

## Syphilis – Treponemal Screen with Reflex to RPR, with Titer and TP-PA Confirmation, as Indicated

### Use

This test comprises a set of serologic treponemal and non-treponemal assays that are used in a “reverse sequence algorithm” as an aid in the diagnosis of syphilis.

**Note:** *This test is **not suitable for monitoring the effectiveness of treatment of prior syphilis-positive patients.** Instead, order the Syphilis – Reactive Plasma Reagin (RPR) with Reflex to Titer, as Indicated assay. Because treponemal antibodies are generally detectable for the lifetime of the patient after syphilis infection, sera from previously treated syphilis patients will remain reactive by treponemal tests; therefore, patients previously positive for syphilis should not be monitored using treponemal tests.*

### Clinical Significance

*Treponema pallidum*, the etiological agent of syphilis, induces the production of at least two types of antibodies in human infection: anti-treponemal antibodies that can be detected by treponemal antigen, and anti-non-treponemal antibodies (reagin) produced as the result of reaction to cellular breakdown due to infection that can be detected by Rapid Plasma Reagin (RPR) antigen. Diagnosis of syphilis relies on the use of two types of serologic tests: non-treponemal and treponemal. The use of only one type of serologic test is generally insufficient for diagnosis because each test has limitations: false-positive RPR results may occur in persons without syphilis and treponemal tests are unable to distinguish between recent and past infections. Non-treponemal test titers usually correlate with current disease activity, and the results are reported quantitatively. Sequential non-treponemal test endpoint titers can be used to monitor effectiveness of treatment. A 4-fold change in titers (e.g., from 1:16 to 1:4 or from 1:8 to 1:32) is considered necessary to demonstrate a clinically significant difference. Non-treponemal tests usually become non-reactive with time after treatment. In some patients, however, non-treponemal antibodies can persist at a low titer for a long period of time (i.e., “serofast reaction”), sometimes for the life of the patient. By contrast, reactive treponemal tests usually remain reactive for the life of the patient. While rare, non-treponemal tests can also produce false negative results, particularly during tertiary syphilis, due to the “prozone effect” whereby the reaction is overwhelmed by excess antibody.

Traditional serologic screening for syphilis initially uses non-treponemal testing with confirmation of reactive results using a treponemal test; however, reverse sequence algorithms, which initially use treponemal testing, with confirmation of reactive results using a non-treponemal test, are gaining popularity, and offer certain advantages analytically and clinically. Because the treponemal screen cannot differentiate active versus previously treated infections, all screen-reactive specimens are reflexed to a nontreponemal test with titer to define those with active infections. False-positive screen results (i.e., initial reactive treponemal screen with negative reflexed non-treponemal test) are resolved by further testing using a second treponemal test.

Further background information, fact sheets, statistics and educational resources may be found at the OSDH Infectious Disease Services [website](#).

### Methodology

- BioPlex 2200 Syphilis Total Antibody Assay for detection of treponemal IgG and IgM antibodies
- Gold Standard Diagnostics AIX 1000 Rapid Plasma Reagin (RPR) Automated Test for detection of non-treponemal antibodies
- Fujirebio Serodia TP-PA Agglutination Assay for detection of *T. pallidum* antibodies

