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TECHNIQUE OF DAVIES-HINTON FLOCCULATION [1] 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 and the flocculation tests with which we have compared it. At present it is being used by the Maassachusetts 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 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, which may be made in sufficient quantity to last a month, provided it is stored in the refrigerator at a temperature of 8° - 10° C.

2. 20% gum acacia, which is prepared by diluting two parts of 30% gum acacia,* with one part of physiologic salt solution. This should be kept in a refrigerator and discarded when it becomes cloudy.

3. 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 which are clear (without hemolysis) and which have been inactivated at 55° C. for 30 minutes. The rather remote possibility of a zonal effect

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*The gum acacia (containing 4.5% of sodium chloride) may be purchased from the Eli Lilly Co. in 100 cc. ampoules.

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

If all of these tubes show a negative Hinton test, the pooled serum is suitable for use and should 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 tests.

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 of the clearer Hinton-negative serums of that day and re-testing by the "rapid" method in the four amounts indicated above. The rapid method consists in shaking the four tubes containing the serum and Hinton indicator for three minutes, then placing them in a serologic bath at 37° C for 30 minutes, centrifuging them for ten minutes at high speed (about 2000 revolutions per minute) and then reading the results. If all the tubes are negative, the serum is suitable for testing spinal fluid.

PROCEDURE

In a suitable rack set up two tubes (one behind the other), measuring 10 mm. x 100 mm., for each spinal fluid, and two tubes for controls. Label each tube.

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.

Mix the 20% gum acacia with the previously tested clear Hinton-negative human serum in equal parts; for example, if ten spinal fluids are to be examined, 1.5 cc. of the serum and 1.5 cc. of the 20% gum acacia mixed together will be enough to make up for these specimens. Pipette 0.2 cc. of this mixture into each tube of the first row (include control) and then pipette 0.2 cc. of Hinton indicator into these same tubes. If several spinal fluids are to be examined at one time, the freshly mixed acacia-serum mixture may be added to an equal amount of Hinton indicator just prior to use, and 0.4 cc. of this mixture pipetted into each tube of the first row.

Into each tube of the second row, including the control, pipette 0.2 cc. of the acacia-serum mixture and 0.6 cc. of Hinton indicator. If so desired, one part of the acacia-serum mixture may be added to three parts of Hinton indicator just prior to use, and 0.8 cc. of this mixture measured into each tube of the second row.

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

Incubate in a water bath at 37° C. for 16 hours, taking care that the water level is slightly above that of the contents of the tubes. Do not allow water to drop into the tubes.

Centrifuge at approximately 2000 revolutions per minute; then read the tests in front of a window or other 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.

Report the tests as follows:

Positive, if in either tube there is flocculation that is definitely visible. Positive tests should not be centrifuged a second time, because this may change the reaction to negative on truly positive specimens.

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

Negative, if the original ground-glass appearance persists in both tubes. Each negative test should be centrifuged a second time for five minutes, read again and reported as negative if the ground-glass appearance persists; otherwise it 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 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.

REFERENCES

1. A Modification of the Hinton Test Applied to Spinal Fluid, J. A. V. Davies, Am. Jour. Clin. Path., Vol. 7, No. 3, p. 240, May, 1937.

J. A. V.Davies, Boston

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