

Chapter 4

QUALITY CONTROL

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Note. The forms included in the appendix are not meant as replacements for existing forms that may already meet CLIA guidelines, but are merely examples of forms that are in compliance with CLIA regulations.

*Deceased 1996

QUALITY CONTROL

GENERAL MEASURES

Quality control measures in syphilis serology are designed to ensure that reliable and reproducible test results are obtained within a laboratory and among different laboratories performing the same tests. Strict adherence to recommended technique and the use of standardized reagents eliminates most technical errors. Departures from predetermined reactivity of control serum samples detects day-to-day variability in testing and indicates any need for corrective action. Occasionally deterioration of reagents causes the variation, but other factors are more frequently responsible. The quality control program in syphilis serology must be under active surveillance by the laboratory supervisor, with document review at least weekly. Secondary review should occur at least monthly by the laboratory director or designee.¹

The quality control measures that are essential for reliable and reproducible test results are as follows.

1. Clean, well-lighted, temperature-controlled laboratories, with adequate space for work, both technical and clerical, and storage including both shelf and refrigerated
2. Proper equipment, instruments, and glassware, all of which meet specifications and are of sufficient quantity and quality for the types and volume of testing performed; records of dates of purchase, repair, maintenance of equipment and instruments, and daily calibration checks
3. Satisfactory cleaning methods for reusable glassware
4. Proper test procedures adapted to the laboratory facilities and the qualifications of the personnel performing tests
5. Current techniques available for reference and followed strictly without modification for compliance with CLIA 88
 - a. A procedure manual must be written in compliance with, and to meet the intent of, the Approved Guideline GP2-A3 Clinical Laboratory Technical Procedure Manual, second edition, National Committee for Clinical Laboratory Standards (NCCLS), and be available at the workbench²
 - b. The procedures should be reviewed annually and the review results documented³
 - c. A copy of a discontinued procedure must be maintained for 2 years thereafter, recording initial date of use and retirement date⁴
6. Careful and precise measurements of specimens and reagents

7. Periodic reading of tests to maintain uniform reading levels by all laboratory personnel
8. Control of reagents
 - a. Chemicals and distilled water should be of high quality, and solutions should be prepared according to the directions specified for each technique
 - b. Adequate evaluation of new lots of reagents for standard reactivity must be done before they are placed in routine use
 - c. There should be proper preparation, labeling, and storage of reagents. Substandard, deteriorated, or outdated reagents should be discarded
 - d. Documentation of steps in the preparation of new lots of reagents and control serums, and maintenance of evaluation records of each lot must be done. Reagents should be dated when prepared or opened and placed in service
9. Maintenance of reference serum samples with established reactivity patterns for each test performed. Include these each time serologic testing is performed to provide a stable baseline and day-to-day consistency.
10. Acceptable test specimens and identification. Ensure acceptability by correct collection and labeling, prompt transmission to the laboratory, and proper storage conditions and processing methods
 - a. All specimens must be accompanied by a requisition form that includes patient identification, name of physician or authorized person ordering the tests, and the tests or assays requested⁵
 - b. The disposition of all unacceptable specimens should be documented in the patient's report or the laboratory's quality improvement records
 - c. Specimen requisitions must be retained for at least two years
 - d. At a minimum, serum and body fluid specimens should be retained for 24 hours, and stained slides for direct microscopic examination should be retained for 7 days
11. Provision of daily worksheets designed for recording the specimen numbers, results of all tests performed, control results, lot numbers of reagents, reagent titers, room temperature, and the worker's initials. As tests are read, record the results of all controls and specimens, not just the interpretation or final report. These records should be retained for a minimum of 2 years.

