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Kinton tests

U.S.P.H.S.

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RECENT SIGNIFICANT CHANGES IN THE TECHNIQUE OF THE KAHN FLOCCULATION TEST FOR SYPHILIS

In 1927 we described a glycerol cholesterol flocculation test for syphilis based on our observation that Wassermann positive serums completely agglutinated particles of cholesterol suspended in a glycerinated hyper-tonic saline solution which contained the merest trace of beef muscle extract, while Wassermann-negative serums left such a suspension visibly unchanged.[1] Briefly, the test was carried out as follows: first, an indicator (commonly called antigen) was prepared by adding a very small amount of the ether-insoluble, alcohol-soluble fraction of beefsteak muscle to a 0.7% solution of cholesterol in absolute alcohol. One part of this solution was mixed with fourteen parts of a 5% solution of sodium chloride, and then fifteen parts of a 30% solution of glycerol were added. Five-tenths cc. of the resulting cloudy mixture, which we called glycerinated indicator, was then added to 0.1, 0.2, and 0.3 cc. of inactivated serum. The tests were shaken thoroughly, placed for twelve to eighteen hours in a serologic bath maintained at a temperature of about 37° C, and the results were then read as follows: positive, if the tubes showed a few large or many fine clumps of the agglutinated cholesterol suspended in a water-clear solution; negative, if the original turbidity remained.

Since this first description, the test has been modified several times. The earlier changes increased its accuracy and facility, and the later modifications widened the scope of its usefulness.

The first changes were described in 1928.[2] They consisted in raising the temperature of incubation from 37° C to 39° C and in adjusting the indicator or antigen by a relatively simple but somewhat troublesome method of titration. The chief reason for raising

the temperature was that 27° C is a hard temperature to maintain during the summer months or in excessively warm climates.

Even then, the test presented difficulties that decreased its accuracy. Accordingly, a careful study was made to determine the efficacy of approximately 150 lipoidal extracts in the preparation of a better glycerinated indicator. This led to the opinion that beef heart muscle gave the best tests not only because it seemed more sensitive, but also because it afforded better criteria by which to judge a positive reaction, the most important of which is a characteristic ring at the meniscus of the test. This resulted in the so-called second modification which utilized a set-up of four tubes, two of which contained the muscle extract and two the heart extract as a supplementary procedure.[3] It was soon found that the use of heart extract definitely resulted in more accurate tests, and xxxx eliminated xxxxxx the troublesome feature of adjusting the indicator, i. e., titrating the antigen. xxxxxxxxxxxxxxxxxxx Because of the comparison afforded by this dual set-up, a third modification was described in which the technique was reduced to its present and simplest form,[4] -- only one tube containing a glycerinated indicator prepared from beef heart muscle was set up for each serum, instead of three or four; centrifuging those cases which were doubtfully positive was resorted to as an additional aid in deciding between a positive, negative or doubtful test. Moreover, in order that this indicator would give identical results when mixed by different persons, special Erlenmeyer designed. blown flasks were xxxxxx These flasks have an inverted V-shaped ridge in the bottom, which makes it possible to control the rate of diluting the indicator. They may be obtained in xxxxxxx suitable capacities for the preparation of specific amounts of the glycerinated indicator.

This third modification is the Hinton test which was evaluated by the American Committee on the Evaluation of Serodiagnostic Tests for Syphilis and the one which is recommended for general use as a Hinton Test.

Subsequent changes have not been made to replace this third modification but they have been made for special problems in serology and are in the nature of supplementary adaptations to increase the scope of its usefulness. Indeed, the first of these was described in the third modification as the "quick method".[4] Briefly this consists in mixing 0.5 cc. of glycerinated indicator with 0.5 cc. of serum that has been inactivated for half an hour, shaking the mixture for three minutes, then incubating for fifteen minutes at 37° C, instead of sixteen hours as in the regular method, centrifuging for an additional fifteen minutes and reading the result: positive if there is slight to complete clearing of the mixture and the presence of agglutinated masses or a granular flocculate, and negative if there is no change. This quick method was greatly facilitated by certain special characteristics of the Hinton indicator, the most important of which is that the glycerinated indicator (antigen emulsion) retains its potency for at least eight weeks if kept at a temperature of 8° C (electric refrigeration). It is customary with some of our laboratories to prepare the glycerinated indicator only about once each month. This makes it possible to do emergency testing with a dependable reagent at any time during the day or night without the necessity of running controls. Such a facility is quite desirable in testing donors for blood transfusion and in the examination of clinic patients suspected of having primary or secondary syphilis. In our experience, the accuracy of the quick method is about 95% that of the regular method, but this has not been determined by official evaluation.

