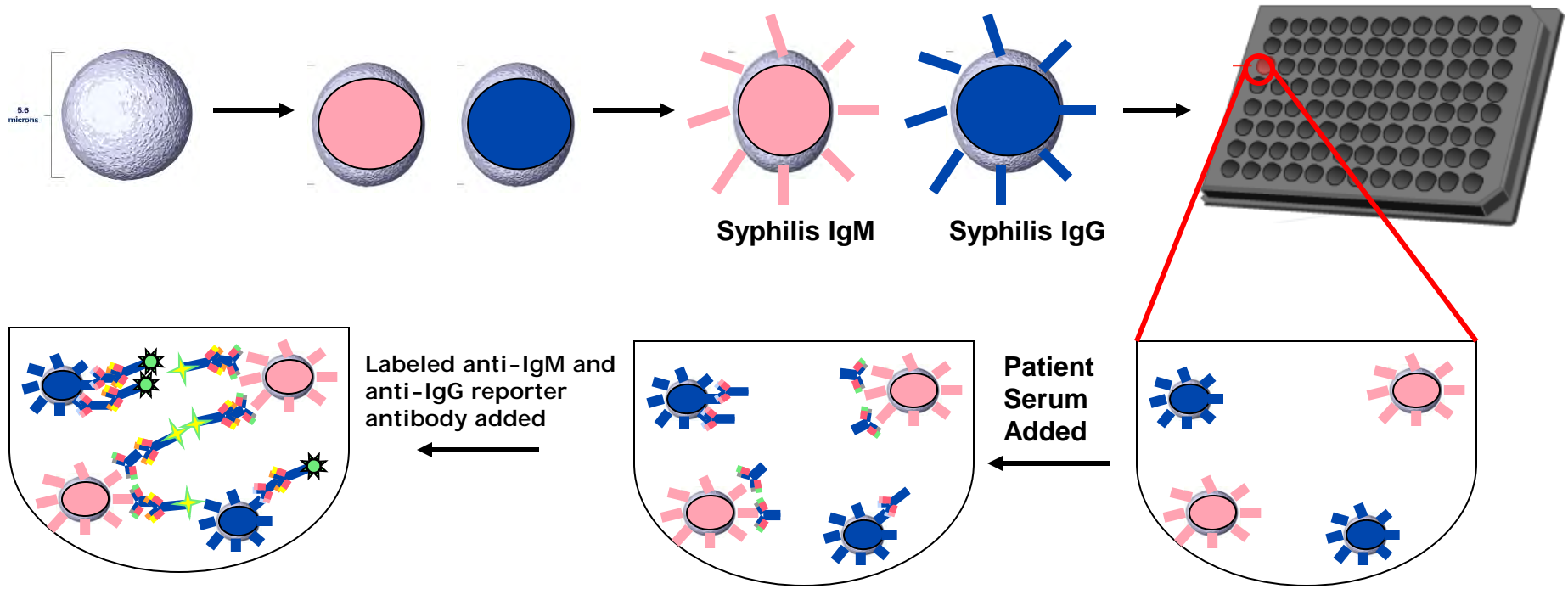




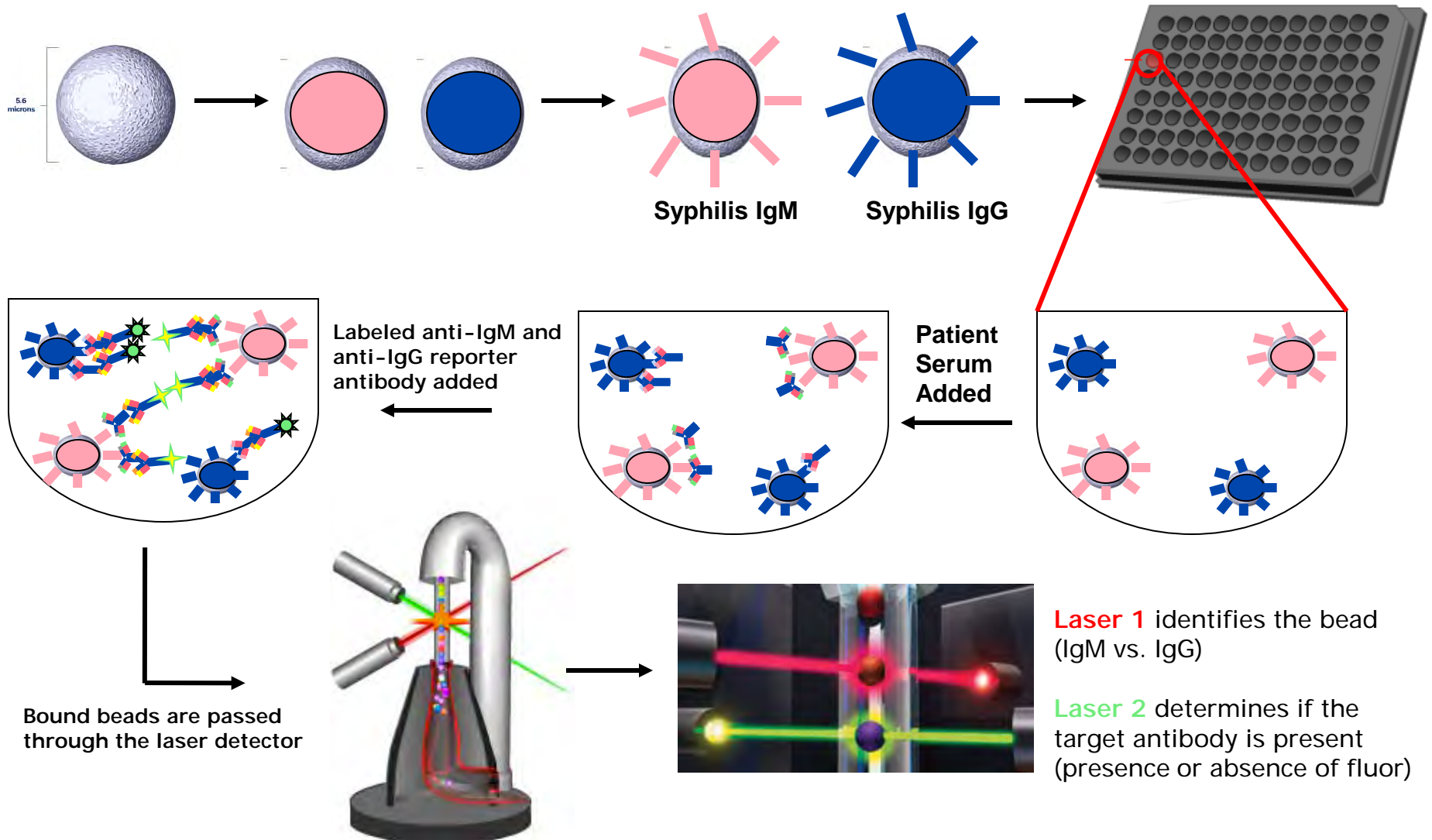
# Treponemal Assays: Multiplex Flow Immunoassays



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