12. Monitoring of all testing records and report forms. All records of testing should be identified with the specimen's laboratory accession number.
 - a. Reports should be easy to read and results correctly reported
 - b. Copies or files of reported results should be retained in a manner that permits prompt retrieval of information
 - c. The laboratory must have a procedure for the immediate notification of a physician or other responsible clinical personnel of a reactive test result
 - d. The laboratory must have defined turnaround times for each of its tests and a policy for notifying the requester when testing is delayed for those tests considered as essential by clinical and laboratory personnel⁶
 - e. Laboratories using a computerized laboratory information system are responsible for: the data produced in their laboratory, including data entry, storage and retrieval; the computer's facility and maintenance; computer procedure manuals, and systems security
 - f. The laboratory must have a system in operation to detect clerical and analytical errors, and to verify highly unusual results that could affect patient management. One common method is review of results by an experienced person¹
 - g. Patient test results and reports, and instrument printouts (when applicable) must be retained for at least two years
13. Participation in a recognized proficiency testing study in which the participating laboratory is provided a means of periodically detecting problems by comparing its results with those of a reference laboratory
 - a. Proficiency testing samples must be integrated within the routine laboratory workload, and analyzed by personnel who routinely perform the tests⁷
 - b. For tests where proficiency testing is not available, performance must be checked with reference material or by split sample analysis with other laboratories or clinical validation by chart review or other suitable documented means
 - c. Proficiency testing records must be retained for at least 2 years
14. Attendance of qualified laboratory personnel at basic and refresher training courses to obtain current information and instruction in new or modified test procedures. A functional in-service continuing education program to meet the needs of the laboratory personnel should be available. Persons performing the serologic tests for syphilis must be evaluated according

to CLIA-88 requirements for moderate or high complexity testing (as appropriate) and qualify.⁸

SPECIFIC MEASURES - REAGENTS

1. Reagent performance and adequacy must be verified before placing the material in service.
2. Commercial reagents and controls must be used according to the manufacturer's directions. If alternative procedures are used, the method must be evaluated with the manufacturer's method to justify the change.
3. The laboratory must use components of reagent kits only with other kits that are in the same lot number, unless otherwise specified by the manufacturer.⁹
4. Results of quality control testing should be retained for 2 years after expiry date of reagents.

NONTREPONEMAL TESTS

It is the responsibility of the laboratory to ensure that reagents are of good quality and standard reactivity. Each new lot of cardiolipin antigen for the Venereal Disease Research Laboratory (VDRL) test or antigen suspension for the rapid plasma reagin (RPR) 18-mm circle card, toluidine red unheated serum test (TRUST), and unheated serum reagin (USR) should be checked in parallel with reference reagents to verify that they are of standard reactivity. Parallel testing should be performed on more than one testing day, by using different specimens of graded reactivity for each test period. Tests should be performed in accordance with the techniques described in this manual. *See respective test chapters for specific testing procedures and performance criteria.* Record the results of all check testing.

Because the ability of a reagent to detect antibodies in specimens with low grade reactivity is necessary for the diagnosis of primary syphilis, serum samples of graded reactivity should be used. At least 10 samples ranging in reactivity from weakly reactive to R2 should be included in comparative testing with reference reagent or the previous lot of reagent. Individual specimens of graded reactivity for check testing may be obtained by selecting specimens from the daily tests runs and storing them in the freezer. Reactive serum diluted with nonreactive serum to produce various degrees of reactivity may also be used. If quantitative tests are to be performed, three serum samples that can be diluted at least 1:4 should be included in a quality control panel. Roughness of an antigen can best be detected using fresh serum samples obtained from persons without syphilis. If possible, ten fresh serum specimens from routine test runs should be used for the nonreactive specimens.

The temperature at which the nontreponemal tests are performed is critical to the reactivity of the test, and thus to test results. The optimal room temperature for testing is 76°F (24.42°C) (range 73° - 85°F or 23° - 29°C). All reagents and specimens should be allowed to warm to room temperature before use.

On a daily basis, control serum samples of three levels of reactivity (reactive, weakly reactive or reactive minimal, and nonreactive) are used for qualitative tests to determine if the antigen and test technique are in control. If quantitative tests are performed, then the reactive control or one other serum sample that can be diluted at least 1:4 should be used. For the nontreponemal flocculation tests with serum and the VDRL test with spinal fluid, the antigen suspension to be used each day is first examined with control serum samples. The results obtained with the controls should reproduce the established reactivity pattern. If the results are not acceptable, routine testing should be delayed until optimal reactivity has been established (by preparing another antigen suspension, correcting room temperature, adjusting equipment). If the pattern of reactivity is not acceptable, results of the tests on individual specimens are considered invalid and are not reported.

To determine if the control serum samples are in control they should be evaluated as described below. A reference control serum for the nontreponemal tests that can be diluted $\geq 1:4$ is available from **Technical Services Branch, Centers for Disease Control and Prevention, Atlanta, GA 30333** in limited quantities. This serum is to be used only for the evaluation of a new control serum sample and not as a daily test control.

Note. A daily run shall not exceed 24 hours. Results of controls must be reviewed before reporting test results.

