THE HINTON[1] AND DAVIES-HINTON TESTS FOR SYPHILIS

WILLIAM A. HINTON, M. D., and JOHN A. V. DAVIES, M. D.

INTRODUCTION

In the detection of syphilis the Hinton and Davies-Hinton tests are of special value because of (1) the volume of output by the regular method; (2) quick results in emergencies by the rapid method; (3) the small amounts of blood required by the Davies-Hinton microflocculation test; (4) easy examination of spinal fluids by the Davies-Hinton test.

HINTON TEST FOR SYPHILIS. (THIRD MODIFICATION)

This test requires precision in execution because consistently accurate results cannot be obtained if minor variations in technic are allowed.

EQUIPMENT

1. Test tube racks. To simplify numbering and pipetting serums, these racks should be constructed to hold 10 or 20 tubes in a row.
2. Serum tubes 100 mm. long, with an approximately uniform inside diameter of 11 mm.
3. A water bath for inactivating serums (uncovered).
4. A Wassermann bath (uncovered). The water in the bath should be changed frequently to prevent a deposit from sticking to the outside of the tubes.
5. A centrifuge with a speed of over 2,000 revolutions per minute.
6. A maximum and minimum thermometer.
7. Graduated 100-cc. and 250-cc. cylinders to measure the reagents.
8. Dropping pipettes with rubber bulbs of about 5 cc. capacity for drawing off serums.
9. Serologic pipettes of 1.0 cc. capacity, graduated in tenths to the tip, to measure serums, and 5-cc. or 10-cc. serologic pipettes to measure reagents.

---

[1] The reliability of this test in comparison with others is authoritatively shown in a series of papers by the American Committee on the Evaluation of Serodiagnostic Tests for Syphilis. (This committee has acted impartially. Nevertheless, its figures and statements have been subject to biased interpretations by individual writers. Therefore, a careful study of these original reports is highly desirable in selecting serologic tests for syphilis.) (1, 2, 3, 4)

All inquiries regarding this test could be addressed to Dr. W. A. Hinton, Boston Dispensary, 25 Bennet St., Boston, Mass.

10. Thick-walled Erlenmeyer flasks (5) of 125-cc. or 250-cc. capacity, with an inverted V-shaped ridge blown in the bottom, for mixing glycerinated indicator. This ridge produces two semicircular compartments, each of which holds 3 to 5 cc. These flasks (fig. 1) are not listed in any of the catalogues; ours are especially made by Macalaster Bicknell Co. of Cambridge, Mass.

PREPARATION OF STOCK SOLUTIONS

Stock indicator.—Extract dried, ground, beef-heart muscle (Bacto beef heart, dehydrated, Difco Laboratories) by putting 100 grams[2] of the powder and 400 cc. of anesthesia ether in a wide-mouthed glass-stoppered bottle and shaking thoroughly by hand for 10 minutes. Allow the bottle to stand 5 to 10 minutes so that the solid material may settle out. Then pour as much of the ether as possible through filter paper into an Erlenmeyer flask without pouring out any large quantity of the solid material. Scrape the solid material from the filter paper into the bottle for further extractions. Do not allow the main portion of extracted tissue to dry between extractions. Discard the ether filtrate in the Erlenmeyer flask. Make five separate extractions in all, using 400 cc. of fresh ether and a new filter paper for each. After the final extraction let the tissue dry on the filter paper. Obtain the net weight of this dried residue of ether-insoluble constituents. Place this dried residue in a glass-stoppered bottle with 95 percent ethyl alcohol, using 5 cc. of alcohol to each gram of residue. Extract for 3 days at room temperature (17° to 20° C.), shaking the contents of the bottle vigorously by hand for 5 minutes three times each day. Remove the solid material by filtering into a graduated cylinder; measure the alcoholic extract and transfer to a glass-stoppered bottle. Add cholesterol (using 0.4 gm. to each 100 cc.) and warm at 37° C. in an incubator or water bath, occasionally shaking, until the cholesterol has dissolved. A solution thus prepared is called stock indicator (antigen).