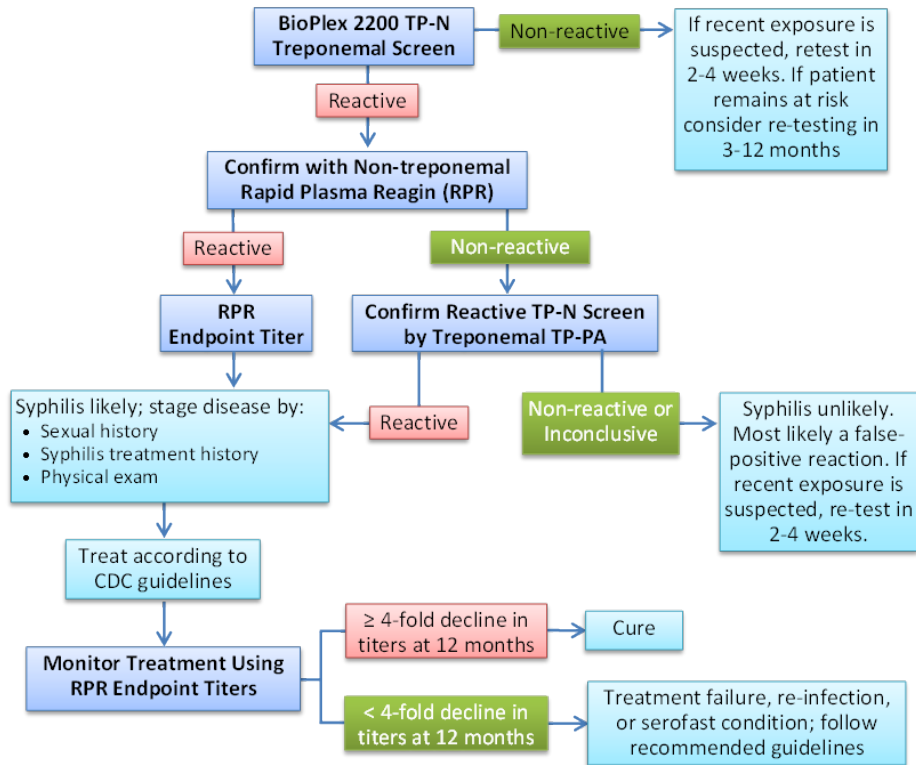
The OSDH PHL primarily uses a reverse syphilis testing algorithm (Figure 1); however, the traditional algorithm (Figure 2) is used to test specimens when the initial treponemal screen is inconclusive. In addition, specimens from patients previously positive for syphilis are tested using the RPR test with reflex to RPR titer when

positive, since these patients typically would remain positive for treponemal antibodies during and post-treatment.

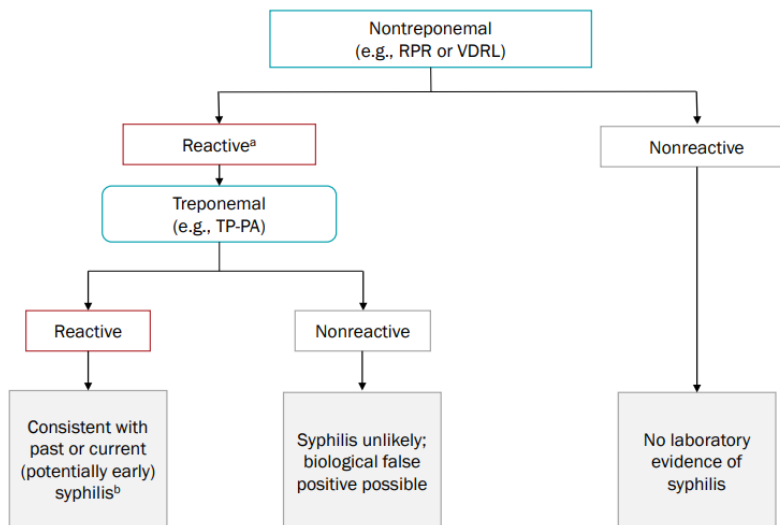
**Reverse Algorithm**

The reverse algorithm (Figure 1) begins with a treponemal test, often an automated immunoassay. No further testing is performed with a nonreactive treponemal test result. By contrast, a reactive result reflexes to a non-treponemal test (e.g., RPR), and if this is reactive, a semiquantitative endpoint titer is performed to establish a baseline for diagnosis and monitoring. Specimens producing discordant treponemal/ non-treponemal test results (i.e., reactive treponemal screen and nonreactive RPR) are subjected to a second treponemal test (TP-PA) to confirm the initial treponemal screen.