Evaluation of Control Serum Samples

1. Criteria of Acceptability

- a. Using reference antigens, test results with new serum control samples should be identical to those obtained with reference standard control serum samples.
- b. The nonreactive control serum sample must not show any degree of flocculation or roughness with reference antigen. The Reactive control serum sample must show definite flocculation, whereas the reaction of the weakly reactive or minimally reactive control serum sample must show a slight degree of flocculation (small clumps) with the reference antigen.

2. Procedure for Testing

- a. Prepare and store the control serum samples according to the manufacturer's instructions.
- b. Perform tests for which patterns are to be established on the new control serum samples with antigens that reproduce reactivity patterns on standard control serum samples.
- c. Repeat tests on 2 additional days, using a different set of the new control serum samples on each testing day.

- d. Compare results obtained in the three testing runs.
 - 1) If identical results are obtained from the reference and the new control serum samples, no further testing is necessary.
 - 2) If the results are discrepant, perform additional testing to establish the reactivity pattern.

Controls must be properly labeled as to content, lot number, date of preparation, and expiration date.

TREPONEMAL TESTS

As with the nontreponemal tests, it is the responsibility of the laboratory to ensure that reagents for the treponemal tests are of good quality and standard reactivity. Each new microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP) kit should be tested in parallel with a previous kit to verify that the new lot is of standard reactivity. Antigen, sorbent, and conjugate for the fluorescent treponemal antibody-absorption (FTA-ABS) and the fluorescent treponemal antibody-absorption double staining (FTA-ABS DS) tests should be conducted in parallel with a standard reagent to verify that the new reagent is of standard reactivity. Parallel testing should be performed on more than one testing day, by using different serum samples of graded reactivity for each test period. Tests should be performed in accordance with the techniques described in this manual. *See respective test chapters for specific testing procedures and performance criteria.* Record the results of all check testing. Reference reagents for the FTA-ABS and FTA-ABS DS tests are available from **Technical Services Branch, Centers for Disease Control and Prevention (CDC), Atlanta, GA 30333**. The availability of these reagents is restricted, reagents are to be used only when check testing new lots of commercial reagents or when problems with a current reagent are suspected.

For check testing of the reagents used in the treponemal tests, 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 $\geq 2+$ reactivity, and 4 nonreactive serum samples. If necessary, prepare reactive serum samples of various levels of reactivity by diluting reactive serum samples with nonreactive serum samples. These pooled samples may be substituted for some of the individual serum samples.

For the daily quality control of the FTA-ABS, FTA-ABS DS, and MHA-TP procedures, the control serum samples are included in the test run. If the pattern of reactivity is not acceptable, results of the tests on individual specimens are considered invalid and are not reported. *Methods for evaluating the controls used in these tests are found in their respective chapters.*

DIRECT FLUORESCENT ANTIBODY TESTS

Fluorescein-labeled conjugates for the direct fluorescent antibody test for *T. pallidum* (DFA-TP) and for the direct fluorescent antibody tissue test for *T. pallidum* (DFAT-TP) should be evaluated in parallel with known reagents and with characterized control antigens. New reagents are evaluated

according to the instructions of this manual and must produce results with reference controls and test specimens that are comparable to those obtained with reference reagents. To determine conjugate specificity, test each conjugate dilution with a *T. pallidum* antigen (FTA-ABS antigen) slide and slides prepared from cultures of *T. denticola*, *B. burgdorferi*, and *Leptospira sp.* **See respective test chapters for specific testing procedures and performance criteria.**

A positive and a negative control must be included with each run of patients specimens.

Positive control slides. *T. pallidum* subspecies *pallidum* slides prepared from *T. pallidum*-infected lesions or testicular impression smears from *T. pallidum*-infected rabbits (**Technical Services Branch, CDC, Atlanta, GA**) or from reconstituted lyophilized fluorescent treponemal antibody-absorption (FTA-ABS) test antigen (**Becton-Dickinson, Microbiology Systems, Cockeysville, MD; Difco, Detroit, MI; INCSTAR Corporation, Stillwater, MN; Pharmacia Diagnostics, Division of Electro-Nucleonics, Fairfield, NJ; Scimedx (BioDx), Denville, NJ; Zeus Scientific, Inc., Raritan, NJ**).

Negative control. Smears made from washed nonpathogenic Reiter treponeme cultures, freshly obtained human mouth treponemes, or washed *T. denticola* cultures may be included.

