

# Serologic Testing for Syphilis with Traditional and Reverse Algorithms

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# You'll leave knowing something about...

- “Treponemal” and “non-treponemal” tests for syphilis
- “Traditional” and “reverse syphilis” screening
- Rapid diagnostic tests for syphilis

# Outline

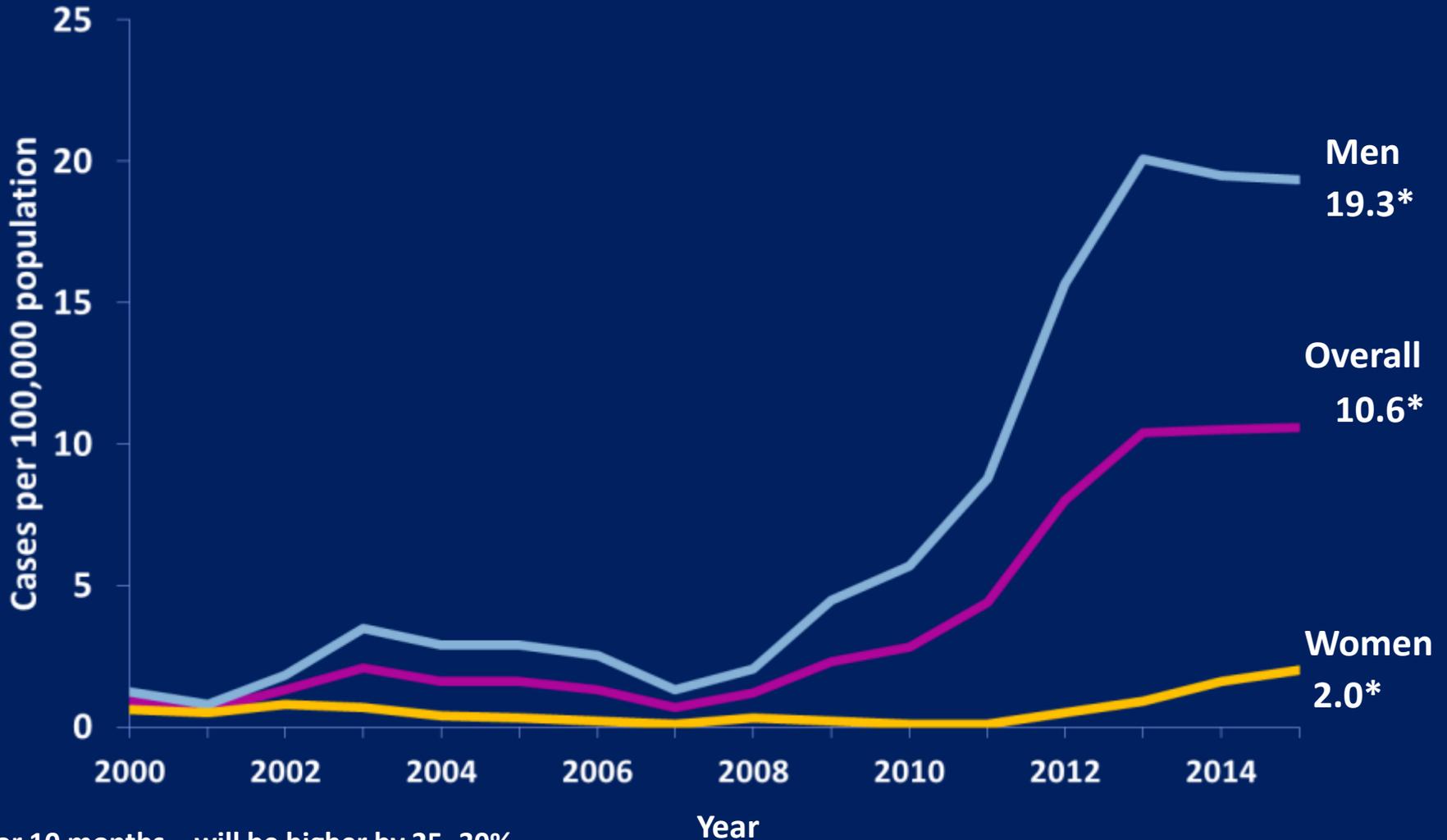
- **Syphilis**
  - **Causative agent**
  - **Key facts about syphilis**
- **Laboratory tests for diagnosis of syphilis**
  - **Non-treponemal tests**
  - **Treponemal tests**
- **Traditional algorithm for syphilis screening**
- **Reverse algorithm for syphilis screening**
- **Interpretation and follow-up**
- **New rapid diagnostic test for syphilis**

# Attribution:

Many slides adapted from...