**Figure 1: Reverse Syphilis Testing Algorithm Used at OSDH PHL**



**Figure 2: Traditional Syphilis Testing Algorithm Used at OSDH PHL**



### Traditional Algorithm

In certain situations where a valid syphilis screening result cannot be generated using the Reverse Algorithm, the OSDH PHL will run the sample using the traditional algorithm. The traditional algorithm for syphilis diagnosis (Figure 2) begins with a non-treponemal (e.g., RPR) test. A reactive result then reflexes to: 1) a semiquantitative endpoint titer to establish a baseline for diagnosis and subsequent monitoring during treatment, and 2) a treponemal test (e.g., TP-PA) to confirm the reactive nontreponemal result. Using the traditional algorithm, a nonreactive non-treponemal test result is consistent with no active syphilis. Reactive results from both the non-treponemal and treponemal tests are consistent with current or past syphilis.

### **Specimen Type**

- Whole blood collected and separated in serum separator tube (SST), or
- Separated serum poured into sterile, plastic, screw-cap tube

### **Minimum Volume/Size**

- 2 mL serum. Draw enough whole blood (~4 mL) to yield the necessary serum volume.  
**Note:** 1 mL of serum is acceptable but this may be QNS if reflexive testing exhausts the sample.

### **Collection Instructions**

Each facility should follow its guidelines for venipuncture collection of blood/serum. See guidance for collection of [Blood in a Serum Separator Tube](#).

### **Common Causes for Rejection**

- Blood collected in tube other than SST
- SST received unspun at 2-8°C, and > 24 hours from DOC
- Specimen received at ambient temperature
- Specimen received at 2-8°C and > 5 days from DOC
- Serum (in transport tube) received frozen and > 14 days from DOC
- SST frozen
- QNS (1 mL allows for treponemal screen testing only)
- Specimen too old for TP-PA; a TP-PA test cannot be performed after 5 days from DOC unless serum is poured off and shipped frozen
- Specimen subjected to > 1 freeze/thaw cycle (TP-PA test only)
- Bacterial contamination
- Extensive hemolysis
- Extensive lipemia

### **Shipping**

- **Storage:** Serum samples may be stored in an SST or in a secondary transfer tube. Samples can be stored at 2–8°C for up to 5 days. For long-term storage (e.g., > 5 days and < 14 days), serum must be transferred to a secondary transfer tube and frozen at –20°C or colder.  
**Please note:** Delays in delivering the sample to the PHL may interfere with the laboratory’s ability to perform secondary (i.e., confirmatory) testing if the screen result is positive.
- **Shipping:** Refrigerated samples should be shipped with sufficient cold packs to keep the sample at 2–8°C. Frozen samples should be shipped on dry ice to maintain a frozen state; if site is unable to provide dry ice for shipment, please call the OSDH PHL at 405-564-7750 at least 24 hours ahead of scheduled submission to request dry ice transport. Place each specimen in an individually sealed bag.

**Turn-around Time**

Within 7 working days from receipt

**Reference Range**

Syphilis treponemal and non-treponemal antibody: Non-reactive

**Reportable Results****Treponemal Screen:**

- Non-reactive
- Reactive
- Inconclusive

**RPR:**

- Non-reactive
- Reactive
- Invalid

**RPR Titer:**

- 1:1 to  $\geq$  1:2048 (at sequential two-fold dilutions)

**TP-PA:**

- Non-reactive
- Reactive
- Inconclusive

**Interpretation****Treponemal Screen:**

- Non-Reactive: A non-reactive treponemal screen result alone suggests the absence of a current syphilis infection; however, it does not exclude the possibility of syphilis infection. *T. pallidum* antibodies may be undetectable in some stages of infection and in some clinical conditions. If recent exposure is suspected, re-draw sample in 2-4 weeks.
- Reactive: Specimens with reactive treponemal screen results are tested by RPR to determine potential current or past infection.
- Inconclusive: Result was in a range above the cut-off for non-reactive specimens, but below that of reactive specimens. Equivocal specimens are tested by RPR.

**RPR:**

- Reactive: Specimens with reactive RPR results are tested by semiquantitative RPR to determine the endpoint titer.
- Non-Reactive: Specimens with non-reactive RPR results are tested by TP-PA to confirm the initial treponemal (TP-N) result.