LABORATORY EQUIPMENT AND SUPPLIES

Equipment

1. All instruments are to be included in a routine maintenance program. Instrument maintenance records must be retained for the life of the instrument.
2. Laboratory equipment should be kept clean and in good working order. Personnel should be instructed on proper use and care of equipment. Records should be maintained on all equipment to monitor accuracy of performance and to determine repair or adjustment needs.

A suggested schedule for care and function checks is shown in Table 4:1.

Thermometers

All thermometers in use should be calibrated against a National Institute of Standards & Technology (NIST), National Bureau of Standards (NBS) certified thermometer. A record should be retained of the deviations inherent in each individual thermometer so correction factors can be established.

Table 4:1. Care and Function Checks for Laboratory Equipment¹⁴

Item	Frequency	Record	Maintenance
Autoclave	With each use	Pressure, Temp.	Use spore strips monthly to monitor contamination.
Water bath	With each use	Temperature	Adjust if necessary, clean monthly
Incubator	With each use	Temperature	Adjust if necessary

Freezer	Daily	Temperature	Adjust if necessary, clean every 6 months.
Refrigerator	Daily	Temperature	Adjust if necessary, clean every 3 months.
Rotator	With each use	Speed	Without a digital readout, count
			number of rotations against your finger for 1/4 minute.
Centrifuge	With each use	Speed	If glass breaks, see chapter 2. Most newer centrifuges record speed automatically. Check brushes and bearings every 6 months.
pH meter	With each use	pH	Use standards: pH 4, pH 7, pH 10. Inspect electrodes each time to see if they are filled and not cracked. Check every 6 months for proper function.
Microscope	With each use	length of use	Clean lens and cover when not in use.
Balance	With each use	Use	Locate free of drafts; clean after use. Check with certified weights every 6 months.
Biological Safety	Every month	Sterility	Expose blood agar plates to air Cabinets
			flow for 1 hr. every month. Check for leaks and rate of air flow every 3 to 6 months by measuring pressure on both sides of filters. Clean filters every 3 months.
Water stills	With each use	Conductivity	Change deionized water cartridges at regular intervals. Check pH periodically to detect

a leaking condenser coil.

NIST traceable thermometers for freezers and refrigerators can be purchased commercially (**Streck Labs, Omaha NE**). These thermometers are encased in protective plastic casings and have attached magnets for adherence to metal doors and shelves.

Pipettors and Tips

With the pipettors available today, 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 the accuracy pipettors, making them lose their initial accuracy. The use of disposable tips from different sources is probably the most common error. For budgetary reasons, pipette tips may be interchanged with those of a less expensive brand. Although the less expensive brand may be satisfactory, the laboratory should verify the accuracy and precision of alternative tips in their system. Commercial kits to check pipettor accuracy are available. Also, manufacturers have procedures for checking the accuracy of their equipment. The gravimetric or spectrophotometric procedures have been the most accepted methods used to calibrate pipettors.^{10,11} These procedures, which use the weight of water or the absorbance of a substance at a given wavelength, should be used only when there are no specific manufacturer's instructions for calibration.

Automatic and adjustable pipetting devices must be checked at specified periodic intervals for accuracy and reproducibility and the results of such testing documented. The calibration of pipetting devices can be contracted out rather than done in house. Damaged pipettors which can not be repaired and properly calibrated must be discarded.¹

Calibrated Needles

Methods for preparation and calibration of stainless steel needles for slide flocculation tests are found in the respective Chapters on VDRL (Chapter 8) and USR (Chapter 9). Needle size and amount delivered are shown in

Table 4:2Table 4:2. Needle specifications for VDRL and USR

Test	Needle gauge	Drop size (µl)	No. drops/ml
VDRL	18	17	60 √ 2
VDRL CSF	21 or 22	10	100√2
USR	18	22	45√1

Glassware

To prevent possible transmission of infectious blood-borne diseases, use disposable plastic- or glassware, whenever possible. In general, disposable labware can be used in the serologic tests for syphilis with the following exceptions:

- 1) VDRL antigen suspension bottles (30 ml) must be made of nondisposable glass.**
- 2) VDRL slides must be glass.**
- 3) VDRL-cerebrospinal fluid (CSF), Kline concavity slide, if available, must be glass.**
- 4) Microscope slides must be glass.**
 - a. For the FTA-ABS tests, precleaned 1"x 3" microscope slides are recommended (Clay Adams, Division of B-D, Oxnard, CA). These slides have a thickness of 1 mm, one frosted end and two etched circles. Caution is advised when using slides having 10 or more wells (or circles) due to the wash-over between wells that can occur during the rinsing and incubation steps of the procedures (Elizabeth Hunter, unpublished communication).**
 - b. For direct microscopic tests (Darkfield, DFA-TP, and DFAT-TP), clean, frosted end slides without etched rings (circles) are suitable.**