ES Theel. Serologic Testing for Syphilis: comparison of the Traditional and Reverse Screening Algorithms. Mayo Clinic, Rochester, Minnesota. October 24, 2012  
Available at  
<http://www.arlingtonscientific.com/assets/mayo-serologic-testing-for-syphilis--comparion-of-algorithms.ppt>. Accessed May 16, 2015

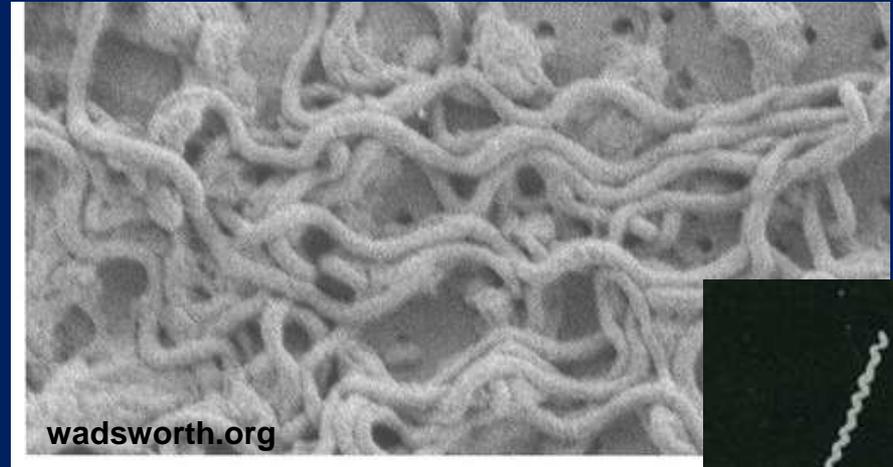
# Rates of early syphilis by sex and year— Oregon, 2000–2015



\*for 10 months....will be higher by 25–30%

# *Treponema pallidum*

- Bacterium
  - “Spirochete”
  - Motile (“corkscrew”)
  - Can’t culture in lab
- Transmission
  - Sexual
  - Trans-placental
  - Percutaneous following contact with infectious lesions
  - Bloodborn
    - Extremely rare



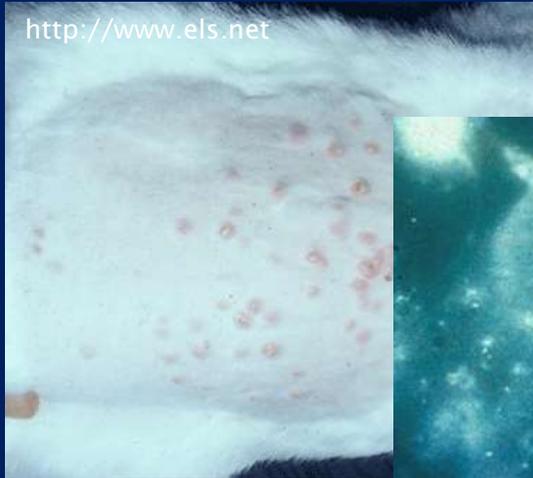
- **Spirochaete**
  - **Spirochaetaceae**
    - **Treponema**
      - *pallidum* (syphilis, yaws), *carateum* (pinta), *denticola*
    - **Borrelia**
      - *burgdorferii/afzelii* (lyme), *hermsii/duttoni/parkeri* (tick-borne relapsing fever)
  - **Leptospiraceae**
    - **Leptospira**
      - *interrogans* (leptospirosis)
  - **Spirillaceae**
    - **Spirillum**
      - *minus* (rat-bite fever)