Reliable results can be assured only if a stock indicator prepared according to the above directions has proved to be accurate in comparative tests with an indicator that is known to be wholly satisfactory as determined by both clinical and serologic means. Materials designated for this test by the Difco Laboratories of Detroit, Mich., have met this critical requirement.

The stock indicator should not be stored in a refrigerator, for chilling will precipitate the cholesterol; if, by inadvertent chilling, the cholesterol should precipitate, it must be redissolved (by heating in a water bath at 37° C.). Stock

[2] The extraction in one operation of larger or smaller amounts of the powder has appeared to yield an inferior reagent.

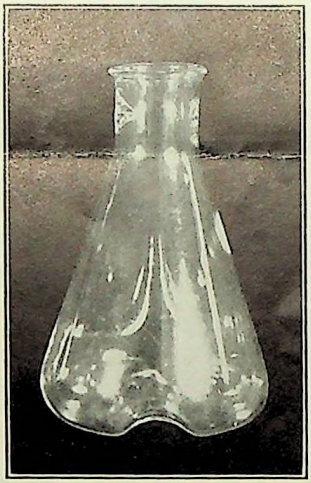

FIGURE 1.

indicator kept in colorless, glass-stoppered bottles at room temperature for more than 2 years has given as good results as that freshly prepared.

Five percent salt solution.—Prepare a 5 percent solution of sodium chloride (C. P.) in sterile distilled water, and add 1.0 gm. of salicylic acid (C. P.) to each 4,500 cc.

The salicylic acid helps to preserve the potency of glycerinated indicator (described later).

Fifty percent solution of glycerol.—Prepare by mixing equal volumes of Baker & Adamson's Glycerin (Reagent)[3] and sterile distilled water.

The 5 percent salt solution and the 50 percent glycerol solution keep indefinitely.

Glycerinated indicator.—These directions for mixing glycerinated indicator should be followed strictly. Thirty cc. is the smallest and 150 cc. the largest amount that can be prepared satisfactorily at one time. If larger quantities are desired, two or more batches should be pooled.

The technic of preparing the glycerinated indicator is as follows:

Pipette one part of cholesterinized heart extract (stock indicator) into one compartment of the Erlenmeyer flask (with the inverted V-shaped ridge), and 0.8 part of the 5 percent salt solution in the other. Use great care in pipetting the salt solution into the flask in order to avoid premature mixture of the two solutions.

Mix by shaking the flask very rapidly from side to side for one minute. Let the mixture stand exactly 5 minutes.

Without further delay add 13.2 parts of the 5 percent salt solution and again shake thoroughly.

Finally, add 15 parts of the 50 percent glycerol solution and shake until the suspension is homogeneous. This mixture is called *glycerinated indicator*. If stored in a glass-stoppered bottle and kept continuously in a refrigerator at a temperature of about 8° C., it will remain unimpaired in strength for at least a month. For this reason some laboratories may wish to prepare enough for more than a day's testing. If this is done, the stock bottle of glycerinated indicator should be well shaken and the amount required for the tests at hand should be poured into another receptacle and the stock bottle of glycerinated indicator returned immediately to the refrigerator. Exposure to ordinary room temperature for more than a few minutes may seriously decrease its potency.

This stability of the glycerinated indicator under appropriate conditions of storage has these advantages:

1. It affords an indicator at all times for rapid tests.
2. It eliminates the frequent preparation of indicator, which is helpful if only a few tests are done at a time.

PERFORMING THE TESTS

1. Centrifuge the blood, if necessary, to aid in separating the serum from its clot; with a long dropping pipette remove the serum (free

[3] This is an especially pure redistilled glycerin.

from blood cells) and deliver into an appropriately labeled serum tube.

To avoid contamination of one serum by another, after each serum has been drawn off, thoroughly rinse the dropping pipette at least three times with sterilized physiologic salt solution. To minimize bacterial contamination, after every 20 serums have been drawn off empty the washing bottle (about 200 cc. capacity) and fill it with fresh salt solution.