Slides for the VDRL and the microscopic tests should be clean and grease free. Reusable slides for the VDRL slide test with serum or CSF must be cleaned after disinfection (Chapter 2). To clean slides, they should be placed in special racks in glassware washing machines. If washing slides by hand, gloves must be worn and slides should be prerinsed, washed, rinsed with distilled water, dried, and, finally, inspected for cleanliness. To free VDRL slides of paraffin rings after disinfection, soak in detergent solution. Glass slides with ceramic rings must be handled gently to prevent flaking of the ring. Avoid prolonged soaking of these slides in detergent solution.

Slides for the VDRL and the microscopic tests may also be cleaned by using an ultrasonic cleaner. After disinfecting slides, fill the cleaning tank with hot water, place slides in holder into tank, and turn on ultrasonic cleaner. Do not overload tank. Clean for 3 minutes and polish dry with gauze. After use, empty tank and wipe dry.

PREPARING CONTROL SERUM AND PROFICIENCY TESTING SAMPLES

Control serum samples of graded reactivity should be included each time serologic testing procedures are performed. Control serum samples of graded reactivity for nontreponemal and treponemal test procedures are available from commercial sources or may be prepared from individual serum samples or serum samples pooled after testing. Reactive serum samples of high titer may be used to prepare spinal fluid controls. A pattern of reactivity should be established for each new lot of control serum obtained from a commercial source, by comparing the new control serum or serum samples with standard control serum or serum samples. Control serum samples of graded reactivity are prepared similarly to serum samples to be submitted to other laboratories for syphilis serology proficiency testing studies, but on a smaller scale. Panels of serum samples of graded reactivity are needed for evaluation of each new lot of reagents and are prepared in a similar manner.

Collecting Serum ^{12,13}

- 1. Using the methods described in Chapter 4 for collection of venous blood, obtain units of nonreactive blood from individual donors. Do not use anticoagulants in collection of blood.**
- 2. Obtain units of highly reactive (1:16 or higher) blood from individual donors with active or treated syphilis. Do not use anticoagulants in collection of blood.**
- 3. Screen units for antibodies against human immunodeficiency virus (HIV) and hepatitis C virus (HCV), and for hepatitis B surface antigen (HBsAg). Use only nonreactive units, whenever possible.**

Note: The individual serum samples or serum sample pools used to prepare control serum or proficiency testing samples should be tested for HIV, HCV and HBsAg. If samples are positive for any virus, or if samples are not tested, this information should be noted on each vial. Proper labeling should ensure that proper safety precautions are taken while handling.

Processing Whole Blood and Storing Serum

- 1. Nonreactive blood**
 - a. Process each donor's sample separately.**

- b. Centrifuge whole blood at 600 x g for 10 minutes and remove serum from the clot. Recentrifuge serum at 600 x g for 10 minutes to remove any red blood cells or particulate matter.**
- c. Remove a sample from each serum; freeze samples and remaining bulk nonreactive sera in individual Pyrex (or equivalent) bottles at -20°C or below.**
- d. When a number of serum samples from nonreactive donors have accumulated for several weeks, thaw samples and test each with the HIV-enzyme immunoassay (EIA), and for hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV). Also perform from the following tests for syphilis: RPR (18 mm circle), VDRL, TRUST, USR, MHA-TP, FTA-ABS, and EIA, the tests done in your laboratory or for which either control serum or proficiency testing is desired. Use only serum samples that have consistently given clear-cut nonreactive results.**

2. Reactive

- a. Process and maintain donor's sample separately.**
- b. Centrifuge whole blood at 600 x g for 10 minutes and remove serum from the clot. Recentrifuge serum to remove any red blood cells or particulate matter.**
- c. Remove samples from each reactive serum for testing; freeze each bulk reactive serum separately in a Pyrex (or equivalent) bottle at -20°C or below.**
- d. Test each sample in the following tests: HIV-EIA, HBsAg, HCV, VDRL, USR, TRUST, RPR (18-mm Card), FTA-ABS, MHA-TP, FTA-ABS DS and any of the ELISA tests currently available for syphilis.**
- e. Record test results of each reactive serum in a record book. Results will be used when preparing dilution pools of graded reactivity for the proficiency testing samples. Discard, properly and safely, the samples that are reactive to HIV, HCV or HBsAg.**

