Syphilis Testing Tools and Interpretation of Results

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Objectives

- Examine the **appropriate Syphilis testing tools** and **confidently interpret** the results.
- Determine the expected **timing** for the onset of detectable **Treponemal and Nontreponemal antibody** (RPR).
- Identify the **causes of false positive and false negative** syphilis test **results.**
- Recognize **index values** that indicate a **weak positive** versus a **strong positive** Syphilis Antibody interpretation.
- Apply the methods for screening and testing for **congenital syphilis.**

RML/LCOK Serologic Syphilis Results

2018-2023(Annualized)

	2022	2023	2018	2019	2020	2021
No. Pts tested	24,749	26,337	25,729	27,282	28,986	40,920
Trep Ab Pos pts	297*	425	598	732	936	1,254*
Inc from prev year	28	128	173	134	204	318
% Reactive Pts	1.20%	1.61%	2.32%	2.68%	3.23%	3.06%

* An increase of 322% when comparing the number of positive pts in 2023(annualized) with 2018

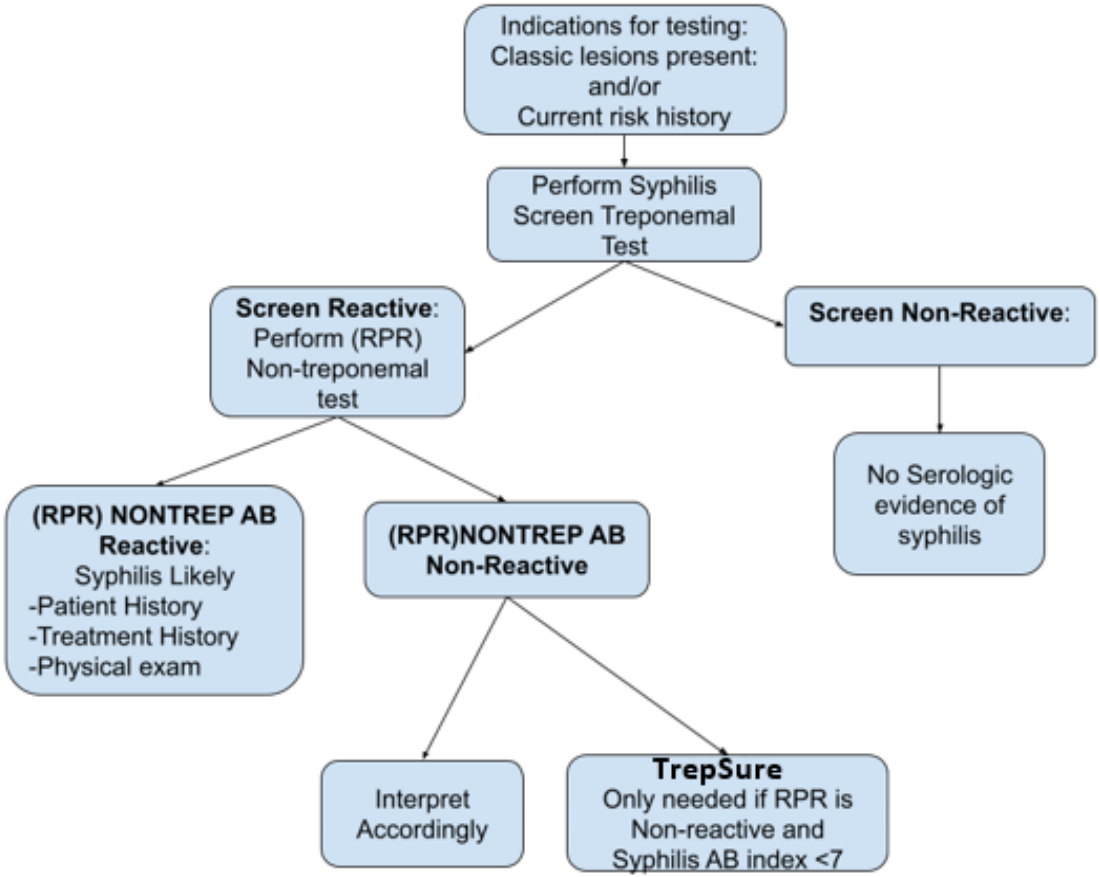
Syphilis

- **Sexually transmitted disease caused by *Treponema pallidum***
- **Stages**
  - **Incubating Stage** (median time is 21 days but will range from 3 to 90 days)
  - **Primary** (e.g. chancre; heals in 3 to 6 weeks)
  - **Secondary (disseminated)** (e.g. diffuse rash is most common; becomes evident 2 to 8 weeks after the appearance of a chancre; spirochetes disseminate widely and achieves it greatest numbers or antigenic load; “great imitator” or “great impostor”)
  - **Early Latent Period** (Following the secondary stage; relapse possible in untreated patient for up to 4 years; 75-90% of relapses occur in first year and probably due to waning immunity; patient is infectious)
  - **Late Latent Period** (Host resistance to reinfection and to infectious relapse; however, pregnant woman can infect her fetus *in utero*)
  - **Tertiary or Late Syphilis** (e.g. affects internal organ; brain, nerves, eyes and heart; develops in one-third of untreated patients; produce clinical illness in 5 to 30 or more years after the initial infection; neurosyphilis, cardiovascular syphilis, gummatous syphilis)
  - **Note: Patients may be completely asymptomatic; ID w/ serology**
- **Congenital Syphilis**
  - Miscarriage
  - Prematurity, low birth weight
  - Stillbirth
  - Death shortly after birth
  - **Untreated** syphilis in pregnant women can lead to infection of the fetus in up to **80% of cases**
