

**SERODIA *TREPONEMA PALLIDUM*
PASSIVE PARTICLE AGGLUTINATION
(TP-PA) TEST**

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SERODIA *TREPONEMA PALLIDUM* PASSIVE PARTICLE AGGLUTINATION (TP-PA) TEST

TEST PRINCIPLES

The Serodia *Treponema pallidum* passive particle agglutination (TP-PA) test is a treponemal test for the serologic detection of antibodies to the various species and subspecies of pathogenic *Treponema*, the causative agents of syphilis, yaws, pinta, bejel, and endemic syphilis. The test is a passive agglutination procedure based on the agglutination of gel particles sensitized with *T. pallidum* antigen by antibodies found in the patient's serum.¹ The test is intended to replace the microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP).

Serum containing antibodies to pathogenic treponemes reacts with gel particles sensitized with sonicated *T. pallidum*, Nichols strain (the antigen), to form a smooth mat of agglutinated gel particles in the microtiter tray well. If antibodies are not present, the particles settle to the bottom of the tray well, forming a characteristic compact button of unagglutinated particles. The unsensitized gel particles control well for each serum should also show this compact button, or the absence of agglutination.

The TP-PA test is used to confirm the reactive results^{1,2} of a nontreponemal screening test for syphilis, such as the Venereal Disease Research Laboratory (VDRL) slide test, or as a diagnostic test in patients with a nonreactive nontreponemal test but with signs or symptoms suggestive of late syphilis.

SPECIMEN COLLECTION AND HANDLING

Specimen

1. Avoid accidental infection when collecting and processing samples³ by observing universal precautions⁴⁻⁷ (Chapter 2).
2. Serum or plasma is an appropriate specimen for the TP-PA test.
3. An acceptable specimen should not contain particulate matter that would interfere with reading test results. Specimens that are excessively hemolyzed, grossly contaminated with bacteria, chylous or otherwise extremely turbid are unsatisfactory. A specimen is too hemolyzed for testing when printed matter cannot be read through it.
Note. Hemolysis may be caused by transporting blood in freezing or extremely hot weather without proper insulation.
4. Not all unsuitable specimens should be discarded or not analyzed. When an unsatisfactory sample is received in the laboratory, notify the requesting physician and discuss whether testing is appropriate for that specimen. If a test result is still desired by the ordering physician, the condition of the sample must be stated on the report, and a notation made on any limitation in test result interpretation.⁸ Results with grossly hemolyzed samples can be reported, if the sample can be diluted beyond the point where hemolysis interferes with the reading and test reactivity still remains.

Collection

The procedures for the collection and processing of venous blood are given in detail in Chapter 3.

1. Serum -- Collect whole blood into a clean, dry tube without an anticoagulant. Allow sufficient time (approximately 20 minutes) at room temperature for specimen to clot.
2. Plasma -- Collect blood in a tube containing EDTA, heparin or sodium citrate as an anticoagulant. Completely fill the tube or collect the blood until the vacuum in the collection tube has been exhausted.
3. Label each specimen with a patient identifier and date.

Handling

1. Centrifuge the specimen at room temperature at 1000 to 2000 x g for at least 5 minutes to sediment cellular elements (Chapter 3).
2. Transfer the serum or plasma into a clean, dry test tube. Observe universal safety precautions in handling serum or plasma specimens.
3. Specimens must be at room temperature (18° - 30°C; 64° - 86°F) at the time of testing.
4. If a delay of more than 5 days is anticipated before testing, freeze the serum specimen at -20°C or below. Plasma specimens must be tested within 48 hours of collection.

MATERIALS

Materials Supplied

Materials included in the Serodia treponemal antibody test kit (**Fujirebio, Inc., Fairfield NJ**) are as follows:

Reagents

1. **Sensitized particles (antigen).** Lyophilized preparation of tanned gelatin particles coated with *T. pallidum*. Reconstituted suspension contains 0.079% sodium azide.
2. **Unsensitized particles (control).** Lyophilized preparation of tanned gelatin particles that are not sensitized with *T. pallidum*. Lyophilized reagent when rehydrated contains 0.079% sodium azide.
3. **Reconstituting solution (liquid).** Used to reconstitute sensitized and unsensitized gelatin particles. Solution contains 0.06% sodium azide.
4. **Sample diluent (liquid).** Used to dilute serum specimens. Solution contains 0.1% sodium azide.
5. **Reactive control (liquid).** Reactive control, prepared from rabbit serum samples, contains antibodies to *T. pallidum*.
6. **Nonreactive control (liquid).** Nonreactive control, contains no antibodies to *T. pallidum*