# Treponemal Assays:

## Advantages

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

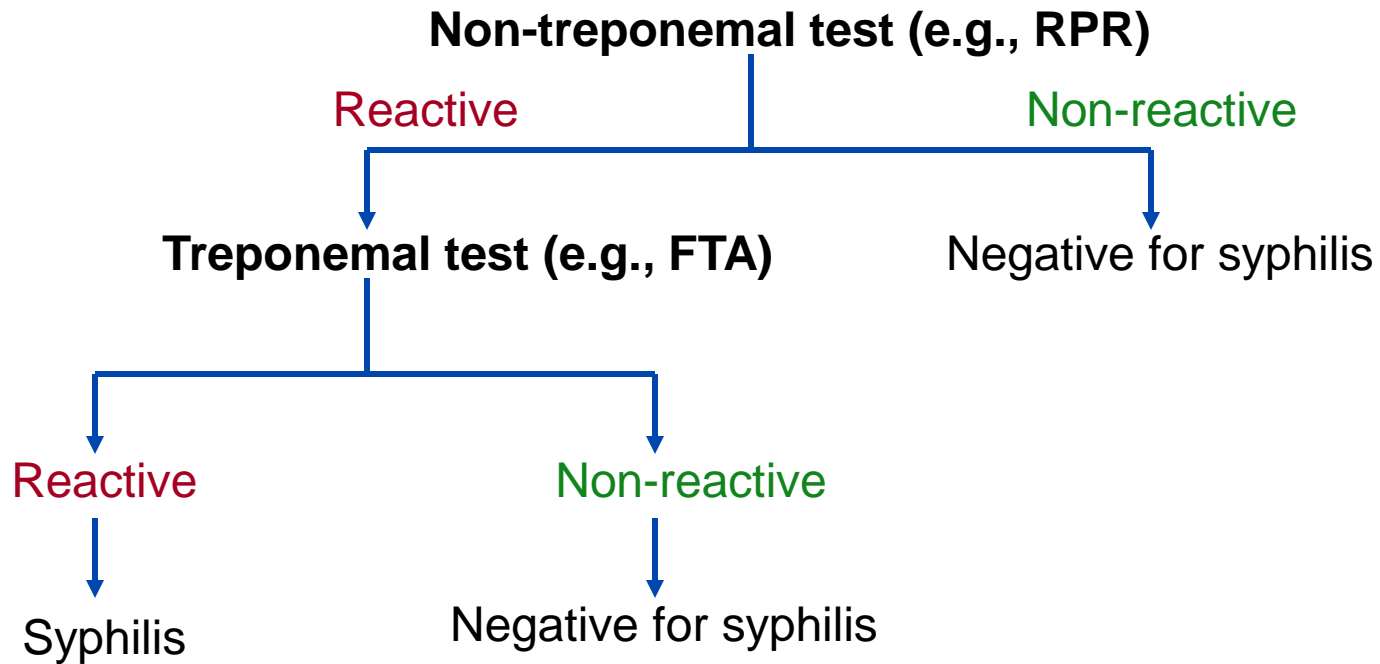
# Treponemal Assays:

## Limitations

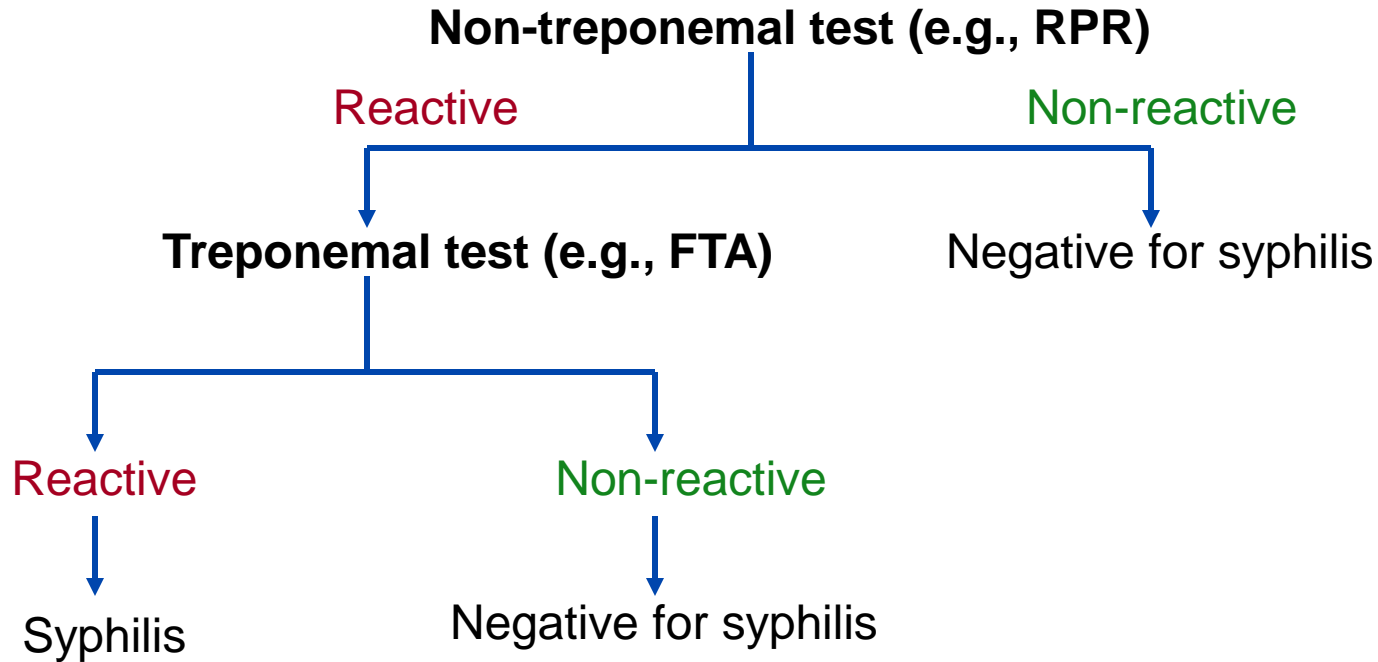
- Remain positive despite treatment
  - **Cannot** be used to monitor response to therapy
- Conventional Methods
  - Subjective interpretation requiring technician expertise to read
- Newer Methods
  - Expensive instrumentation
  - Higher cost/test