  - The **gold standard** for making a diagnosis is **clinical history of the mother** and newborn along with a thorough clinical examination of the newborn. A nontreponemal antibody testing such as RPR and VDRL are the only reliable **serologic** laboratory test for the newborn diagnosis. A very complex diagnosis

Pathogenesis of Syphilis (Treponema pallidum)

- Almost any organ in the body can be invaded especially the CNS
- Number of organisms needed to establish an infection varies with the patient, but it is known from basic research utilizing rabbits that it takes only **4 to 8 spirochetes** to result in a infection.
- Division time of the spirochete is **30-33 hrs**
- Clinical lesions appear when a concentration of approximately **10 million organisms/mg** of tissue is reached
- The **incubation stage** is directly proportional to the size of the inoculum
- Median incubation period is **21 days** but varies from **3 to 90 days** after which the spirochetal load expands enough to result in a chancre
- Followed in 2 to 8 weeks by immunologically mediated signs and symptoms of secondary Syphilis
- Approximately **2/3** of untreated patients will **control or spontaneously clear** the infection and only **1/3** progress to **late Syphilis** in 5 to 30 years.
- During the primary stage, the development of the chancre occurs at the site of inoculation. A painless solitary lesion, does not develop in every case, or it may be so inconspicuous that it goes unnoticed. Chancre heals within 3 to 6 weeks (range 1 to 12 weeks)

Syphilis Testing Algorithm



Syphilis

- **Implementation of the Reverse Algorithm**
  - **Why Screen with Treponemal Antibody (Syphilis Ab) rather than nontreponemal (RPR) assay**
  - **Reasons:**
    - **False positives** occur with the nontreponemal Antibody (RPR); however, if the patient has a **RPR titer of =>1:4** then the **specificity is 98%**
    - **False negatives** with the nontreponemal Antibody (RPR) Assay
      - **Sensitivity is 78% to 86%** in the **primary** stage, **100%** in the **secondary** and **95-98%** in **early latent** disease.
      - **Caution:** However, it is important to note that 25% of untreated patient in the late latent and tertiary stage will over time, become RPR negative.
    - Treponemal Ab/Syphilis Ab (TP-CIA) will reliably **detect infection in all stages and in all Syphilis infected patients**
    - RPR is tedious and requires repetitive pipette motion by techs
    - Expense of time and reagents
    - Significant increase in testing volume
    - Automated treponemal test significantly decreased TAT, tech time, cost and ease of use