2. Heat the serums in the inactivating bath at 55° C. for 30 minutes. Be sure that the level of the water is above the level of every serum and that the temperature is kept at 55° C. or 56° C. throughout inactivation. Serums should not be inactivated before the day of testing. If it is necessary to retest a specimen, use serum freshly separated from the clot.

3. Select all serums that show (*a*) hemolysis, manifested by redness greater than is produced when 0.1 cc. of blood is dissolved in 3.0 cc. of distilled water; (*b*) bacterial contamination, shown by cloudiness; or (*c*) marked opacity from other causes. Place in a separate rack and test, as soon as possible, according to the rapid method (p. 22). This will avoid further deterioration, which by decreasing the sensitiveness of the test and increasing the opacity of the medium makes the test harder to read.

4. Set up the racks with one properly numbered serum tube for each remaining specimen. Tubes should be clear and clean.[4]

5. With a 1.0-cc. pipette, measure 0.5 cc. of each heated (inactivated) serum into the tube that has been labeled for it. (Use a separate pipette for each serum.)

For routine purposes 1 tube is sufficient for each test. If, however, this test is negative and there is reason to suspect syphilis, it is desirable to retest the specimen using 0.1 cc. in 1 tube and 0.5 cc. in the other. We have found that in approximately 1 syphilitic out of 200, the second tube, containing the 0.1-cc. of serum, gives a positive reaction, while the first, which contains the 0.5-cc. of serum, gives a negative reaction.

If less than 10 tests are to be made at a time, use positive and negative controls.

6. Compare the appearance of each pipetted serum with that in the tube from which it was taken. This is to make sure that there has been no error in pipetting or in labeling the tubes.

7. Not more than 30 minutes before incubation, add with a clean 10-cc. pipette 0.5 cc. of glycerinated indicator to each serum.

---

[4] To clean the tubes, rinse them thoroughly as soon after use as possible with tap water, and then fill each with a warm solution of 5 gm. of sodium hydroxide in 1,000 cc. of tap water; allow them to stay in this solution for about 2 hours and wash thoroughly with hot water to remove the alkali. This process usually removes (without the aid of a test-tube brush) any deposit which may have remained from previous use.

8. Pipette 0.5 cc. of the same indicator and 0.5 cc. of the 5-percent salt solution into an empty serum tube. This serves as a control, the use of which will be indicated later (p. 22).

9. Incline the rack to an angle of about 45° and then shake by thrusting it quickly upward and forward, then downward and backward with sufficient speed to cause the fluid to travel halfway up the tubes and make small bubbles. At least 3 minutes of shaking are required for accurate results. If there are enough tests, a shaking machine is desirable.

10. Place the rack in the Wassermann bath at 37° C. and let it remain for 16 hours (conveniently from 5 p. m. to 9 a. m.). Longer incubation makes the tests increasingly hard to read. Do not agitate the tubes before reading.

When the racks containing the tests are removed from the bath, record the readings shown by the bath thermometer as well as those of the maximum and minimum thermometer. The temperature should not fall below 34° nor rise above 39° C.

Reading the results.—The tests should be read within an hour after the incubation has ended. To read them, sit in front of a window but do not face the sunlight. (The light must be good, and for this reason suitable artificial light must be provided on dark days or at night.) In order to determine whether or not there is clearing of the fluid and a ring or band of white flakes or white coarse granules at the meniscus, lift each tube carefully from the rack and hold it at the level of the eye; slant it at an angle of about 45° and view it in the direction of a darkened background on either side of a window or of a suitably placed light. While still viewing it at the same angle, slowly roll the tube between the fingers; this will make a faint ring visible. Finally, gently shake the tube and look for flocculation, evidenced by agglutinated masses or by faint granularity.

The reactions are read and reported as positive (without indicating the intensity of the reaction), negative, doubtful, and unsatisfactory.

Such reports do not confuse physicians by implying that the intensity of the reaction is related to the clinical condition of the patient (6).