Equipment

1. Droppers to deliver 25 µL for delivery of sensitized or unsensitized particles

Materials Required but Not Supplied

Equipment

1. Pipette droppers calibrated to deliver 25 μ L
2. Disposable, clear plastic trays with 8 rows of 12 **U-shaped wells** each
3. 1.0- or 2.0-mL serologic pipettes graduated in 1/100 mL
4. Safety pipetting devices for serologic pipettes
5. 100- μ L and 25- μ L automatic safety pipettes with disposable tips
6. Tray viewer
7. Automatic vibratory shaker
8. Latex gloves, safety glasses, and protective clothing
9. Discard containers and disinfectants

Materials Not Required but Suggested

1. A minimally reactive control that gives 1+ agglutination

CALIBRATION

Pipettors and Tips

With the pipettors currently available, the measurement of small serum volumes is routine. Most manufacturers include in the specifications of their pipettors the accuracy for frequently used microliter volumes. Daily use may affect pipettors, making them lose their initial accuracy. Substituting disposable tips from sources other than the manufacturer of the pipettor, probably is the most common source of error. For budgetary reasons, a less expensive brand of pipette tips may be substituted for those of the manufacturer. Although the less expensive brand may be satisfactory, the laboratory should verify the accuracy and precision of the substitute pipette tips in their test system. Commercial kits for checking pipettor accuracy are available. Also, manufacturers provide procedures for checking the accuracy of their equipment. Historically, the gravimetric or spectrophotometric procedures, which use the weight of water or the absorbance of a substance at a given wavelength, have been the most accepted methods used to calibrate pipettors. These procedures should not be used in place of those specified by the manufacturers.

QUALITY CONTROL

The evaluation of TP-PA kits is the responsibility of the user laboratory. Reagents evaluated as described here must produce results comparable to those obtained with reference reagents.

Reagents

Follow the manufacturers' instructions for rehydrating and storing reagents.

Criteria of acceptability

1. Sensitized and unsensitized particles should be nonreactive when incubated with the sample diluent for 2 hours and overnight.
2. The titer obtained with the reactive control serum supplied with the kit must produce an endpoint titer within ∇ 1 twofold dilution of the titer stated by the manufacturer.

Nonreactive results should be obtained with the 1:40 dilution of the reactive control serum incubated with the unsensitized gel particles.

3. The nonreactive control serum must be nonreactive with the sensitized and unsensitized gel particles, i.e., show no signs of agglutination.
4. Reportable test results (reactive and nonreactive) obtained with individual serum samples should be comparable to those obtained with the reference kit reagents.

Evaluation Procedure

Reference reagents or previous kit that met criteria of acceptability

1. Sensitized particles
2. Unsensitized particles
3. Reactive control serum
4. Minimally reactive control (1+ agglutination)
5. Nonreactive control serum

Test specimens

Test 10 individual serum samples of predetermined reactivity on each of 2 days. The recommended distribution is 3 serum samples with 1+ reactivity, 3 with 2+ reactivity, and 4 nonreactive serum samples. If necessary, prepare reactive serum samples of various levels of reactivity by diluting reactive samples with nonreactive serum samples. These pooled samples may be substituted for some of the individual serum samples.

Testing

The TP-PA reagents from the new and the reference lots are tested on 2 days by using reactive and nonreactive control serum samples from the new kit and from the reference kit and 10 individual serum samples.

1. Assemble the 10 individual serum samples already described.
2. Reconstitute the sensitized and the unsensitized cells according to directions; mix gently to disperse the cells evenly.
3. Assemble the reactive and nonreactive control serum samples supplied in the kit, and the minimal reactive control serum sample supplied by the laboratory.
4. Perform the quantitative test on reactive control and the qualitative test on the minimally reactive and nonreactive controls and individual serum samples. Test all serum samples in parallel, using new and reference reagents. For reagent controls, test the unsensitized and the sensitized cells from both new and reference lots, with their respective sample diluents.
5. Read and record test results after 2 hours and after overnight incubation at room temperature (18° - 30°C, 64° - 86°F).
6. Compare the results obtained with reference and new reagents. Determine whether new TP-PA reagents meet the criteria of acceptability.
7. If results between reagent lots are discordant, additional testing may be necessary.