# Syphilis—a few key concepts

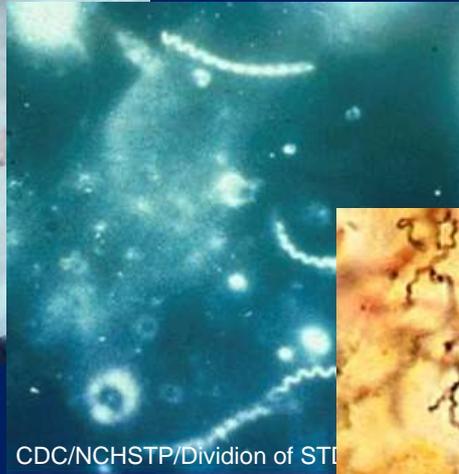
- **Highly infectious**
  - Infectious Dose ~57 organisms
  - Attack rate 1/3
- **Incubation – 21 days (median)**
- **3 clinical stages**
  - **Primary:**
    - Painless sore (chancre) at inoculation site
  - **Secondary:**
    - Rash, fever, lymphadenopathy, malaise
  - **Symptomatic Late/Tertiary:**
    - Dementia, tabes dorsalis, cardiovascular disease



# Lab Diagnosis—uncommon methods



**Rabbit Infectivity Test (RIT)**



**Dark field microscopy**



**Immunostaining**

**Polymerase Chain Reaction (PCR)**



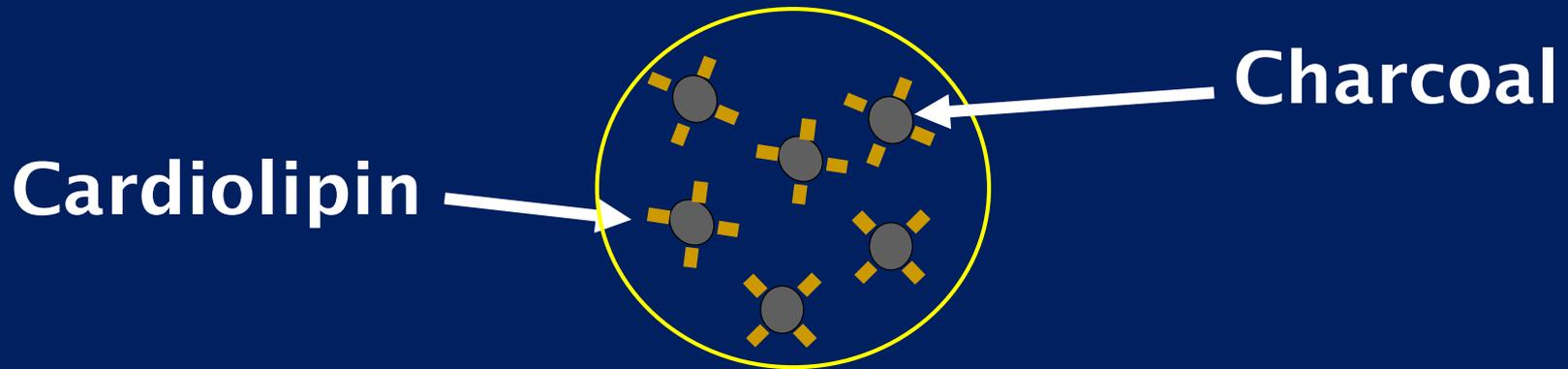
## Lab Diagnosis—common methods

- **Serology (tests for antibodies produced upon syphilis infection)**
  - **Mainstay for syphilis testing**
  - **Two kinds**
    - **Non-treponemal**
    - **Treponemal**