Positive reactions (recorded+).—At or a little above the level of the meniscus there is a ring or band, approximately 0.2 to 1.5 mm. wide, of white coarse granules or flakes of lipoids, slightly to moderately but not strongly adherent to the wall of the tube.[5] By gentle shaking, the ring or band is loosened and the particles scattered so that they are visible as agglutinated masses in a clear fluid or as somewhat coarse granules in a cloudy field.

[5] Hemolyzed serums that are also bacterially contaminated frequently produce a whitish ring which is strongly adherent to the tube.

Negative reactions (*recorded* −).—Usually there is at most only slight clearing but no ring, band, or floccules. However, with some indicators, the negatives may show a "scummy" nongranular deposit of lipoids on the wall of the tube extending from the meniscus downward for from 1 to 3 mm.

Doubtful reactions (*recorded* ±).—Centrifuge for 10 minutes at high speed (about 2,000 revolutions a minute) (1) those tubes which on gentle shaking showed only slight granularity beyond that observed in the control tube (containing 0.5 cc. of indicator and 0.5 cc. of the 5 percent salt solution) and (2) those tubes which showed only a slightly flaky or slightly granular ring. If, as a result of the centrifuging, there is definite clearing, and on top a thin layer of lipoids which shaking breaks up into fine flakes or coarse granules, the test is recorded as ± and reported as doubtful; if these changes have not taken place, it is reported as negative.

RAPID METHOD

This procedure may be used whenever there is urgent need for a quick report, for example, in testing donors for transfusion and as an aid in the diagnosis of suspected primary and secondary syphilis. Although this method has not been officially evaluated it is, according to our experience, from 98 percent to 99 percent as accurate as the regular method. Because of this slight margin of error, it is recommended that the results be checked by the regular method on part of the serum whenever possible. It is our custom to test all specimens selected under number 3, page 20, by the rapid method.

TECHNIC OF RAPID METHOD

Heat the serums for 3 minutes at 60° C. Pipette 0.5 cc. of the serum into a serum tube and add 0.5 cc. of the glycerinated indicator; shake according to direction 9, page 21; place in the Wassermann bath at 37° C. for 20 minutes; centrifuge at about 2,000 r. p. m. for 10 minutes; read and record as follows:

Positive (*recorded* +) if there are plainly visible flakes at the top of the fluid, and a well-marked flocculate (precipitate) is seen on shaking.

Negative (*recorded* −) if centrifuging has caused no change, provided the specimen shows no decided hemolysis (see under 3, p. 20), or no marked bacterial contamination.

Unsatisfactory if the specimen is hemolyzed or bacterially contaminated, unless the reaction is strongly positive. This interpretation is necessary, because even the moderately hemolyzed or bacterially contaminated serum of a known syphilitic usually gives a negative reaction.

DAVIES-HINTON MICROFLOCCULATION TEST[6]

In order to facilitate carrying out this test (7), specimens of blood should be collected in special rubber-capped glass collection tubes (8) which are then placed in labeled glass tubes that measure approximately 100 mm. in length and 15 mm. in diameter, such tubes being ordinarily called Wassermann tubes. The collection tubes are made of ordinary glass tubing and measure 80 mm. in length by about 2.5 mm. in inside diameter. Over each end there should be a flexible rubber cap such as is used to stopper small bottles of vaccine or serum.[7] The capacity of the tube is about 0.3 cc.

LABORATORY PROCEDURE

Number the Wassermann tubes containing the specimens and place them in a serologic rack. Take each specimen in its collection tube from the rack, remove the rubber cap from one end, and loosen the clot from the wall of the tube with the stylet of an 18-gage needle or with some other stiff wire. Replace the rubber cap on the collection tube and return it to the numbered Wassermann tube; centrifuge at high speed for 10 minutes. Examine each specimen so handled to see if the serum is clear and well separated from its clot; if the serum is not clear, recentrifuge for another 10 minutes. If this still does not clear it, make a note of the condition of the serum. Place the rack containing the clear specimens (clot downwards) in a serologic water bath kept at 56° C., preferably for 30 minutes, or if in a hurry 20 minutes, having first filled the Wassermann tubes with water from the water bath. Next, remove the rack from the water bath, pour the water from the tubes, remove the cap from the serum end of each collection tube and notch it just above the junction of clot and serum with a file. Hold the collection tube horizontally, break it, and discard the part of the tube that contains the clot. Proceed with the part containing the clear serum as follows:

1. Drain into another glass collection tube enough serum so that the length of the column of serum is about 2.5 cm., and drain into a second similar glass tube enough serum so that the column is 0.5 cm. to 1.0 cm. long.