# Syphilis Screening Algorithms: Traditional versus Reverse Screening

# Traditional Algorithm



# Traditional Algorithm

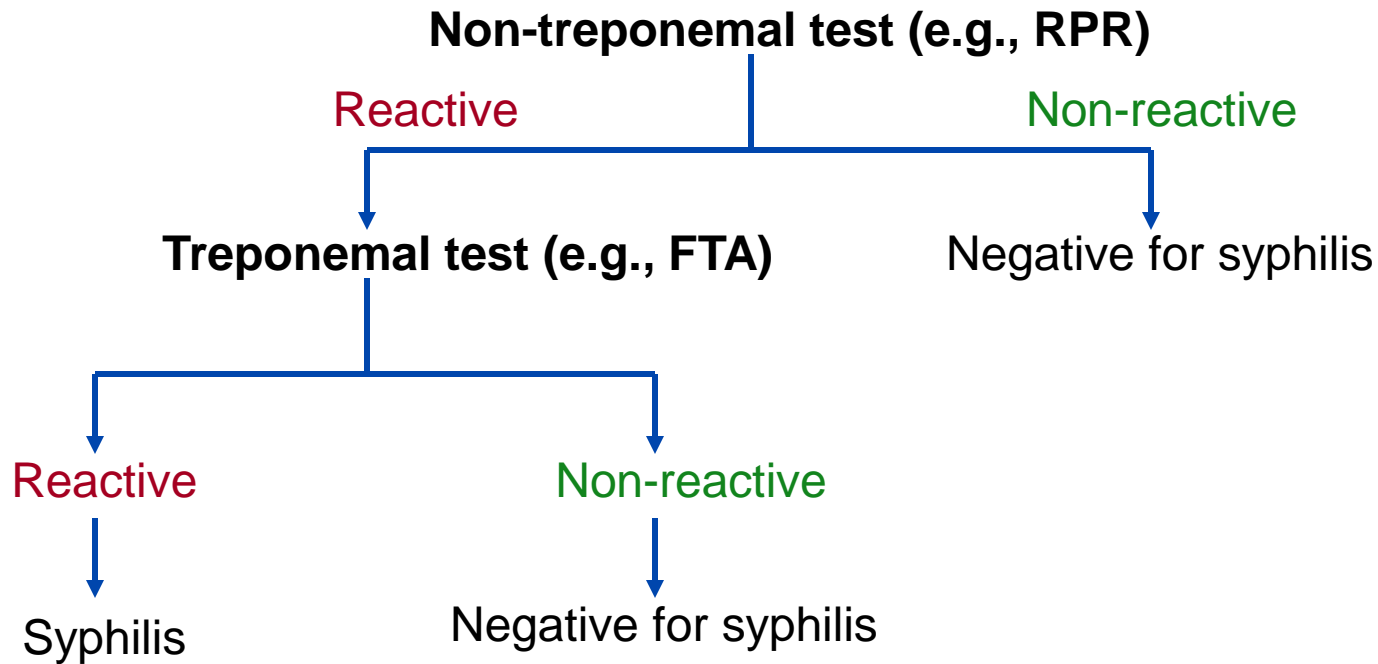


## Advantages:

- Results show good correlation with disease status
- Rapid, inexpensive screening method
- Excellent option for laboratory with small throughput
- Recommended by the CDC