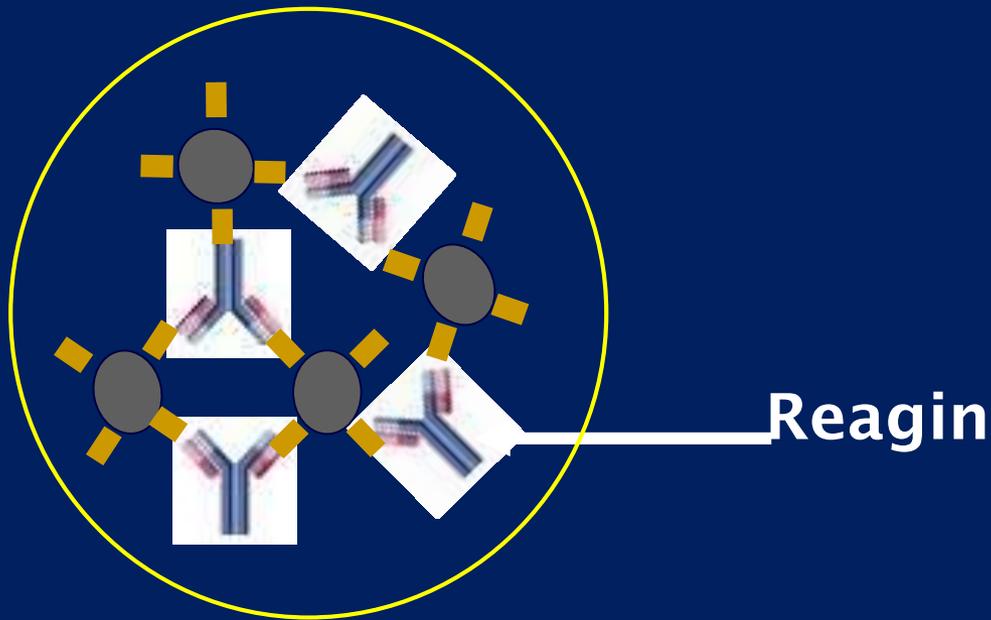
## Non-treponemal serologic tests

- *T. pallidum* causes cells to release cardiolipin
- Reagin = antibody to cardiolipin
- Non-treponemal tests measure levels of reagin:
  - Rapid Plasma Reagin (RPR)
  - Venereal Disease Research Laboratory (VDRL)
  - Toluidine red unheated serum test (TRUST)

- **RPR and VDRL are agglutination assays**
- **Reagent is carbon particles+cardiolipin**
- **No reagin present, no agglutination**



# Reagin present...agglutination of the charcoal

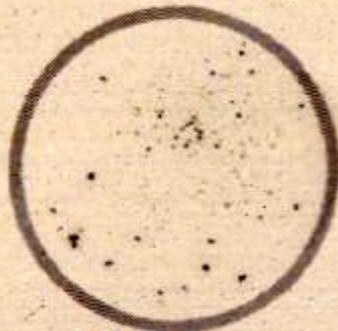


**MACRO-VUE®  
RPR Card Test  
18 mm CONTROL**

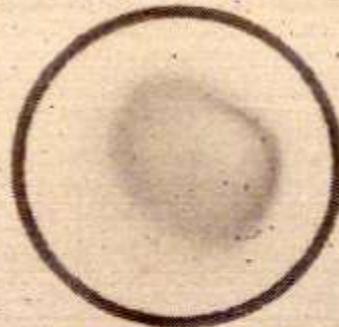
*A Brewer Diagnostic Card*

U. S. PAT. NO. 3,074,853

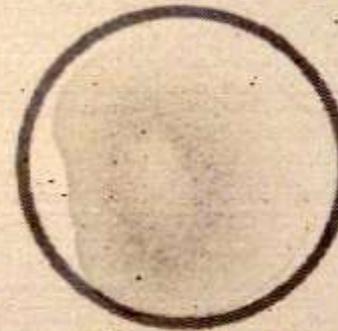
**FOR IN VITRO DIAGNOSTIC USE**



**Reactive**



**Nonreactive**



**Reactive  
(Minimal to  
Moderate)**

5011

Becton Dickinson Microbiology Systems  
Div. of Becton Dickinson and Co., Cockeysville, MD USA