2. With a capillary pipette, add to the first tube glycerinated Hinton indicator equal to the amount of serum in the tube as estimated by the combined length of the column of serum and indicator.

---

[6] This test in its single official evaluation (2) in 1936 stood highest, and when done for the sake of comparison in our own laboratory in the 1937–38 evaluation, it was 100 percent specific and 0.5 percent more sensitive than the regular Hinton test.

[7] These rubber caps may be obtained from the West Company, 1117 Shackamaxon St., Philadelphia, Pa. Specify No. 3 vial stoppers in No. 68 stock.

Care should be taken not to allow air to separate the serum and Hinton indicator, which would prevent mixing.

The capillary pipette consists of a piece of glass tubing about 10 or 12 cm. long and 1 cm. in diameter. One end is tapered to a capillary tip about 1 mm. or less in diameter; the other end is capped with a rubber bulb. Provided no serum is drawn into the capillary pipette, it need not be rinsed between specimens.

3. To the second tube, with the same capillary pipette, add diluted Hinton indicator equal to 5 times the amount of the serum as estimated by the combined length of the column of serum and indicator.

4. Mix the serum and the Hinton indicator in each tube by tilting the liquid toward alternate ends of the tube 10 ~~or 15~~ times.

5. Cap both ends of the two collection tubes, return one to the original numbered Wassermann tube and the other to a Wassermann tube correspondingly numbered, and place the rack in a 37° C. serologic bath for 16 hours, having first filled the Wassermann tubes with water from the bath.

A beaker of water, maintained at 37° C. in a bacteriologic incubator, may be used instead of the serologic bath. If speed is desired, the tests may be incubated for only 30 minutes without apparent loss of accuracy.

6. Remove the rack from the water bath and centrifuge the tubes for 5 minutes at approximately 2,000 revolutions per minute.

7. Read the results with a low-power objective of the microscope. The light should be cut down by lowering the condenser so that aggregates at the meniscus can be seen readily. Moreover, the stage of the microscope should be tilted at an angle of about 30° from the horizontal and the tube placed under the lens so that the meniscus is uppermost. Readings are designated as follows:

Positive, if there are definite, discrete, compact clumps at the meniscus in *either* tube. Gentle thumping of the tube may help float the clumps into view.

Doubtful, if there are a few small clumps at the meniscus. In such cases, the clumps should be broken up by thumping the tube with a finger and the tube recentrifuged for 3 minutes. The test is reported "doubtful" if small clumps are again visible at the meniscus and "positive" if large, compact clumps are present in either tube.

Negative, if there are no clumps at all. Amorphous, cloudy, granular material at the meniscus should be interpreted as negative.

Unsatisfactory, if badly hemolyzed or contaminated specimens give no marked flocculation in either tube.

The technic described above requires about 0.1 cc. of serum; smaller amounts of serum (0.05 cc. or more) may be handled in the same way in capillary tubes[7]

[7] Capillary tubes of these specifications may be obtained from Friedrich & Dimmock, Millville, N. J.

measuring 10 or 11 cm. in length and 1.25 to 1.5 mm. in inside diameter, except that the tubes are sealed with a small gas flame instead of rubber caps.

DAVIES-HINTON FLOCCULATION TEST OF CEREBROSPINAL FLUID

Although this test has not been officially evaluated, it has, in our experience, proved to be far easier to perform and more efficient than the Wassermann (*10*) and those flocculation tests with which we have compared it. This test is now being used by the Massachusetts Department of Public Health.