# Traditional Algorithm



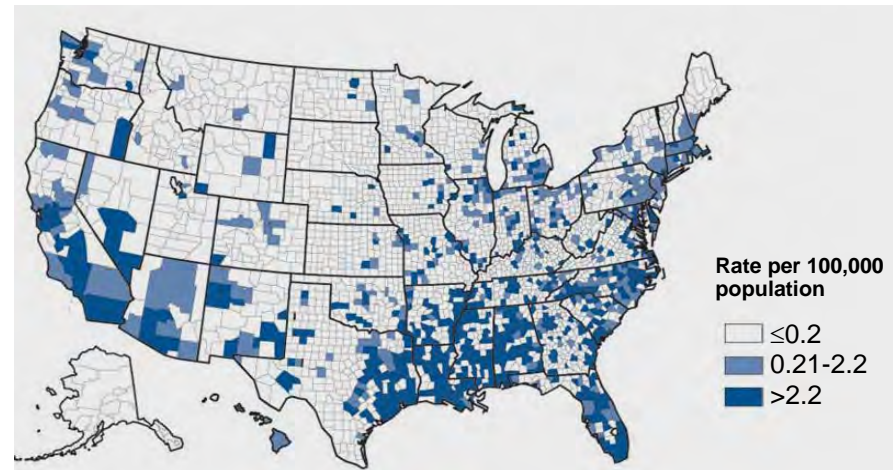
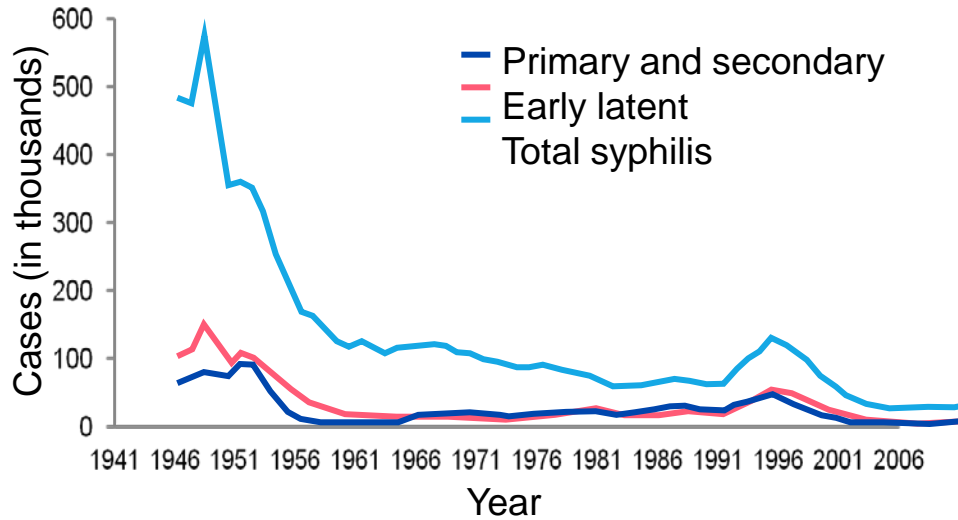
## Disadvantages:

- Manual (RPR) and subjective interpretation
- Screening method is non-specific and *may* lead to false-positive results
- Not suitable for high throughput laboratories
- Potentially lower sensitivity for detecting early syphilis and late/latent disease