Syphilis - Serologic Testing
Interpret Cautiously with Clinical History Consideration

- **True Positives**
  - **Majority of patients will be serologic positive upon presentation of Chancre**
- **True Negatives**
  - **Interpret in light of clinical history**
  - **Consider incubation stage with timing, immunocompetence, coinfections (HIV)**
- **False Positives**
  - **Syphilis Ab/Treponemal Ab (e.g. Pregnant women, dialysis, IV drug abuse, etc)**
    - **TrepSure or TPPA** are excellent assays to **adjudicate discordant results** within the reverse sequence algorithm as to determine a true positive when the Syphilis AB assay has a low Ab index (1.0 to 7.0) and the Non-Treponemal (RPR) is nonreactive or weakly positive (<1:4)
  - **Nontreponemal Ab/RPR** [e.g. SLE and other connective tissue diseases, autoimmune diseases, pregnancy, IV drug abuse, Infections (viral, tuberculosis, malaria), medical conditions (lymphoma, endocarditis, etc.)]
- **False Negative**
  - **Early disease** (small minority will be NR with onset of Chancre but will turn serologically positive by 2-3 weeks)
  - **25% of patients with advanced stage Syphilis will have a negative RPR**
  - **Immunosuppression (e.g. HIV pts, treatment)**

Interpretation of Weak Antibody Index (≤7.0)

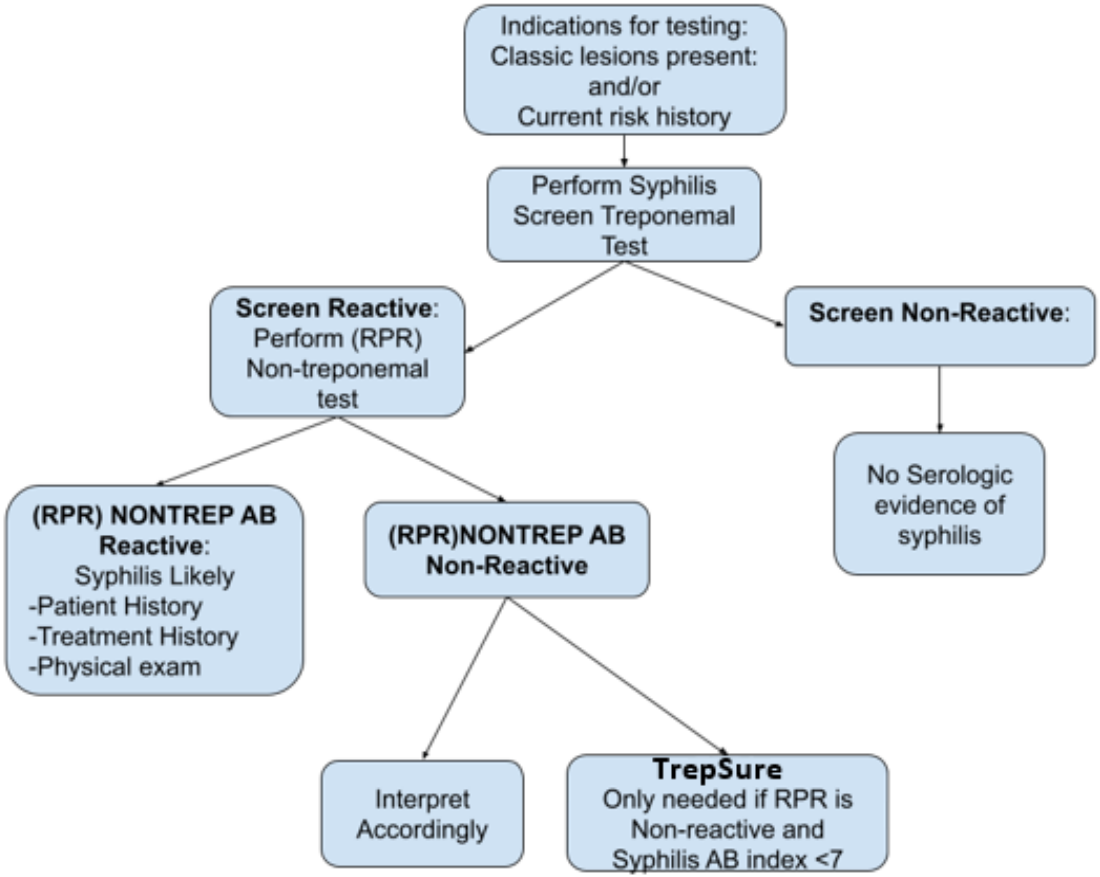
- **Weak Activity** index of **1.0 - ≤3.0** (89 patients)
  - **27.0%** (24 pts) were confirmed **Reactive (R)** by TPPA/TrepSure
  - **70.8%** (63 pt) were **Non-Reactive (NR)** by TPPA/TrepSure (Majority between index of 1.0-2.0)
  - **2.2%** (2 pts) were **inconclusive** which suggests retesting in 2-3 weeks
- **Weak Activity** index of **>3.0 - 7.0** (93) patients)
  - **87.1%** (81 pts) were confirmed **Reactive (R)** by TPPA/TrepSure
  - **9.7%** (9 pts) were **Non-Reactive (NR)** by TPPA/TrepSure (interpret in light of clinical history)
  - **3.2%** (3 pts) were **inconclusive** which suggests retesting in 2-3 weeks