The two most important changes designed to broaden the usefulness of the test were made almost simultaneously in 1937 by Davies. I shall briefly consider first his micro-flocculation test,[5] which can be accurately done with even somewhat less than 0.3 cc. of blood in contrast to the regular method which requires at least 1.5 cc. To facilitate the execution of this test, he uses special rubber-capped glass collection tubes into which the blood is drawn from the patient. He uses similar tubes instead of test tubes in carrying out the test xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx, or if only .05 cc. of serum can be recovered, he uses small capillary tubes without impairing the accuracy of the results. He xxx reads the tests with a microscope. This test, the importance of which is already manifest in our own State work, affords a reliable method of testing the blood of infants and others from whom it is impossible to get more than a few drops of blood. Despite this great advantage it may not appeal to the technician as much as it should, because it requires some special technical skill and more time in its execution. The reliability of this method has already been shown in one official evaluation.

Davies' other modification xxxxxxxxxxxxxxxxxxxxxxxxxxxxxx ed the of the Hinton test ix extendxxx iii usefulness to the detection of syphilitic spinal fluid.[6] To carry out this test he merely adds a mixture of equal parts of 20% gum acacia and Hinton-negative human serum to the spinal fluid and then the Hinton indicator. Although xxxx the of this test efficiency has not been determined by an official evaluation, demonstrated its reliability has been xxxxxx in a number of large hospitals in Massachusetts. In an unpublished statistical study by

Marquis of the Peter Bent Brigham Hospital in Boston, it was found to be slightly more sensitive than the Wassermann test and definitely more specific.

This in brief is an outline of the changes in technique which have taken place in the evolution of the Kahn test up to this time. Its comparative efficiency in our own hands and in the hands of other workers may be found in a series of authoritative articles[7] published in Venereal Disease Information by the Committee on the Evaluation of Serodiagnostic Tests for Syphilis.

References

1. A Glycerol-Cholesterol Precipitation Reaction in Syphilis, W. A. Hinton, Boston Med. & Surg. Jour., June, 1917.
2. The Hinton Glycerol Cholesterol Agglutination Reaction, Modifications in Technique (Second Communication), W. A. Hinton and G. G. Stuart, Jour. of Lab. & Clin. Med., St. Louis, April, 1919.
3. The Hinton Glycerol Cholesterol Reaction for Syphilis, Second Modification, W. A. Hinton and A. Bern, New England Jour. of Med., May, 1926.
4. Hinton Test for Syphilis, Third Modification, W. A. Hinton, Jour. of Lab. & Clin. Med., St. Louis, Nov., 1932.
5. A Microflocculation Test for Syphilis, John A. V. Davies, Jour. of Lab. & Clin. Med., June, 1937.
6. A Modification of the Hinton Test Applied to Spinal Fluid, John A. V. Davies, Am. Jour. of Clin. Path., May, 1937.
7. First Evaluation: Evaluation of Serodiagnostic Tests for Syphilis in the U.S., Report of Results; Ven. Dis. Inf., Vol. 16, No. 6, June, 1935; Second Evaluation: Efficiency of State and Local Laboratories in the Performance of Serodiagnostic Tests for Syphilis, Ven. Dis. Inf., Vol. 18, No. 1; Third Evaluation: A Comparative Study of Serodiagnostic Tests for Syphilis as Performed by 50 State Laboratories in the U. S., Ven.Dis. Inf., Vol. 18, No. 6, Aug. 1937.