[illegible]-HINTON FLOCCULATION TEST*

In order to facilitate carrying out this test[7], specimens of blood should be collected in special rubber-stopped glass collection tubes[8] which are then placed in labelled 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 60 mm. in length by about 4.5 mm. in inside diameter. Over each end is a flexible rubber cap such as is used to stopper small bottles of vaccine or serum.** The capacity of the tube is about 0.3 cc.

there should be

Laboratory procedure. -- Number the Wassermann tubes containing the specimens and place them in a suitable 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-gauge 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 ten 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 ten minutes. If this still does not clear it, note should be made of the condition of the serum

Place the rack containing the clear specimens (clot downward) in a suitable serologic water bath preferably for thirty minutes, or if in a hurry twenty minutes, at 56° C., having first filled the Wassermann tube with water from the water bath. Next, remove the rack from the

---

* This test in its single official evaluation[2] in 1936 stood ignored, and when done for the sake of comparison in our own laboratory in the 1937-38 evaluation, it was 100 per cent specific and 0.6 per cent more sensitive than the regular Hinton test.

** These rubber caps may be obtained from the Best Company, 1117 Buchanan St., Philadelphia, Pa. Specify #2 vial stoppers in #68 stock.

water bath, pour the water off 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. Handle the part with the clear serum as follows:

1. Drain into one glass tube (similar to the collection tube described above) enough serum so that the length of the column of serum is about 1.5 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.

> 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 xxxx into the capillary pipette, it need not be rinsed between specimens. Care should be taken not to allow air to separate the serum and Hinton indicator, which would prevent mixing.

3. To the second tube, with the same capillary pipette, add diluted Hinton indicator equal to five 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 ten or fifteen 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 sixteen 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 ~~xxxxxxxxxxxxxxx~~ thirty minutes ~~xxxx~~ without apparent loss of accuracy.

6. Remove the rack from the water bath and centrifuge the tubes for five minutes at approximately 2,500 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 of the sediment 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 three 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 ~~xxxxxx~~ 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* measuring 10 or 11 cm. in length ~~xxxxxxxxxxxxxxxxxxxxxxxxxxx~~ and 1.25 to 1.5 m.m. in inside diameter, except that the tubes are sealed with a small gas flame instead of rubber caps.

---

* Capillary tubes of these specifications may be obtained from Fried & Limbock, Millville, N. J.

LaVilla-Hinton Flocculation Test on 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 falsely 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 page 4) which may be made in sufficient quantity to last a month, provided it is stored in the refrigerator at a temperature of 6° 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 thirty 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 five minutes, placing them in a serologic bath at 37° C. for 10 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 three days' supply. These should be kept in a refrigerator at 8° to 10° C. Pooled serum should be kept no longer than three 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 selecting 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 per cent, which is prepared by diluting two parts of 30 per cent gum acacia,* 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 one 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:

---

* The gum acacia (containing 4.5 per cent of sodium chloride) may be purchased from the Eli Lilly Company in 100 cc. ampules.

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

Tube 2. 0.6 cc. of physiologic salt solution, plus 0.1 cc. of 20% 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 thirty minutes, centrifuge at approximately 2,000 revolutions per minute for ten 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 per cent gum acacia with the previously tested Hinton-negative human serum in equal parts; for example, if ten spinal fluids are to be examined, 3.0 cc. of the serum and 3.0 cc. of the 20 per cent 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 0.1 cc. of the freshly mixed acacia-serum, plus 0.1 cc. of Hinton indicator.

Tube 2. - 0.6 cc. of physiologic salt solution, plus 0.1 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 thirty minutes, centrifuge at high speed (approximately 2,000 revolutions per minute) for five 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. x 100 mm. for each spinal fluid, and two tubes for controls. Label each tube.

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 specific-serum mixture into each tube of the first row (include 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 specific-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 homogeneous.

5. Incubate the tests in a serologic bath at 37° C. for sixteen 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 five 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 top near the bottom with a finger of the other hand. By such a procedure, any flocculen at the meniscus are dispersed downward and are easily visible. If the controls show any flocculen 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, and why this is necessary we do not know.

Doubtful, if either of the tubes shows questionable flocculation which centrifuging a second five 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 five 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.