Note on 2 Rare Occasion: The Anti-Syphilis Antibody index is >7, RPR is NR and the TPPA/Trepsure is NR indicating a false positive. In the last 15 months (approx 36,000 tests) have had two(0.00555%) such patients. One a patient at delivery and the other a patient on dialysis. When the Syphilis Antibody Screening results does not fit the clinical history, CHALLENGE THE RESULTS!!!!!!!!!

Untreated Syphilis

- In **Untreated disease** the Treponemal Ab will be positive and the Non-Treponemal Antibody (RPR) titers will reach their **highest titer** during the secondary and early latent stages and **decline thereafter;** however, starting such patients on recommended therapy, there will be those that will become **"serofast",** that is the RPR/NonTreponemal titer will stop and hold at **less than 1:4**, regardless of the number of courses of treatment.
- Over time, at least **25% of untreated persons** become Non-Treponemal Antibody (RPR) **NonReactive (NR)**.
- Interpret results with a **thorough clinical history and examination**

Syphilis Testing Algorithm



Syphilis

- **Interpretation and further testing**
  - **Automated treponemal screening with TP-CIA (Syp Ab)**
    - **Negative - <1.0 index** (**caution**: Consider timing of seroconversion and even other medical conditions causing delay of positive antibody even though Chancre exists; Gold Standard is Clinical History)
    - **Weak positive (1.0-7.0 index)** followup testing with RPR. If RPR is Reactive (R) it is titered and patient reported positive if the RPR is NR and Syphilis Antibody/Treponemal Antibody **≤7.0**, a **TrepSure** will be performed
    - **Positive - >7.0 index.** RPR will be performed and titered if Reactive
  - **Further testing for weak positive Treponemal Ab**
    - RPR (Nontreponemal) and titer if positive. Pt reported as positive
    - **TrepSure** (replaced TPPA) is performed if Syp Ab has an **index of 1.0 to 7.0** and Non-Treponemal Antibody Assay is Non-Reactive (NR)
      - High Specificity