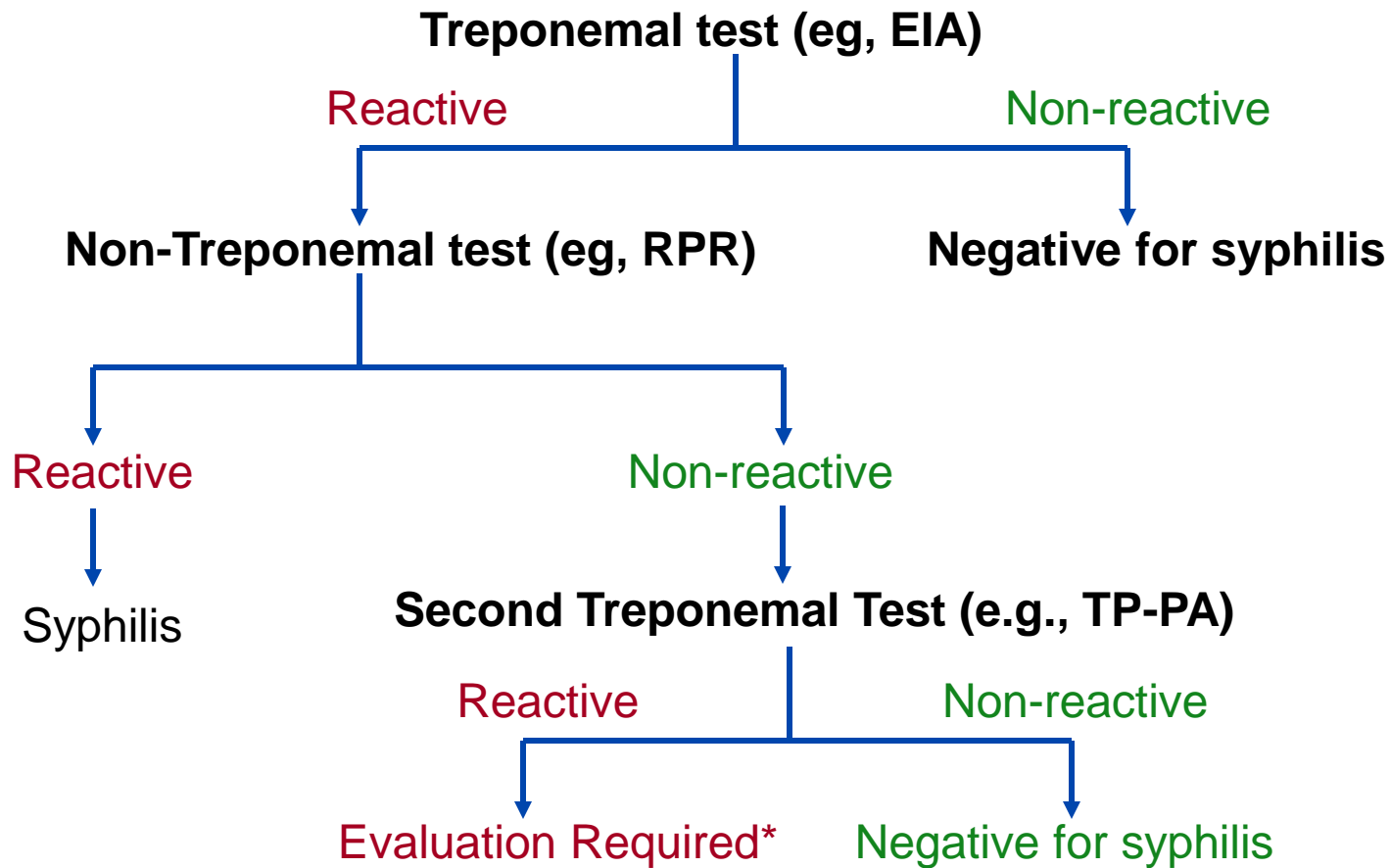
# The Traditional Syphilis Algorithm:

## If it works, why change it?

- Incidence of disease impacts the positive predictive value of the assay



# Reverse Algorithm



# Reverse Algorithm: Advantages

- Automated treponemal screening assays are available (i.e., EIA, MFI)<sup>2</sup>
  - > 500 sera/9 hr shift by MFI vs. ~ 200 sera/9 hr shift by manual methods
- Objective interpretation of results
- Results from EIA or MFI can be interfaced with LIS
- Specific screening test for anti- *T. pallidum* antibodies
- Potentially increased detection of patients with early syphilis<sup>3</sup>:
  - Among 560 patients with lesions, 18 (3.2%) were EIA (+), DFA (+) and RPR (-)
  - Among 9,137 patients with EIA (+), RPR (-) results, 54 became RPR (+) on follow-up testing

# Reverse Algorithm: Limitations

- Higher cost/sample
- Higher assay complexity
- Increased detection of patients with screen (+), RPR (-) results<sup>4,5</sup>:
  - CDC - ~56% of EIA reactive samples are non-reactive by RPR
  - How do we interpret these results?

# Reverse Syphilis Screening Algorithm: Result Interpretation

## Case #1

- 37-year-old with HIV
- Presents to primary care physician with a 2-week history of fatigue, intermittent fever and new rash on palms and soles
- Previously resolved genital lesion
- Syphilis serology ordered
  - Syphilis IgG by EIA: **positive**
  - RPR: **positive**, titer of 1:64

# Reverse Syphilis Screening Algorithm: Result Interpretation

## Case #1 Conclusion

- No further testing needed on this sample
- **Interpretation:** "Untreated or recently treated syphilis." Follow CDC treatment guidelines<sup>4</sup>
- For treatment follow-up:
  - Samples can be tested directly by RPR.
  - A 4-fold decrease in RPR titers (eg, 1:64 to 1:16) is interpreted as response to therapy

# Reverse Syphilis Screening Algorithm:

## Result Interpretation

### Case #2

- 23-year-old female
- Evaluated during first-trimester, routine pregnancy visit
- Previously healthy
- Syphilis serology ordered
  - Syphilis IgG by EIA: **positive**
  - RPR: **negative**
  - Second treponemal test, TP-PA: **negative**