# Non-Treponemal Test 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 Test Limitations

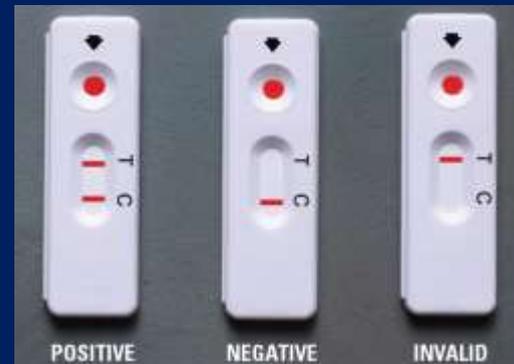
- Results are subjective
  - Intra- and Inter-laboratory variability
- False positives (lower specificity)
  - lupus, pregnancy, viral hepatitis
- Might be negative (lower sensitivity) in very early syphilis and late syphilis even if never treated
- Low “throughput” = can’t be “batched”

# Treponemal serologic tests

- Syphilis ⇒ Antibodies against *T. pallidum*
- Tests detect 'treponeme specific' antibodies
  - Fluorescent treponemal antibody absorption test (FTA-ABS)
  - Microhemagglutination assay (MHA)
  - *T. pallidum* particle agglutination (TP-PA)
  - Enzyme Immunoassay (EIA)
  - Immunochromatographic strips (ICS...point of care tests)



FTA-ABS



ICS

# Treponemal Test Advantages

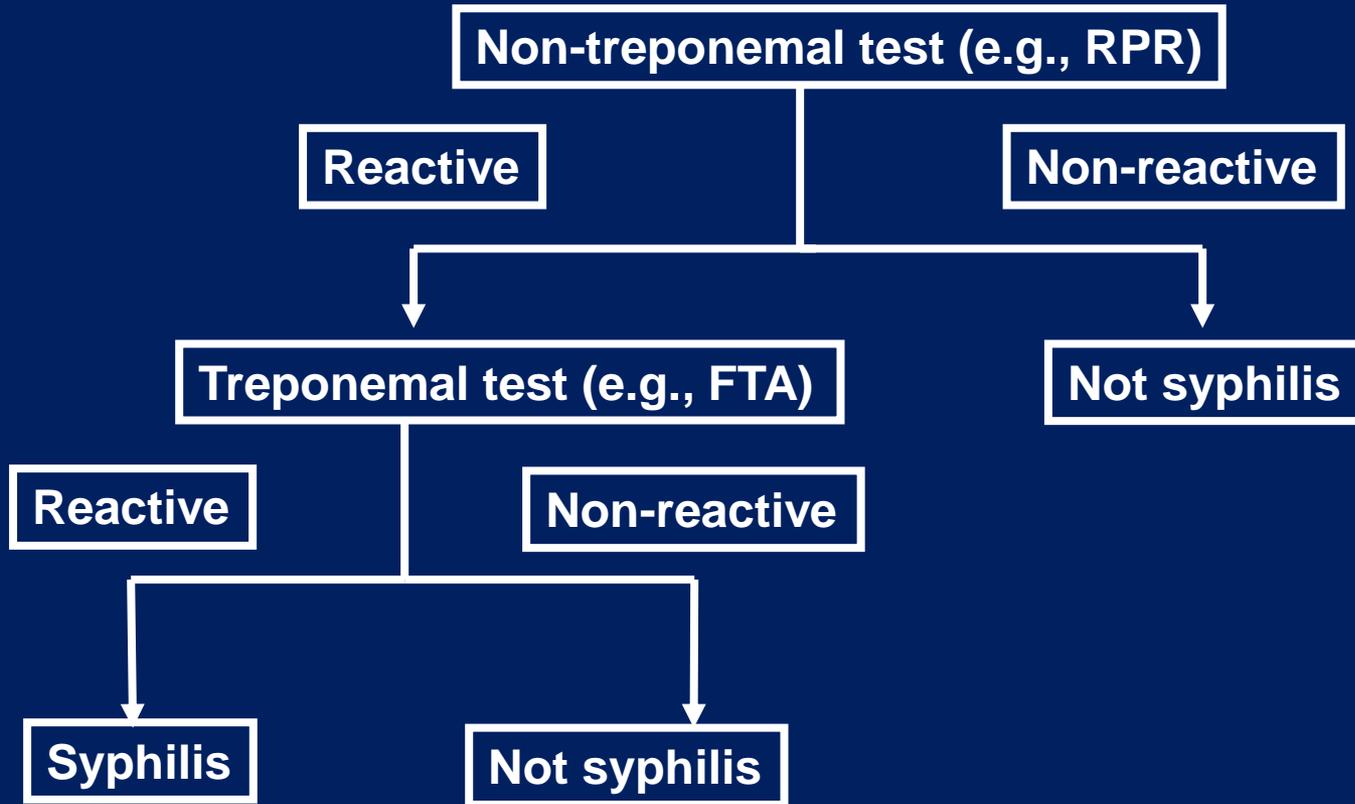
- Few false positives (high specificity)
- Fewer false negatives (more sensitive) especially during early and late syphilis
- Objective result interpretation
- Automation option
- High throughput = “batchable”
- High reproducibility/precision