Syphilis Testing Results and potential Interpretations

- **Syphilis Ab/Treponemal Ab is Nonreactive(NR)**
  - Most probable the patient does **NOT have Syphilis** but **interpret cautiously** considering the **incubation stage** with the timing, coinfection, immunocompetence and clinical history
- **Syphilis Ab/Treponemal Ab Reactive (R) & RPR Reactive (R) with Titer**
  - **A**ctive case of Syphilis; however, check clinical history to determine if pt has been treated
  - Caution: RPR will be Reactive for 1 year in primary, 2 years in secondary and >5 years in tertiary
- **Syphilis Ab/Treponemal Ab (Ab index of ≤7) Reactive (R) & RPR Nonreactive (NR)**
  - TrepSure is performed and **NR**:
    - Then the Syp Ab is reported as a **false positive**
  - TrepSure is performed and **R:**
    - **Very Early stage** disease
    - Patient has been possibly **Successfully Treated** for Syphilis at a very early stage some time earlier
- **Syphilis Ab/Treponemal Ab (Ab index of ≤7) Reactive (R) and RPR Reactive (R) at <1:4**
  - Most probable an **early onset** of a Syphilis infection
- **Syphilis Ab/Treponemal Ab (Ab index of >7) Reactive (R) & RPR is Nonreactive (NR) or an RPR titer of <1:4** (Note: Treponemal Ab at **>7** is considered a **true Treponemal positive antibody,** NO need for TrepSure:
    - Patient has been **Successfully Treated** for Syphilis
    - **Late Latent Syphilis** with RPR NR (25%) or serofast at <1:4
    - **Tertiary Syphilis** with RPR NR (25%) or serofast at <1:4
- **Syphilis Ab (Ab index of >7 Syphilis Ab/Treponema) Reactive (R) & RPR is Reactive (R) at a RPR titer of ≥1:4**
  - Stage the patient with an active Syphilis infection with clinical history

Review Significance of Results

Syphilis Ab	RPR	TrepSure/TPPA	Interpretation
NR	NP	NP	No serologic evidence of Syphilis Infection; History
R >7	R ≥1:4	NP	Reactive; History consistent with active case of Syphilis
R <7(1.0 - 3.0)	NR	NR	False positive; pregnant; clinical history critical
R <7(1.0 - 3.0)	R (1:2)	NR	False positive; worked up for lupus; history critical
R <7	R (1:2)	R	Early primary stage disease; history critical
R <7(1.0 - 3.0)	NR	Inconclusive	Retest in 2-3 weeks; history critical
R >7	NR	NP	Previous infection but successfully treated; history*
R >7	R <1:4	NP	Late treated disease; serofast; history critical
R >7	NR	NP	Late untreated disease; 25% lose RPR ab; history critical
R >7	NR (prozone)	NP	Strong History of primary Syphilis; Pt with RPR prozone
R >7	NR	NR	False positive; dialysis patient and also a pregnant pt, History Critical!!!!!

*NOTE: Following successful treatment, RPR will be Reactive for 1 year in primary, 2 years in secondary and ≥5 years in tertiary

R = Reactive; NR = NonReactive; NP = Not performed

Congenital Syphilis Considerations

- Infection of the fetus **in utero** can occur at any stage of infection in any untreated or inadequately treated mother.
  - Most likely to occur during the **spirochetemia** of early Syphilis
  - Infection of the fetus **before the fourth month of gestation is unusual**; therefore, early spontaneous abortion is unlikely to be a result of Syphilis
  - Depending on the **severity of the infection**, late abortion, stilllbirth, neonatal death, neonatal disease or latent infection may be seen
  - **Adequate treatment** of the mother usually, **but not always**, ensures that the fetus will not be infected
  - Important **issues for consideration: 1.)** How does the neonate's RPR compare with mothers, **2.)** Has the mother been adequately treated. **3.)** Was the treatment <30 days or >30 days before delivery, **4.)** What is the stage (e.g. primary, secondary, tertiary, etc) of the mother, **5.)** If RPR is positive or mother suspicious for Syphilis, is there a plan to perform a RPR every 2-3 months on newborn?
  - **Serologic testing (e.g. reverse algorithm) of the mother** is always warranted at delivery, especially in high risk patients.
  - Giving **penicillin** to the neonate is almost **risk-free** when given to all neonates born to syphilitic mothers, regardless of whether the mother was treated during pregnancy