TEST PROCEDURES

Preparing Test Reagents

Rehydrate the lyophilized reagents with the reconstituting solution supplied, according to the manufacturer's instructions. Store lyophilized and rehydrated reagents at 2° - 8°C. Discard reagents if they become contaminated or fail to demonstrate proper reactivity. Mix reconstituted reagents thoroughly before use to ensure a homogeneous suspension.

1. Sensitized gel particles (antigen). Reconstitute each 5 x 20 test kit antigen vial with 0.6 mL of reconstituting solution. Reconstitute the 55 test kit vial with 1.5 mL, the 110 test kit vial with 3.0 mL, and the 300 test kit vial with 8.0 mL of reconstituting solution. Allow suspension to stand 30 minutes before using. Use reconstituted antigen within 7 days.
2. Unsensitized gel particles (control). Reconstitute each 5 x 20 test kit vial with 0.6 mL of reconstituting solution. Reconstitute the 55 test kit vial with 1.5 mL, the 110 test kit vial with 3.0 mL, and the 300 test kit vial with 8.0 mL of reconstituting solution. Allow suspension to stand 30 minutes before using. Use reconstituted unsensitized cells within 7 days.

Performing the Test

Qualitative test

1. Add 100 µL of sample diluent to the first well of a U-bottomed microtiter plate and 25 µL to the second, third and fourth well for each sample to be tested, including the nonreactive control.
2. Add 25 µL each of the sample to be tested (patient sample or nonreactive control) to the first well, which contains 100 µL of sample diluent (1:5 dilution).
3. Mix, by alternately depressing and releasing the pipettor button, at least eight times. Transfer 25 µL to the second well; mix thoroughly. Repeat through well 4, discarding 25 µL from well 4 (dilution 1:40).
4. An example of the TP-PA procedure is presented in Table 14:1.
5. Record the control and the serum specimen numbers on the daily worksheets (the numbers correspond to the tray and well numbers, respectively).
6. Test the patient and control serum samples. Store residual serum samples at 2° - 8°C; retest on the same day if necessary. The refrigerated serum samples must reach room temperature before testing.
7. For the reactive control, prepare twofold dilutions of the reactive control serum (1:20 dilution) in sample diluent to exceed the endpoint titer established for the reactive control in each lot number (e.g., 1:80, 1:160, 1:320, 1:640). In the A (top) row of the tray, place 100 µL of sample diluent in well A1 and place 25 µL of sample diluent in tray wells A2 through A10. Place 25 µL of reactive control serum into well A1, using a 25-µL automatic pipette, mix 6 to 8 times, and transfer 25 µL of this mixture to well A2. Continue mixing and transferring in 25-µL quantities through well A10. Discard 25 µL of the mixture from well A10.
8. Place 25 µL of sample diluent in wells A11 and A12. These will be the reagent controls.
9. Add 25 µL of reconstituted sensitized gelatin particles to row A, wells A4 through A10 and well A11. Add 25 µL of sensitized erythrocyte working dilution to the

fourth well of each patient serum sample and the nonreactive control. The final serum dilution in each test well is 1:80.

10. Add 25 μL of reconstituted unsensitized gelatin particles to wells A3, A12, and the third well of each patient sample and the nonreactive control. The final serum dilution in each control well is 1:40.
11. Place the tray on an automatic vibratory shaker for 30 seconds or tap the sides of the trays gently to mix; stack the trays and cover with an empty tray.
12. Incubate the trays undisturbed at room temperature ($18^{\circ} - 30^{\circ}\text{C}$) for at least 2 hours. The incubation period may be extended to overnight with no apparent change in reactivity.

Quantitative

It is recommended that samples that have nonspecific agglutination in the unsensitized particle control be confirmed in the quantitative assay.

1. Place 100 μL of sample diluent in the first well of the row and 25 μL in wells 2 through 12.
2. Add 25 μL of serum to the first well, using a 25- μL automatic pipette; mix 6 to 8 times.
3. Transfer 25 μL of the diluted serum to well 2 and mix well. Transfer 25 μL to well 3. Repeat the procedure through well 12 to obtain a serial two-fold dilution. Discard the extra 25 μL from the last well (well 12).
4. Place 25 μL of unsensitized particles in well 3, and 25 μL of sensitized particle in wells 4 to 12, using the droppers supplied with the kit.
5. Mix the contents of the wells thoroughly by placing the microtiter trays on an automatic vibratory shaker for 30 seconds, or tap the sides of the tray gently. Cover the plate and allow it to incubate undisturbed for 2 hours or overnight.
6. Read the titer as the reciprocal of the last dilution giving 1+ agglutination.