# Treponemal Test Limitations

- **Remain positive for life**
  - **Cannot be used to monitor response to therapy**
- **Conventional (older) versions (e.g. FTA-ABS, TP-PA)**
  - **Subjective interpretation like non-treponemal tests**
- **Newer versions**
  - **Expensive instrumentation**
  - **Higher cost/test**

# Syphilis Screening Algorithms: Traditional versus 'Reverse'

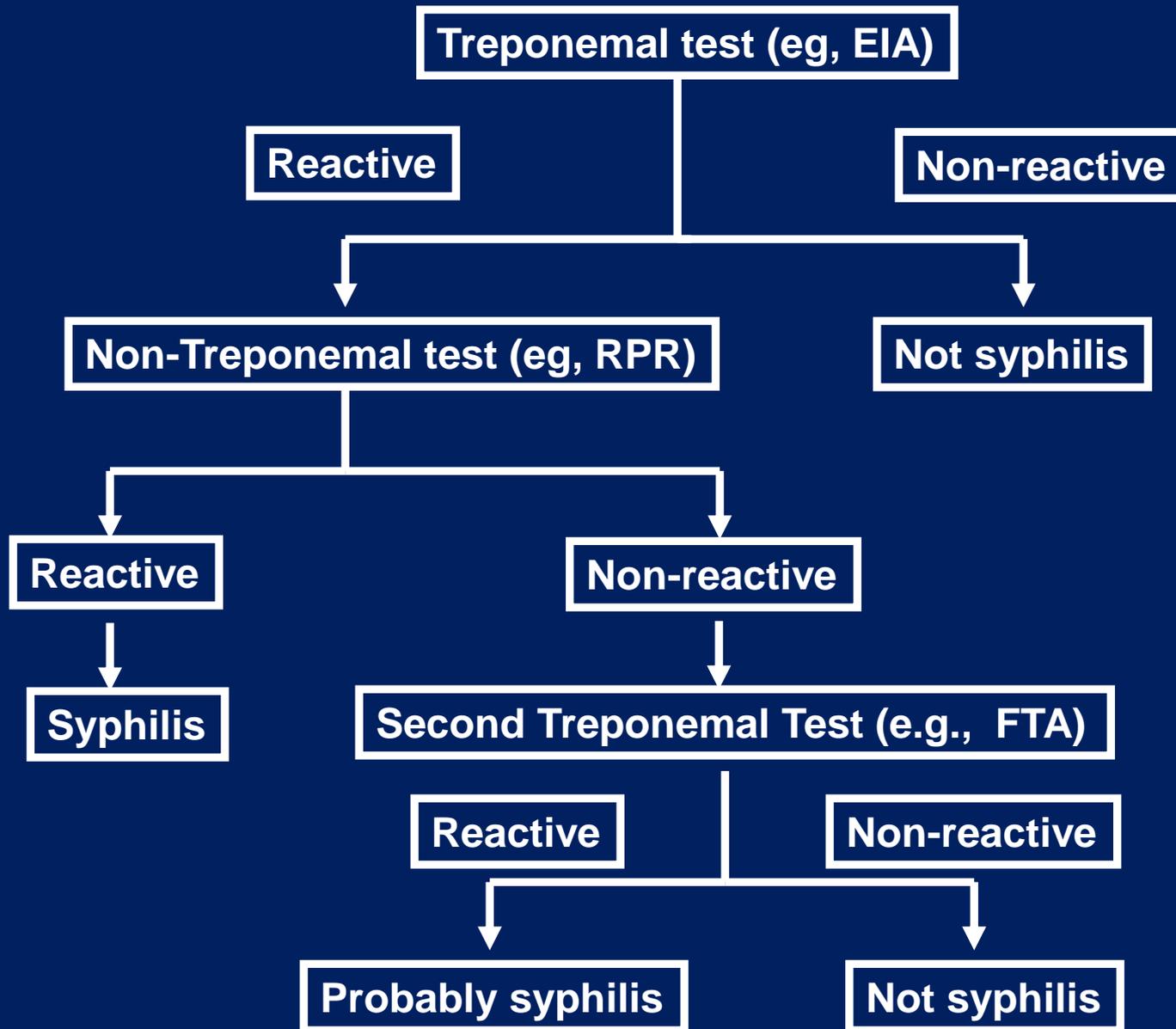
# Traditional Algorithm



# Traditional algorithm pros and cons

- **Pros**
  - **Familiar**
  - **One confirmation test, typically done reflexively, leads to clear result**
  - **Rapid, inexpensive**
  - **Recommended by CDC**
- **Cons**
  - **Manual**
  - **Subjective interpretation**
  - **False-positives**
  - **False negatives, especially late syphilis**

# Reverse Algorithm



# Reverse algorithm pros and cons

- **Pros**
  - **Objective**
  - **Can be batched for high volume labs**
  - **Recommended by public health agencies in Europe and Canada**
  - **More sensitive and more specific...more cases of syphilis diagnosed and treated**
- **Cons**
  - **Unfamiliar**
  - **Cost**
  - **Complexity – often second confirmatory test needed, not yet typically done reflexively**
  - **Disfavored by CDC**

# Interpreting reverse algorithm

## Case #1

- 37-year-old man with HIV
- 2-weeks of fatigue, fever and rash on palms and soles
- Previously resolved genital lesion
- Syphilis IgG by EIA: positive
- RPR: positive, titer of 1:64

# Interpreting reverse algorithm

## Case #1 Conclusion

- **Untreated or recently treated syphilis**
- **Follow treatment guidelines**
- **No further testing needed on this sample**
- **For follow-up after treatment**
  - **RPR titers only, should fall 4-fold (2 dilutions, e.g. 1:64 to 1:16)**