Preparing Serum Dilutions for Control Serum or Proficiency Testing Samples

1. Preparation of nonreactive pools

- a. Select the nonreactive serum samples that give clear-cut nonreactive results in the VDRL, RPR, MHA-TP, or FTA-ABS tests and process as follows:**
 - 1) Thaw and mix thoroughly by gently swirling the bottles.**
 - 2) Heat each serum sample at 56°C for 30 minutes. (Heating nonreactive sera reduces the possibility of rough nonreactive reactions in a VDRL test.)**
 - 3) Combine individual nonreactive serum samples into a single nonreactive pool.**
 - 4) Mix the nonreactive pools by inverting the bottles at least 10 times, avoiding foaming, and store at 2° - 8°C overnight.**
 - 5) Remove a sample from the nonreactive pool and retest to ensure nonreactivity in the VDRL and FTA-ABS tests. For unknown reasons,**

pooling some nonreactive serum samples into large pools may cause staining greater than N, but less than I+ in the FTA-ABS tests.

- b. Remove the nonreactive serum pool from the refrigerator (2° - 8°C). A previously frozen pool can be thawed by holding it at 2° - 8°C over a weekend, or it may be thawed on the day it is to be used by placing it in a 37°C water bath.
- c. Mix serum samples by inverting the bottles at least 10 times (avoiding foaming) and remove coarse suspended particles (which could clog the sterilizing filter) by passing the samples through a clarifying Millipore type filter.
- d. Filter the clarified serum sample again, using sterile technique. Filters of porosity 5.0 μ, 1.2 μ, 0.8 μ, 0.65 μ, and 0.45 μ may be stacked or used individually, starting with the largest porosity, for clarifying and sterilizing serum sample pools.

Note: Serum showing ≤1+ reactivity in the FTA-ABS tests may be used in a pool for diluting purposes only. Serum samples showing stronger reactions in the FTA-ABS and MHA-TP tests may be pooled for a treponemal test reactive pool that gives reactive results in the FTA-ABS and MHA-TP tests only. Pools with definite nonreactive VDRL, RPR, MHA-TP, and FTA-ABS test results are needed for individual nonreactive controls or proficiency testing samples.

2. Preparation of bulk reactive serum

- a. Remove reactive serum samples from the freezer and thaw by placing in a 37°C water bath. A single reactive serum sample, if large enough, is used to prepare all dilutions for the control serum or proficiency testing samples. If several individual reactive serum samples must be pooled to obtain a sufficient quantity, they should be mixed thoroughly by placing the serum pool in an Erlenmeyer flask or a wide-mouth bottle having a capacity 3-5 times the volume of the pool. Mix by rotating the flask on a mechanical rotator for 1 hour at approximately 100 rpm (avoid foaming).
- b. Filter the clarified serum pool again, using sterile technique. Millipore type filters of porosity 5.0 μ, 1.2 μ, 0.8 μ, 0.65 μ, and 0.45 μ may be stacked or used individually, starting with the largest porosity, for clarifying and sterilizing serum pools.

- 3. Preparation of dilution pools for control serum and proficiency samples**
 - a. Prepare preliminary dilutions of reactive serum in the selected nonreactive serum pool, using the reactivity results obtained when the bulk reactive serum was pretested (Table 4:3). Dilutions should be carried out far enough (usually 1:400 or 1:500) and close enough together to find the minimal reactive or reactivity level for all tests. At this time, retest the sample of the nonreactive pool.**

- b. Perform all tests included in the proficiency testing program or for which control serum samples are desired.
- c. Select appropriate dilutions that give graded reactivity in each test procedure. It may be necessary to prepare intermediate dilutions of reactive serum in nonreactive serum in order to obtain the desired reactivity.
- d. Calculate the amount of each serum dilution to be prepared. The amount will be determined by the quantity needed for each day's testing, the length of time during which controls will be used, and the type of storage facility available. If making similar calculations for proficiency testing samples, the amount will depend on the number of participating laboratories and the use of single or duplicate samples.
- e. Prepare serum pools of the calculated volume needed for use in the shipment by diluting reactive serum in nonreactive serum. Add 0.01% total volume of gentamicin sulfate for each milliliter of serum if samples are to be shipped or stored for more than 2 days.