Table 14: 1. **Outline and Example of TP-PA**

Row & Well	Reactive Control (Dilution)	Sample Diluent (μL)	Sensitized Cells (μL)	Unsensitized Cells (μL)	Final Serum Dilution	Reading	Interpretation
A1	1:5	100	-	-	1:10		

A2	1:10	25	-	-	1:20		
A3	1:20	25	-	25	1:40	-	NR
A4	1:40	25	25	-	1:80	2+	R
A5	1:80	25	25	-	1:160	2+	R
A6	1:160	25	25	-	1:320*	1+	R
A7	1:320	25	25	-	1:640	-	NR
A8	1:640	25	25	-	1:1280	-	NR
A9	1:1280	25	25	-	1:2560	-	NR
A10	1: 2560	25	25	-	1:5120	-	NR
Row & Well	Serum Dilution	Sample Diluent (μL)	Sensitized Cells (μL)	Unsensitized Cells (μL)	Final Serum Dilution	Reading	Interpretation
A11	-	25	-	25	N/A	-	NR
A12	-	25	25	-	N/A	-	NR
Row & Well	Nonreactive Control (Dilution)	Sample Diluent (μL)	Sensitized Cells (μL)	Unsensitized Cells (μL)	Final Serum Dilution	Reading	Interpretation
B1	1:5	100	-	-	1:5		
B2	1:10	25	-	-	1:10		
B3	1:20	25	-	25	1:40	-	NR
B4	1:40	25	25	-	1:80	-	NR
Row and Well	Patients= Sera (Dilution)	Sample Diluent (μL)	Sensitized Cells (μL)	Unsensitized Cells (μL)	Final Serum Dilution	Reading	Interpretation
C1	1:5	100	-	-	1:5		
C2	1:10	25	-	-	1:10		
C3	1:20	25	-	25	1:40	-	NR

C4	1:40	25	25	-	1:80	- or +	NR or R
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R = Reactive results, NR = Nonreactive results, N/A = Not Applicable, * = Endpoint titer of control serum in example.

Method for Determining Daily Volume of Cells

Calculation of volume of sensitized particles:

Assume that 10 unknown serum samples are to be run, in addition to the necessary controls.

<u>Wells</u>	<u>Contents</u>	<u>Number</u>
A4 - A10	Reactive control serum titration	7
B3, A12	Nonreactive control and diluent control	2
C4 - G4; C8 - G8	Patient serum samples	<u>10</u>
Total		19

The number of wells requiring sensitized particles, e.g., 19, X 0.025 mL of sensitized particles/well = total volume of sensitized particles, or mL = 19 X .025 = 0.475.

Calculation of the volume of unsensitized particles:

<u>Wells</u>	<u>Contents</u>	<u>Number</u>
A3, A12, B3	Reagent controls	3
C3 - G3, C7 - G7	Patient serum sample controls	<u>10</u>
Total		13

The number of wells requiring unsensitized particles, e.g., 13, X 0.025 mL of unsensitized cells/well = total volume of unsensitized particles needed, or mL = 13 X .025 = 0.325.

Calculation of the volume of serum diluent needed.

1.	Reactive Control. 100 μ L + (0.25 μ L x 9 wells) =	0.325 mL
2.	Reagent controls. 0.25 μ L x 2 wells =	0.050 mL
2.	Nonreactive control. 100 μ L + (0.25 μ L x 3 wells) =	0.175 mL
3.	Samples. [100 μ L + (0.25 μ L x 3 wells)] x 10 =	<u>1.750 mL</u>
		2.300 mL

READING AND REPORTING RESULTS

1. Read the settling patterns of the gelatin particles by using an angled mirror (tray viewer) to visualize the patterns from below. Readings are scored on a scale of - to 2+ agglutination; the degree of agglutination is judged according to the criteria in Table 14:2.
2. The endpoint dilution of the reactive control is the last dilution reading 1+. The reactive control serum should not vary more than ∇ 1 doubling dilution from the endpoint titer established for that serum. The dilutions are expressed in terms of the final serum dilution obtained after the addition of all reagents.
3. The nonreactive control serum should not react at the 1:80 serum dilution with sensitized cells.
4. Reagent controls. Both sensitized particles (antigen) and unsensitized particles, when tested in serum diluent alone, should give nonreactive test results.