**RPR Titer:**

- Specimens with endpoint titers  $\geq$  1.1 are consistent with syphilis infection, either current or past. Patients should be evaluated clinically to identify signs, symptoms, or prior history of infection.
- RPR titers can be used to monitor a patient's response to treatment. RPR titers usually become non-reactive with time, following successful treatment. Because RPR titers are semi-quantitative and methodology can differ, tracking the progression of titers as part of treatment should only be done using specimen submissions to the same testing facility.
  - $\geq$  4-fold decline in titers at 12 months signifies successful treatment.
  - $<$  4-fold decline in titers at 12 months indicates treatment failure or reinfection, or "serofast" condition

**TP-PA:**

- Reactive: A reactive TP-PA test result, together with a reactive treponemal screen and nonreactive RPR, is

consistent with past or potential early syphilis infection.

- Non-Reactive: A non-reactive TP-PA result, together with a reactive treponemal screen and non-reactive RPR, is considered inconclusive for syphilis infection, likely signifying a false positive treponemal screening or potentially an early infection.
- Inconclusive: Patients with reactive treponemal screens, non-reactive RPR, and inconclusive TP-PA results should have blood drawn in 2-4 weeks for re-test.

### **Limitations/Interferences**

- Bacterial contamination, icteric, lipemic, hemolyzed, or heat-inactivated samples may cause erroneous results and should be avoided.
- In accord with all diagnostic methods, a final diagnosis should not be made on the result of a single test but should be based on a correlation with all laboratory test results and other clinical findings. When results are inconclusive, clinicians should inquire about previous syphilis infection and treatment, and if early syphilis is possible. Patients with inconclusive results should be retested in two to four weeks.

### **Treponemal Screen:**

- All treponemal tests tend to remain reactive for the life of a treponeme-infected individual, even after treatment. Treponemal antibody titers correlate poorly with disease activity; therefore, they should not be used to evaluate response to therapy. Because of the persistence of reactivity, treponemal tests are of no value in determining relapse or re-infection in a patient who has had a reactive TP-PA result.
- False positives, especially in low prevalence populations, and false negatives may occur.
- Interference may be encountered with certain sera containing non-specific and/or unidentified reactive substances.
- Test results from specimens obtained from immunosuppressed patients should be interpreted with caution.
- Assay interference due to possible circulating antibodies against pinta, yaws, and bejel has not been evaluated.

### **RPR:**

- False negative reactions may occur, particularly in tertiary syphilis but also in early primary and late latent stages.
- Rarely, false negative results arise from a phenomenon called “prozone effect”, whereby the reaction is overwhelmed by excess antibody.
- False positive reactions occur occasionally in samples from individuals with a history of drug abuse, with febrile illness, women who are pregnant or with diseases such as lupus erythematosus, malaria, vaccinia, mononucleosis, leprosy, viral pneumonia, and after smallpox vaccinations.
- Pinta, yaws, bejel, and other treponemal diseases produce positive reactions in this test

### **TP-PA:**

- TP-PA may be reactive in a small percentage (< 1%) of normal or healthy persons; these false positive results are often transient, and their cause is unknown.
- TP-PA may be reactive in persons from areas where yaws or pinta, bejel, and other treponemal diseases were, or are, endemic.
- Samples from patients with HIV, leprosy, toxoplasmosis, *H. pylori*, cardiovascular disease, and drug addiction may react, on occasion, causing false positive or indeterminate results.
- TP-PA is less sensitive than the fluorescent treponemal antibody absorption (FTA-ABS) test in untreated primary syphilis but compares favorably in all other stages of syphilis.

### **CPT Codes**

Combinations of 86780, 86592 and 86593 depending on testing performed.

TPN Screen: 86780; RPR Qualitative and Quantitative: 86592/86593; TP-PA Confirmation: 86780

**Notes**

These tests are approved for *in vitro* diagnostics use by the U.S. Food and Drug Administration.

Do not use this test for monitoring effectiveness of treatment of previously positive syphilis patients; rather order *Syphilis – Reactive Plasma Reagin (RPR) with Reflex to Titer, as Indicated*, which uses a qualitative RPR assay and reflexes to a quantitative RPR if positive on the qualitative assay.