Table 4:3. Example of Results Obtained with Prepared Serum Dilutions

Dilution	Reactive Serum (ml)	Nonreactive Serum (ml)	Test Results	
			RPR	VDRL
1:25	0.1	2.40	R	R
1:50	0.5	2.45	R	R
1:100	0.01	0.99	R	R
1:150	0.01	1.49	R	R
1:200	0.01	1.99	R	R
1:250	0.01	2.49	R	R
1:300	0.01	2.99	R	Rm
1:350	0.01	3.49	R	W
1:400	0.01	3.99	Rm	Wm
1:450	0.01	4.49	N	N
1:500	0.01	4.99	N	N

R = reactive; W = weakly reactive; N = nonreactive; m = minimal. According to the example, a set of serum dilutions suitable for the RPR and the VDRL might be selected as follows:

Control 1, dil 1:200 Gives clear-cut R result, saving on reactive pool

Control 2, dil 1:350 Intermediate in VDRL, but no help in RPR

Control 3, dil 1:400 Intermediate in RPR, but too critical to be sole intermediate control in VDRL

Control 4, Nonreactive control should be nonreactive pool only

- f. Mix each serum pool thoroughly by placing the serum pool in an Erlenmeyer flask or a wide-mouth bottle having 3-5 times the volume of the pool; rotate the flask on a mechanical rotator for 1 hour at approximately 100 rpm (avoid foaming).**
 - g. Test a sample from each serum pool in the proficiency testing program or for which control serum samples are desired. If necessary, adjust the pool to higher or lower reactivity, and retest a sample of the adjusted pool.**
 - h. Store serum pools at 2° - 8°C for not more than 1 week before dispensing into the appropriate tube or vial. For longer storage, freeze at -20°C or below.**
 - I. Serum pools (if frozen, thaw at refrigerator temperature or in a 37°C water bath) are sterilized by Millipore type filtration using a 0.45 μ filter pad. Observe strict sterile technique to prevent bacterial contamination of the pools.**
 - j. Dispense samples, using a dosed system and observing sterile technique, into 5-ml Wheaton vials, approximately 2 ml per vial for proficiency testing serum samples. For control serum samples, dispense quantities of each dilution, enough for one testing period, into properly labeled tubes; stopper tightly.**
4. Sterility testing of tubed serum samples (for proficiency testing samples)
- a. Place all tubed samples in a 35°C incubator for 24 hours and examine each tubed sample for bacterial contamination.
 - b. Remove two complete sets of tubed samples and place the contents of each sample into a tube of thioglycollate.
 - 1) Incubate one set of inoculated tubes of thioglycollate at 25°C for 14 days and the other set at 35°C for 14 days.
 - 2) Examine tubes for bacterial contamination.
 - c. Remove an additional complete set of tubed samples and leave at room temperature for 14 days. Examine for bacterial contamination.
 - d. Store all remaining tubed samples at 2° - 8°C until ready to package and ship.

ESTABLISHING PATTERN OF REACTIVITY FOR CONTROL SERUM SAMPLES

Nontreponemal Tests

1. Remove a set of the new control serum samples from the freezer, thaw, and mix thoroughly.
2. Perform tests on the new control serums for which patterns are to be established with antigen suspensions that reproduce reactivity patterns on standard control serum samples.
3. Repeat tests on 2 additional days, using a different set of the new control serum samples on each testing day.
4. Compare results obtained in the three testing runs.
 - a. If the results are identical, no further testing is necessary.
 - b. If the results are discrepant, perform additional testing to establish the reactivity pattern.

Note. Commercial control serum samples are evaluated similarly before being routinely used.

5. Routine use of control serum samples
 - a. Each day that tests are to be performed, remove one set of control serum samples from the freezer, thaw, and mix thoroughly. The control serum must be at room temperature when tested.
 - b. Check the reactivity of the test antigen suspension with the control serum samples as described in the particular test technique.
 - c. Do not use an antigen suspension that does not reproduce the established reactivity pattern of the control serum samples.
 - d. Check other parameters; i.e., room temperature, needle, rotator speed.

Treponemal Tests

1. Control serum samples for the FTA-ABS and FTA-ABS DS tests
 - A. Selecting and preparing reactive and nonspecific serum samples for use as controls
 - 1) For the reactive control serum sample, select samples from individual syphilitic donors that are reactive 4+ when diluted in PBS and are no less

than 3+ to 4+ when diluted in sorbent. High-titered serum samples should be diluted in nonreactive serum to keep minimally reactive (1+) control dilution at or below 1:500.