5. Retest serum samples with readings of ∇ . Retest samples with nonspecific agglutination in the control particles as described below.
6. Report as "reactive" a serum showing agglutination of 1+ or higher with sensitized particles (antigen), provided there is no agglutination with unsensitized gelatin particles (Table 14:3).
7. Report as "nonreactive" a serum showing no agglutination with sensitized particles and unsensitized particles (Table 14:3).

Table 14:2. Criteria for Determining Degree of Agglutination

Degree of Agglutination	Reading	Interpretation
Agglutinated particles spread out, covering the bottom of the well uniformly	2+	Reactive
Definite large ring with a rough multiform outer margin and peripheral agglutination	1+	Reactive
Particles concentrated in the shape of a compact ring with a smooth, round outer margin	\pm	Indeterminate
Particles concentrated in the shape of a button in the center of the well with a smooth round outer margin	-	Nonreactive

Retesting Specimens Giving Nonspecific Agglutination

If a serum specimen has agglutinated both the sensitized and unsensitized particles, retest the serum as follows:

1. Place 0.95 mL of reconstituted Unsensitized Particles in a 12 x 75-mm test tube.
2. Add 50 μ L of specimen into the tube and mix thoroughly. Incubate at room temperature (15° to 30°C, 64° - 86°F) for 20 minutes.
3. Centrifuge for 5 minutes at 1,000 - 2,000 x g. Remove the supernatant (absorbed 1:20 diluted sample) carefully, and place 50 μ L in well 3 of the microtiter plate.

Table 14:3. Reporting System for TP-PA

Test Readings		Report
Initial	Repeat	
2+	Repeat not necessary	Reactive
1+	Repeat not necessary	Reactive
∇^*	$\geq 1+$	Reactive
∇	$< 1+$	Nonreactive
N	Repeat not necessary	Nonreactive
NS**	Repeat with absorption	Nonspecific agglutination

*Readings of ∇ occur occasionally, possibly because of low antibody titer or technical factors; serum showing this level of reactivity should be retested. Readings of ∇ on retest are reported as indeterminant.

**Occasionally a sample may agglutinate both the sensitized and unsensitized particles. The serum should be retested following the absorption procedure given below. If the sample is only agglutinated in the unsensitized particles, repeat the qualitative test with the serum unabsorbed.

4. Place 25 μ L of sample diluent in wells 4 to 12. Prepare serial doubling dilutions of absorbed sample from well 3 through well 12.
5. Place 25 μ L of unsensitized particles in well 3, using the droppers supplied with the kit.
6. Place 25 μ L of sensitized particles in wells 4 to 12, using the dropper supplied in the kit.
7. Mix the contents of the wells for 30 seconds on an automatic vibratory shaker, or gently tap the side of the plate to mix the contents.
8. Cover the plate and let it incubate at room temperature (15° - 30°C) for 2 hours or overnight.
9. Report as "inconclusive, nonspecific agglutination in serum control" (Table 14:4) if absorption of the serum with unsensitized particle does not remove the nonspecific agglutination and serum sample is only one doubling dilution greater than with unsensitized cells or the agglutination with sensitized particles is at the same dilution as with unsensitized particles.

Table 14:4. **Interpretation of Retesting of Nonspecific Agglutination Results**

Serum	1:40	1:80	1:160	1:320	1:640	Report
Sensitized particles	-	2+	2+	1+	-	Reactive
Unsensitized particles 2+	-	-	-	-	-	
Sensitized particles	-	2+	1+	-	-	Inconclusive
Unsensitized particles 2+	-	-	-	-	-	
Sensitized particles	-	1+	-	-	-	Inconclusive
Unsensitized particles	1+	-	-	-	-	

CALCULATIONS⁹

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

TP = True Positive, the number of individuals who test reactive and have syphilis.

FN = False Negative, the number of persons who test nonreactive and have syphilis.

TN = True Negative, the number of persons who test nonreactive and do not have syphilis.

FP = False Positive, the number of persons who test reactive and do not have syphilis.

Table 14:5. Sensitivity of the TP-PA by Stage of Untreated Syphilis

Stage	Primary	Secondary	Latent	Specificity
Clinical Trials	100 ^a	100	ND	ND
CDC Study	88 ^b	100	100	95 ^c

^aAll sera were RPR and VDRL reactive.

^bNonreactive sample was FTA-ABS nonreactive, VDRL was weakly reactive.

^cFour of the reactives were from patients with Rheumatic fever.