# Interpreting reverse algorithm

## Case #2

- 23-year-old female
- First-trimester pregnancy screening
- Previously healthy
- Syphilis IgG by EIA: positive
- RPR: negative
- Second treponemal test, FTA: negative

# Interpreting reverse algorithm

## Case #2 conclusion

- False positive EIA
- Not syphilis
- No further screening at this time
- Consider screening again at 28 weeks and delivery if syphilis prevalent in community

# Interpreting reverse algorithm

## Case #3

- 50-year-old Somali immigrant
- Kidney transplant evaluation
- No known history of syphilis or treatment
- Syphilis IgG by EIA: positive
- RPR: negative
- FTA: positive

# Interpreting reverse algorithm

## Case #3 Conclusion

- Possible latent syphilis
- Evaluate and treat according to current guidelines
- Consider lumbar puncture if neurologic symptoms consistent with late neurosyphilis

# Interpreting reverse algorithm

## Case #4

- 30-year-old inmate
- Past history of treated syphilis (10 years prior)
- Syphilis IgG by EIA: positive
- RPR: negative

# Interpreting reverse algorithm

## Case #4 Conclusion

- Consistent with successfully treated syphilis
- No additional testing needed

# Summary

- Syphilis usually diagnosed by serology
  - Non-treponemal (e.g., RPR, VDRL)
  - Treponemal (e.g., FTA, TP-PA, EIA, MFI)
- Traditional Algorithm
  - Non-treponemal test (RPR) first
  - 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

# Summary

- **Reverse Algorithm**
  - **Treponemal test first**
  - **Confirm with RPR**
  - **If RPR negative, use different treponemal ‘tiebreaker’ test**
  - **Advantages**
    - **High volume throughput**
    - **More sensitive, same specificity**
  - **Limitations**
    - **Result interpretation can be challenging**
    - **‘Tiebreaker’ test not yet reflexive in most labs**