- 2) For the nonspecific control serum, select samples from individual nonsyphilitic donors that are reactive 2+ or greater when diluted in PBS and nonreactive when diluted in sorbent.
- 3) To prepare control serum samples, process serum samples as described in Collecting Serum and in Processing Whole Blood and Storing of Serum Samples; prepare enough for 2-3 months.
- 4) Confirm the reactivity of reactive and nonspecific serum samples diluted 1:5 both in PBS and in sorbent.
- 5) Dispense into properly labeled tubes enough of the reactive and the nonspecific serum samples for one testing period (0.3 to 0.4 ml); stopper tightly.
- 6) Combine in sets and store in the freezer.

B. Pretesting for a minimally reactive (1+) control

- 1) Heat standard control serum samples and new reactive serum samples at 56°C for 30 minutes.
- 2) To determine the range of reactivity of the new reactive serum sample, prepare serial twofold dilutions of the serum (1:100 to 1:3200) in PBS.
- 3) Add 0.03 ml of each serum dilution to correspondingly labeled antigen slides.
- 4) Set up standard controls for the FTA-ABS test.
- 5) Read and compare the reactivity of each dilution against the minimally reactive (1+) control.
- 6) Select the dilutions of the new reactive serum sample that approximate the degree of fluorescence of the standard minimally reactive (1+) control serum sample.
- 7) Prepare and test additional intermediate dilutions in the indicated range to obtain one whose reactivity is identical to that of the standard Minimally Reactive (1+) control serum sample currently in use.

C. Establishing pattern of reactivity

- 1) Remove a set of new control serum samples and a set of standard control serum samples from the freezer: thaw, mix thoroughly, and heat at 56EC for 30 minutes.
- 2) Reactive control serum sample.
 - a) Dilute the new reactive serum 1:5 both in PBS and in sorbent.
 - b) Prepare three dilutions of reactive serum in PBS: the dilution selected in the pretesting that corresponds to the standard minimally reactive (1+) control serum sample; one dilution slightly above the selected dilution and one dilution slightly below the selected dilution.
- 3) Perform tests on the new control serum sample and the standard control serum sample according to the directions for testing controls in the FTA-ABS or FTA-ABS DS technique.
- 4) Verify reactivity by repeat testing on at least 2 additional days; use a different set of new control serum samples on each testing day.
- 5) Compare results obtained in the three test runs.
 - a) If the results are identical, no further testing is necessary.
 - b) If the results are discrepant, perform additional testing to establish the reactivity pattern.

2. Establishment of reactive control serum sample for MHA-TP

Although the MHA-TP kit has a reactive control serum sample with an established endpoint titer, some serologists have titered and established additional reactive controls from their serum bank. These controls could serve as the adjunct control to the kit reactive control serum sample to solve problems that occasionally arise. To properly titer and establish an endpoint on an intra-laboratory control, it would be necessary to test this serum sample quantitatively on several occasions with several kits. The procedure would be the same as that used for titering the kit-reactive control serum sample, i.e., dilutions of 1:80, 1:160, 1:320.

Spinal Fluid Venereal Disease Research Laboratory (VDRL) Test

1. Select an individual serum sample or a serum sample pool that is reactive in the spinal fluid test when diluted 1:80 or higher in 0.9% saline.
2. Dispense small quantities, enough for one testing period of the reactive undiluted serum, into labeled tubes and stopper tightly.

3. After 3 or more days' storage, thaw one tube of the reactive serum and mix thoroughly.
4. Prepare serial dilutions of the serum in 0.9% saline starting at 1:80. The final dilution used will depend on the titer of the serum sample being used for the control.
5. Test the serum dilutions in parallel with standard spinal fluid controls using the VDRL-CSF technique.
6. Select three serum dilutions that produce reactive, minimally reactive, and nonreactive test patterns, respectively. For example, the 1:80 dilution may be used as the reactive control while the 1:320 dilution may give a minimally reactive result and the 1:1280 dilution may give a nonreactive result.
7. Confirm the reactivity pattern of these three dilutions by testing in parallel with standard controls on at least 3 different testing days. Use a different tube of new control serum each test day.

Note. The procedures for establishing patterns of reactivity on control serum and proficiency testing samples may also be used to evaluate commercial control serum samples or commercially prepared proficiency testing samples.

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