Specimens of spinal fluid for this test should not be cloudy because of bacterial contamination, which is likely to give falsely negative tests, nor admixed with more than a trace of blood, because in rare instances an excess of blood can cause a false positive reaction. These facts must be borne in mind when interpreting the results, for even if the specimen has been cleared by centrifuging, these statements still hold.

The test requires the following materials:

1. *Glycerinated Hinton indicator* (see p. 19), which may be made in sufficient quantity to last a month, provided it is stored in the refrigerator at a temperature of 8° to 10° C.

2. *Hinton-negative human serum*, which may be obtained by pooling serums that remain after performing the routine serologic tests. Select for this pooling only Hinton-negative serums that are clear (without hemolysis) and that have been inactivated at 55° C. for 30 minutes. The rather remote possibility of a zonal effect may be ruled out by testing the pooled serum in the following amounts:

Tube 1.—0.5 cc. serum and 0.5 cc. Hinton indicator.

Tube 2.—0.1 cc. serum and 0.5 cc. Hinton indicator.

Tube 3.—0.1 cc. serum and 1.0 cc. Hinton indicator.

Tube 4.—0.1 cc. serum and 2.0 cc. Hinton indicator.

Do a "rapid test," which consists in shaking the four tubes containing the serum and Hinton indicator for 5 minutes, placing them in a serologic bath at 37° C. for 30 minutes, centrifuging them for 10 minutes at high speed (about 2,000 revolutions per minute) and then reading the results. If all the tubes are negative, as shown by the absence of floccules, the serum is suitable for testing spinal fluid. The pooled serum should then be passed through a Berkefeld "N" filter and collected under sterile conditions in rubber-stoppered bottles, so that each contains not more than 3 days' supply. These should be kept in a refrigerator at 8° to 10° C. Pooled serum should be kept no longer than 3 weeks and should be thrown away sooner if it becomes cloudy. Old serum is likely to give falsely negative reactions.

Laboratories that test only a few spinal fluids at a time may find it more convenient to prepare only enough serum for the day by select-

ing one or two clear Hinton-negative serums of that day and retesting by the "rapid method" in the four amounts as indicated above.

3. *Gum acacia, 20 percent*, which is prepared by diluting two parts of 30 percent gum acacia[8] with one part of physiologic salt solution. This solution of acacia should be kept in a refrigerator and discarded when it becomes cloudy. In order to avoid accidental contamination of a large amount of the gum acacia, 5 to 10 cc. lots (sufficient for not more than 1 week's testing) should be put into small bottles or test tubes, autoclaved at 15 pounds pressure for 15 minutes, and then rubber-stoppered with sterile precautions. A preliminary test should be made on a sample of the lot, as follows:

Tube 1.—0.6 cc. of physiologic salt solution, plus 0.1 cc. of 20 percent gum acacia, plus 0.2 cc. of Hinton indicator.

Tube 2.—0.6 cc. of physiologic salt solution, plus 0.1 cc. of 20 percent gum acacia, plus 0.6 cc. of Hinton indicator.

Mix well by shaking with the hand, incubate in a serologic bath at 37° C. for 30 minutes, centrifuge at approximately 2,000 revolutions per minute for 10 minutes, and read. If either of the above tests is positive, as shown by the formation of floccules, the entire lot of gum acacia should be discarded.

Performing the tests.—Mix the 20-percent gum acacia with the previously tested Hinton-negative human serum in equal parts; for example, if 10 spinal fluids are to be examined, 3 cc. of the serum and 3 cc. of the 20-percent gum acacia mixed together will suffice for these specimens.

Before setting up the tests proper, perform a "rapid test," as follows:

Tube 1.—0.6 cc. of physiologic salt solution, plus 0.2 cc. of the freshly mixed acacia serum, plus 0.2 cc. of Hinton indicator.

Tube 2.—0.6 cc. of physiologic salt solution, plus 0.2 cc. of the freshly mixed acacia serum, plus 0.6 cc. of Hinton indicator.