INTERPRETATION OF RESULTS

1. Reactivity in the TP-PA test with serum specimens also reactive with a cardiolipin test suggests current or past infection with a pathogenic treponeme.
2. Nonreactivity in the TP-PA test with serum specimens that are reactive in a cardiolipin test is referred to as a biologic false-positive nontreponemal test result and does not indicate syphilis. False-positive nontreponemal test results are seen in certain acute and chronic infections, following immunizations, in autoimmune diseases, and in intravenous drug addiction.
3. Nonreactivity in the TP-PA with serum specimens that are also nonreactive in a cardiolipin test usually indicates the absence of infection, except in persons incubating syphilis in which the TP-PA may be falsely nonreactive. A reactive TP-PA may also support the diagnosis of late syphilis or long-standing late latent syphilis in the small proportion of such cases in which the nontreponemal test has become nonreactive but infection is suspected because of the clinical presentation or a history consistent with syphilis infection.

ACCEPTABLE VARIATIONS

1. Microtitration trays other than those recommended may be satisfactory but should be evaluated before use.
2. Automatic safety pipettes delivering 25 µL may be used to deliver sensitized and unsensitized gelatin particle suspensions.
3. Diluters of 25-µL capacity may be used instead of automatic safety pipettes delivering 25 µL to make serum dilutions.

SOURCES OF ERROR

1. If microtitration trays other than those recommended are used for the test, the trays should be evaluated carefully. The appearance of the reading pattern, the titers of the reactive control and the test results may differ from those observed with the recommended tray.

2. If flimsy, flexible trays or those with V-shaped or flat-bottom wells are used, the cells will not settle in proper patterns.
3. If incubation occurs in an area where trays are subject to vibration, particles may not settle properly.
4. If trays are not stored in protected packages or if trays are incubated uncovered, lint and dirt may interfere with the settling pattern.
5. If even minute errors are made in pipetting specimens or controls because automatic pipettes are used inaccurately, the test results may be irreproducible or erroneous.
6. If the plate is tapped too vigorously in mixing the contents, specimens may spill or become cross-contaminated.
7. If outdated reagents are used, results may be invalid or erroneous. The laboratorian may be subject to legal action if outdated kits are used.
8. If kits are stored improperly, e.g., in the freezer or unrefrigerated, the test results may be altered.
9. Serum or plasma samples that contain excessive free bilirubin may give erroneous results.

TEST LIMITATIONS

1. All treponemal tests tend to remain reactive following treponemal infection; therefore, the TP-PA test should not be used to evaluate response to therapy. Because of the persistence of reactivity, probably for the life of the patient, the treponemal tests are of no value in determining relapse or reinfection in a patient who has had a reactive TP-PA test result.
2. False-positive TP-PA test results may occur in association with other underlying illnesses.
3. The TP-PA test may be reactive in persons from geographic areas where yaws or pinta was, or is, endemic.
4. Serum specimens from patients with rheumatic fever, leprosy, autoimmune diseases, and drug addiction may react, on occasion, with tests detecting anti-treponemal antibodies.
5. Nonspecific agglutination, although not common, may or may not be resolved by retesting absorbed serum against sensitized and unsensitized gelatin particles.

REFERENCES

1. Deguchi M, Hosotsubo H, Yamashita N, Ohmine T and Asari S. Evaluation of gelatin particle agglutination method for detection of *Treponema pallidum* antibody. Journal of the Japanese Association of Infectious Diseases 1994; 68:1271-7.
2. Jaffe HW. The laboratory diagnosis of syphilis. Ann Intern Med 1975;83:846-50.
3. National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture. 2nd ed. Villanova, PA: NCCLS Publication H 3-A2, 1984.
4. Centers for Disease Control. Update on hepatitis B prevention. MMWR 1987;36:353-60,366.
5. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other blood-borne pathogens in health-care settings. MMWR 1988;37:377-82,387-8.

6. Centers for Disease Control. Recommendations for prevention of HIV transmission in health care settings. MMWR 1987;36(supp. 2)::1-18.
7. National Committee for Clinical Laboratory Standards. Tentative Standard M29-T2. Protection of laboratory workers from infectious disease transmitted by blood, body fluid and tissue. Villanova, PA: NCCLS, 1991.
8. Department of Health and Human Services, Health Care Financing Administration. Clinical Laboratory Improvement Amendments of 1988; Final Rule. Federal Register 1992 (Feb. 28):7183 [42CFR 493.1703 (c)]
9. National Committee for Clinical Laboratory Standards. Document I/LA18-A. Specifications for immunological testing for infectious diseases; Approved guideline. Villanova, Pa: NCCLS, 1994.