Testing for Congenital Syphilis

- **Non-Treponemal Antibody Assay (RPR).**
  - Same test as that used on mother and ideally performed by same laboratory
  - Maternal history of treponemal and nontreponemal(RPR) test results along with medical history
  - Clinical findings of congenital syphilis
  - Consider a maternal co-infection with HIV and Syphilis
- **Strongly suspect congenital syphilis in neonates who have a positive RPR titer**
  - ≥ 4-fold higher than maternal titer
  - < 4-fold higher than maternal titer but **mother was inadequately treated**
  - RPR titer of ≥1:16
- **In asymptomatic children,** the likelihood of diagnosis of congenital syphilis is based on the semi-quantitative nontreponemal serologic titer (≥1:16) and the adequacy of the maternal treatment.
- **False-positive RPR results may be cause by:**
  - Medical conditions such as **pregnancy, Lupus or other autoimmune diseases, or IV drug use,** lymphoma. Endocarditis
  - **Dialysis**
  - Viral infections such as Epstein-Barr virus, hepatitis, varicella, or measles
  - Other infections such as tuberculosis, malaria

Note: **Maternal anti-Syphilis Ab/Anti-Treponemal ab can be detected in the baby up to 15-18 months of age**

Review Significance of Assays

- **Syphilis Antibody Screen (Treponemal Antibody Assay)**
  - Screen patients
  - Remains positive for life in 90% of successfully treated patients , especially those treated late
  - Consider the antibody index in relationship to the timing and clinical history
  - Caution: When index is **<7** a percentage will be false positive **particularly in pregnant patients** who have an index of **1-3. When the index is 1-<3 the percentage of false positive is 70.8% and 3 to 7 it is 9.7%.**
  - LabCorp Oklahoma (formerly RML) confirms weak positives (index of 1 - 7) that are RPR Non-Reactive (NR) with TrepSure (replaced TPPA)
  - **Maternal Syphilis antibodies**/Treponemal Ab from the infected mother can be **detected in a newborn** for up to **15-18 months**
- **RPR (Nontreponemal Ab)**
  - **Confirms** the Syphilis Treponemal Ab positive result. Positive will be titered
  - An indicator of the degree of activity of a patient with a Syphilis infection
  - **When treated**, the patient will remain positive for up to **one year for primary infection**, **two years for secondary infection and 5 years or greater for late Syphilis**
  - Follow **decreasing titers starting 3-6 months** following treatment.
  - Helpful for monitoring the efficacy of therapy. **Failure** of the titer to **decrease fourfold or become negative** suggests a persistent infection or reinfection or even a **false positive.** Consider serofast when stuck on <1:4
  - Important in assisting in the **diagnosis of Congenital Syphilis**
  - **Not specific** for Syphilis (e.g. weak pos/false positive in some cases of SLE, pregnancy, viral infections, etc.)
    - Detecting anticardiolipin antibody (Cardiolipin cholesterol lecithin)
- **TrepSure (replaced TPPA)**
  - **Adjudicate discordant results** when Syphilis Ab (Treponemal antibody) is weakly positive (<7.0 index) and RPR is NR
  - Treponemal antibody assay - **Specific for Syphilis**
- **FTA (Fluorescent Treponemal Antibody Assay)**
  - Replaced for screening by the CIA or EIA Treponemal Antibody Assay (Syphilis Treponemal Antibody)
- **VDRL (Venereal Disease Research Laboratory);** Nontreponemal antibody assay
  - Preferred for testing **CSF**
  - False positives with blood contamination
  - **Negative result** on CSF rules out **neurosyphilis** in late disease but not in early disease