Shake the tubes thoroughly with the hand, incubate in a serologic bath at 37° C. for 30 minutes, centrifuge at high speed (approximately 2,000 revolutions per minute) for 5 minutes, and note the results. If floccules are present in either of the tubes, the mixture is unsuitable for use and the ingredients thereof should then be investigated separately. On the other hand, if both tubes show no floccules, one may proceed with the test, as follows:

In a suitable rack set up two tubes (one behind the other), measuring 11 mm. by 100 mm. for each spinal fluid, and two tubes for controls. Label each tube.

---

[8] The gum acacia (containing 4.5 percent of sodium chloride) may be purchased from the Eli Lilly Co. in 100-cc. ampules.

1. Pipette 0.6 cc. of the first spinal fluid into the first tube of the first row and the same amount of it into the corresponding tube of the second, and continue in this way with each specimen of spinal fluid. Pipette 0.6 cc. of physiologic salt solution into each control tube.

2. Pipette 0.2 cc. of the acacia-serum mixture into each tube of the first row (including the control) and then pipette 0.2 cc. of Hinton indicator into these same tubes.

3. Into each tube of the second row, including the control, pipette 0.2 cc. of the acacia-serum mixture and then 0.6 cc. of Hinton indicator.

4. Thoroughly and vigorously, either by hand or with a shaking machine, shake the rack containing the tests so that the contents become completely homogenous.

5. Incubate the tests in a serologic bath at 37° C. for 16 hours, taking care that the water level is slightly above that of the contents of the tubes.

6. Centrifuge all of the tubes including the controls for 5 minutes at approximately 2,000 revolutions per minute; then read first the controls and then the tests in front of a window or a suitable artificial light. Hold the tube at the top with one hand and tap near the bottom with a finger of the other hand. By such a procedure any floccules at the meniscus are dispersed downward and are easily visible. If the controls show any floccules at all, all of the tests should be declared unsatisfactory; otherwise, report the results as follows:

Positive, if in *either* tube for a given specimen there are definitely visible floccules. Positive tubes should not be centrifuged a second time, because this may change the reaction to negative on truly positive specimens; but all others should be centrifuged twice. Why this is necessary we do not know.

Doubtful, if either of the tubes shows questionable flocculation which centrifuging a second 5 minutes does not amplify.

If the original ground-glass appearance persists in *both* tubes of a given specimen, they should be centrifuged a second time for 5 minutes, read again and reported as *negative* if the ground-glass appearance persists; otherwise, the result should be reported as positive or doubtful, depending upon the visibility of the floccules.

Unsatisfactory, if the tubes containing spinal fluid that was originally turbid from bacterial contamination show no clearly visible particles but a cloudiness that is distinctly greater than that in the control tube; or if the test is positive in the presence of contamination with more than a trace of blood.

219231°—10——3

REFERENCES

(1) Cumming, H. S., and others: The evaluation of serodiagnostic tests for syphilis in the United States: Report of results. Ven. Dis. Inform., 1935, 16: 189.

(2) Parran, Thomas, and others: The efficiency of State and local laboratories in the performance of serodiagnostic tests for syphilis. Ven. Dis. Inform., 1937, 18: 4.

(3) ——— A comparative study of serodiagnostic tests for syphilis as performed by 39 State laboratories. Ven. Dis. Inform., 1937, 18: 273.

(4) Hinton, W. A.: The Hinton test for syphilis. Third modification. J. Lab. & Clin. Med., 1932, 18: 198.

(5) ——— A flask for diluting antigens used in serum tests for syphilis. Am. J. Clin. Path., 1933, 3: 41.

(6) ——— Syphilis and its treatment. New York, the Macmillan Co., 1936, p. 28.

(7) Davies, J. A. V.: A microflocculation test for syphilis. J. Lab. & Clin. Med., 1937, 22: 959; Ven. Dis. Inform., 1937, 18: 187.

(8) ——— A method of collecting small blood specimens with special reference to microflocculation tests for syphilis. J. Lab. & Clin. Med. 1938, 23: 1206.

(9) ——— A modification of the Hinton test applied to spinal fluid. Am. J. Clin. Path., 1937, 7: 240.

(10) Marquis. Am. J. Syph., Gonor. & Ven. Dis., 1939, 23: 738.