# Reverse Syphilis Screening Algorithm:

## Result Interpretation

### Case #2 Conclusion

- **Interpretation:** “Probable false-positive screening test. Negative for syphilis.”
- False-positive serologic tests are not uncommon during pregnancy and confirmatory testing is often required
- Syphilis IgM testing **not** recommended for routine pregnancy screening

# Reverse Syphilis Screening Algorithm:

## Result Interpretation

### Case #3

- 50-year-old immigrant from Somalia
- Pre-kidney transplant evaluation
- Syphilis serology ordered
  - Syphilis IgG by EIA: **positive**
  - RPR: **negative**
  - TP-PA: **positive**

# Reverse Syphilis Screening Algorithm: Result Interpretation

## Case #3 Conclusion

- **Interpretation:** "Historical and clinical evaluation required."
- During evaluation with provider, patient indicates no *known* history of treatment for syphilis.
- Patient treated for *possible* latent syphilis

# Reverse Syphilis Screening Algorithm:

## Result Interpretation

### Case #4

- 30-year-old inmate
- Past history of syphilis (10 years prior)
- Syphilis serology ordered
  - Syphilis IgG by EIA: **positive**
  - RPR: **negative**
- **Interpretation:** “Past, successfully treated syphilis. No further testing for syphilis required.”

# Reverse Syphilis Screening Algorithm:

## Summary

Patient history	Treponemal screen	RPR	2 <sup>nd</sup> treponemal <sup>a</sup>	Interpretation	Follow-up
Unknown history of syphilis	NEG	NA	NA	No serologic evidence of syphilis	None, unless clinically indicated (eg, early syphilis)
Unknown history of syphilis	POS	POS	NA	Untreated or recently treated syphilis	See CDC treatment guidelines; follow RPR titers
Unknown history of syphilis	POS	NEG	NEG	Probable false-positive screening test	No follow-up testing, unless clinically indicated
Unknown history of syphilis	POS	NEG	POS	Possible syphilis (eg, latent) or previously treated syphilis	History and clinical evaluation required
Known history of syphilis	POS	NEG	POS or NA	Past, successfully treated syphilis	None

<sup>a</sup>Second treponemal test should be TP-PA or a different method than screening test  
For CDC treatment guidelines see <http://www.cdc.gov/std/treatment/default.htm>

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# Conclusions

- Syphilis is typically diagnosed by serologic means
- Two main classes of syphilis serologic tests:
  - Non-treponemal (e.g., RPR, VDRL)
  - Treponemal (e.g., FTA, TP-PA, EIA, MFI)
- Traditional Algorithm
  - Non-treponemal test first
    - Screen by RPR
    - If RPR positive use treponemal test to confirm
  - Advantages
    - Recommended by CDC
    - Cost-effective
    - Suitable for most lower throughput labs
  - Limitations
    - May miss very early or late/latent infection

# Conclusions

- Reverse Algorithm
  - Treponemal test first
    - Screen by EIA or MFI
    - Screen positive samples tested by non-treponemal test: RPR
    - EIA/MFI and RPR discordant samples should be tested by a second treponemal test: TP-PA
  - Advantages
    - Allows for automation and increased sample throughput
  - Limitations
    - Result interpretation can be challenging
  - Good communication with providers is critical

# References

- 1 Magnuson HJ, et al. Inoculation of syphilis in human volunteers. *Medicine (Baltimore)* 1956;35:33-82.
- 2 Binnicker MJ, et al. Treponema-specific test for serodiagnosis of syphilis: comparative evaluation of seven assays. *J Clin Microbiol* 2012;49:1313-1317
- 3 Mishra S, et al. The laboratory impact of changing syphilis screening from the rapid-plasma reagin to a treponemal enzyme immunoassay: a case study from the greater Toronto area. *Sex Transm Dis* 2011; 38:190-196
- 4 CDC. Discordant results from reverse sequence syphilis screening: five laboratories, United States, 2006-2010. *Morb Mortal Wkly Rep* 2011;60:133-137
- 5 Binnicker MJ, et al. Direct comparison of the traditional and reverse syphilis screening algorithms in a population with a low prevalence for syphilis. *J Clin Microbiol* 2012; 50:148-150



## Questions & Discussion