8-4

HINTON TEST FOR SYPHILIS
(Third Modification)

Increasing emphasis placed upon serum-tests in the control of syphilis demands continuous research to improve them. In the course of such investigations, it was necessary to make a third modification of the Hinton Test for syphilis. Though, in its modified form, as presented here, the test has been greatly simplified, it none the less requires great precision in its execution because, as is true of any sensitive method, consistent and accurate results can not be obtained if minor variations in technique are allowed to creep into the routine.

EQUIPMENT

(1) Test tube racks holding 20 to 160 tubes. To simplify the numbering and pipetting of the tests, these racks should be constructed to hold ten or twenty tubes in a row.

(2) Serum tubes 10 mm. inside diameter and 100 mm. long. The diameter of the tubes should be approximately uniform.

(3) A water bath for heating serums, kept at 55°C.

not be stored in a refrigerator, for chilling will precipitate the cholesterol. If, by inadvertent chilling, the cholesterol should precipitate, it must be redissolved before use.)

Such cholesterinized heart extracts have been kept in our laboratories in colorless glass-stoppered bottles, at room temperature, for a period of more than two years.

Cholesterol obtained from Merck and from the Digestive Ferments Company have given identical results when compared. All of the many cholesterinized heart extracts prepared in accordance with these directions have given almost precisely the same results.

(2) A 5% solution of sodium chloride (C.P.) in sterile distilled water, to which 1.0 gm. of salicylic acid should be added to each 3000 cc. Hereafter, a solution so prepared will be referred to as 5% salt solution. The salicylic acid not only helps to preserve the potency of glycerinated indicator (described later), but also minimizes slight changes which occur with some serums when the test is negative.

(3) A 50% solution of glycerol, prepared by mixing equal volumes of the glycerol (C.P.) and distilled water.

Several liters of the 5% sodium chloride solution and the 50% glycerol solution may be made up at a time.

TECHNIQUE OF THE TEST

(1) With a long dropping pipette, remove each serum from its clot and deliver into an appropriately labelled serum tube. This dropping pipette should be <u>thoroughly</u> rinsed at least three times with sterilized physiological salt solution after each serum, to avoid contamination of one serum with

another, and to minimize bacterial contamination which will occur if the salt solution is not sterile and is not frequently changed.

(2) Heat the serums in the inactivating bath at 55°C. for one-half hour. Be sure that the level of the water in the bath is above every serum.

> Extreme care should be taken to keep the water at 55°C. throughout the entire period of inactivation, as erroneous results may be obtained when the temperature falls even one or two degrees below this point. Inactivation at a temperature above 56°C. decreases the strength of the reactions. It is preferable to inactivate serums just before testing, as serums which give strongly positive reactions when tested a few hours after removal from the clot occasionally give negative or weak reactions when kept for even as short a time as a day.
>
> Should it be necessary to retest a specimen, always use serum freshly separated from the clot, rather than that which has been inactivated 24 hours previously.

(3) Set up the racks with one properly numbered serum tube (10 mm. X 100 mm.) for each specimen to be tested. Be sure that the tubes are clear and clean.

> To clean the tubes, fill them with a warm solution of 5 grams of sodium hydroxide in 1000 cc. of tap water; allow to stand for about two hours, and wash <u>thoroughly</u> with hot water to remove the alkali. This process completely removes any deposit of cholesterol which may have remained from previous use. (Boiling in this caustic solution corrodes the tubes.) Sterilize in a hot-air oven. This will minimize the possibility of bacterial growth, which may occur because of prolonged incubation of the test.

the time of reading, the reaction is designated as unsatisfactory, provided it has neither the characteristic ring or band, nor the visible precipitate of a positive reaction. It is important to remember that the serum of a known syphilitic, if showing even moderate hemolysis, will very often give a negative reaction.

Doubtful reactions (recorded ±)

The slightest granularity seen on shaking, beyond that observed in the control tube (containing 0.5 cc. of indicator and 0.5 cc. of the 5% salt solution), or the slightest flaky or granular ring, is to be viewed with suspicion. In such cases, centrifuge for 10 minutes, at high speed, the tubes* which show the suspicious reactions. Centrifuging causes the particles of an indefinite precipitate to cohere so as to be easily visible. The reaction is considered doubtful if at the meniscus a thin film of lipoids is formed which, on shaking, breaks up into fine flakes or coarse granules that persist as a precipitate even after vigorous shaking. When centrifuging does not markedly intensify the precipitate, the reaction is called negative.

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*Also centrifuge all tubes having serums that are somewhat opaque in themselves, but are not opaque because of bacterial contamination or hemolysis, since opacity may mask a doubtful reaction. It is important to restrict the use of the centrifuge for this condition--otherwise doubtful reactions will very frequently occur with the serums of non-syphilitic persons.

A QUICK METHOD THAT DETECTS MOST OF THE POSITIVE REACTIONS

Whenever there is immediate need for a report, the tests in question may be incubated in the water bath for one hour, at 37°C., or for two hours in the incubator, at the same temperature, and then centrifuged at high speed for ten minutes. The presence of a definite precipitate visible on shaking indicates a probable positive reaction. A negative reaction so obtained is not conclusive: The tests in question must be returned to the water bath for an additional 15 hours, or to the incubator for 18 hours, after which the reading and the interpretation are made as if the test had been conducted in the routine manner.

SIGNIFICANCE OF THE TEST

A positive Hinton reaction almost always indicates syphilis. Nevertheless, a single positive reaction must not be the basis for a diagnosis of syphilis unless supported by definite clinical evidence of the disease. Whenever a single positive reaction is unaccompanied by definite clinical signs of syphilis, checks should be made by tests on at least two additional specimens

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appear, however, there is a question as to the significance of this unyielding ("persistent") positive Hinton reaction. I do not feel that the test has been in use long enough to offer an answer to this question. I do believe, however, that continuous clinical observation is necessary in all such cases, and that most of them should have some type of antisyphilitic treatment as well. My own experience, as well as that of others, shows that the positive Hinton reaction almost always persists a third longer than the Wassermann, and in many instances more than twice as long. Inasmuch as negative Wassermann reactions often occur prematurely during treatment, as demonstrated by frequent serologic or clinical relapses, or both, the persistence of a positive Hinton reaction would indicate a superiority of the Hinton test as an aid in