

## Syphilis – Reactive Plasma Reagin (RPR) with Reflex to Titer, as Indicated

### Use

This test comprises a set of serologic qualitative and quantitative non-treponemal assays that are used as an aid in monitoring patients with prior syphilis results. The test has utility in monitoring the effectiveness of treatment by comparing sequential RPR titer results from the same patient. RPR titer requests on screen-negative specimens require advance notice to the OSDH PHL. Also, such requests must be clearly indicated on the [OSDH PHL Test Requisition Form \(ODH 419\)](#).

### Clinical Significance

*T. 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.

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.

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.

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

### Methodology

- Gold Standard Diagnostics AIX 1000 Rapid Plasma Reagin (RPR) Automated Test for detection of non-treponemal antibodies

Specimens from patients previously positive for syphilis are tested using the RPR test with reflex to quantitative RPR to determine the endpoint titer when positive.

### 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.

## 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 > 7 days from DOC
- Serum (in transport tube) received frozen and > 14 days from DOC
- SST frozen
- QNS
- Bacterial contamination
- Extensive hemolysis
- Extensive lipemia

## Shipping

- **Storage:** Serum samples may be stored in an SST or in a secondary transfer tube. Do not store or transport at ambient temperature. Samples can be stored at 2–8°C for up to 7 days. For long-term storage (e.g., > 7 days and < 14 days), serum specimens must be transferred to a secondary transfer tube and frozen at –20°C or colder.
- **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 non-treponemal antibody: Non-reactive

## Reportable Results

### RPR:

- Non-reactive
- Reactive

### RPR Titer:

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

## Interpretation

### RPR:

- Reactive: Specimens with reactive RPR results are tested by quantitative 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

### **Limitations/Interferences**

- Bacterial contamination, icteric, lipemic, hemolyzed, or heat-inactivated samples may cause erroneous results and should be avoided.
- Patients with inconclusive results should be retested in two to four weeks.
- Since test methodologies can vary by laboratory, titer results from consecutive samples from the same patient should be obtained from the same testing laboratory.
- 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.

### **CPT Codes**

RPR Qualitative and Quantitative: 86592/86593

### **Notes**

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