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PROCESS OF PRODUCING TEST FLUID FOR THE PROCEDURE OF TEST FOR SYPHILIS

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4 Claims. (Cl. 167—78)

This invention relates to a process of producing test fluid for the procedure of test for syphilis.

The world-famed test for syphilis introduced by Wassermann is a very elaborate and complicated procedure, requiring enormous amount of energy and accuracy of skill of those who are engaged in its performance, and moreover the result of test can be read only after the lapse of about 24 hours.

In order to overcome this difficulty, its substitute in the form of precipitation test has been introduced, of which the most well known are those devised by Meinicke, Sachs-Georgi, Kahn, and Murata, etc. Some workers, however, are inclined to think that these tests are perhaps not as specific and accurate as the Wassermann reaction. In these precipitation tests, the test can be completed after the lapse of at least 30 minutes, and usually requires 2 to 3 hours. Moreover, in any of the heretofore practised tests for syphilis, comparatively large amount of blood must be taken from the patient, and it must be separated into serum.

This invention has for its object to produce a new test fluid by which the procedure of test for syphilis can be extremely easily effected even by those unskilled in the art. The special advantages of this test are that with only one drop of blood and a simple technique, with a little practice, it can be made within a few minutes' time in one's own examining room. The result can be read clearly by the coloring. The accuracy of this test compares very well with those of Wassermann, Meinicke, Sachs-Georgi, Kahn, Murata, etc., and it can also be performed with blood serum, spinal fluid, or serous exudate obtained from vesicles.

The process according to this invention consists in first obtaining a mixture of alcohol and animal's heart muscle or other muscle in a finely divided condition, maintaining said mixture at temperature of from 40° to 75° C. or more for a comparatively long period of time, for instance about two weeks or more, in a hot water bath, then after cooling adding thereto cholesterin, and further adding thereto a bluish coloring matter, for instance, crystal violet or its group, Azure II or its group, methylene blue or its group, etc., said coloring substances being used severally or in admixture.

The mode of carrying out this invention will now be described more in detail. Heart muscle obtained from an animal, for instance beef heart, is ground several times by means of an ordinary meat grinder, after removing the fat, fibrous tissue, and blood vessels. The ground muscle is then treated by 95% alcohol. A suitable container, with a tightly fitting cork stopper, containing the muscle and alcohol in the proportion of 20 grams to 100 c. c. is placed in a hot water bath maintained at temperature of from 40° to 75° C., preferably of 60° C., and kept therein at least for two or three weeks, and preferably for seven to ten weeks or more. During this period the contents should be shaken regularly—once in the morning and again in the evening every day. Then, the contents are cooled, filtered, and kept in a dark place as stock solution.

To 100 c. c. of the stock solution prepared as above, 0.2 gram of cholesterin is added and dissolved by shaking in a hot water bath (56° C.) for about 10 minutes. For convenience, this is called "Fluid No. 1".

With another portion of the stock solution a 5% solution of gum benzoin is made. This is called "Fluid No. 2".

Now, prepare a 1% alcoholic solution of crystal violet. This is called "Fluid No. 3".

Similarly prepare a 1% alcoholic solution of Azure II. This is called "Fluid No. 4".

To 100 c. c. of fluid No. 1 are added 5 c. c. of fluid No. 2, and they are mixed thoroughly.

1 c. c. of fluid No. 3 and 1 c. c. of fluid No. 4 are mixed well together, and 1.2 c. c. of this mixture are added to the above-mentioned mixture of fluid No. 1 and fluid No. 2. The purplish blue fluid thus obtained is the finished product, and is now ready to be used for the procedure of test for syphilis.

It is to be understood that heart muscle obtained from any other animal, such as horse, rabbit, ram, guinea pig, etc., may be conveniently used. The amount of the dyes used may be varied according to their effectiveness, and any other coloring matter may be used instead of or in addition to the above-mentioned coloring matter.

The product of this invention is used in the procedure of the test for syphilis in the following manner:—

A drop (about 0.03 c. c.) of the blood to be tested is obtained from the patient's finger tip or the lobe of his ear and is placed on the concavity of a so-called hollow slide somewhat similar to that commonly used in making hanging-drop preparation but with a bigger sized concavity. A drop (0.05 c. c.) of 3.5% sodium chloride solution is added before the blood has clotted, and is stirred with a corner of an ordinary glass slide. To this is now added a drop (0.03 c. c.)

of the diluted test fluid produced according to this invention. 1 part of the test fluid diluted with 3 parts of 2.5% sodium chloride solution will give the best result. After shaking for about 3 minutes, the slide is now examined under the microscope with magnification of about 50X. The positive reaction is evidenced by the appearance of beautifully purplish blue colored clumps amongst the red corpuscles in the field, while if the reaction is negative no such changes occur and the red blood cells alone can be recognized. If the reaction is very strongly or strongly positive, one is able to see with the naked eye, after 2 or 3 minutes' shaking, or even in the course of shaking, the appearance of the purplish blue particles. However, when the reaction is only weakly or doubtfully positive, one only sees under the microscope the tiny purplish blue bodies.

From the foregoing, it will be seen that, in the procedure of test employing the test fluid produced according to this invention, only a drop of blood will do for the purpose. It is not necessary to separate blood into serum, and with a simple technique, the test can be made within a few minutes' time in one's own examining room, and the result can be read distinctly by coloring matter under the microscope. These advantages indicate that the invention brings much progress in the art in contradistinction to any of the heretofore practised tests for syphilis.

What we claim is:—

1. A process for the production of test fluid for use in testing for syphilis, comprising preparing a mixture of alcohol and animal's heart muscle in finely subdivided condition, maintaining said mixture at a temperature of from 40° to 75° C. for at least two weeks, cooling the resultant mixture and dissolving cholesterin therein, and then adding bluish coloring matter comprising crystal violet to the product.

2. A process for the production of test fluid for use in testing for syphilis, comprising preparing a mixture of alcohol and animal's heart muscle in finely subdivided condition, maintaining said mixture at a temperature of from 40° to 75° C. for at least two weeks, cooling the resultant mixture and dissolving cholesterin therein, and then adding bluish coloring matter comprising Azure II to the product.

3. A process for the production of test fluid for use in testing for syphilis, comprising preparing a mixture of alcohol and animal's heart muscle in finely subdivided condition, maintaining said mixture at a temperature of from 40° to 75° C. for at least two weeks, cooling the resultant mixture and dissolving cholesterin therein, and then adding bluish coloring matter comprising methylene blue to the product.

4. A process for the production of test fluid for use in testing for syphilis, comprising preparing a mixture of alcohol and animal muscle in finely subdivided condition, maintaining said mixture at a temperature of from 40° to 75° C. for at least two weeks, dissolving cholesterin in the resultant product, and then incorporating in the product bluish coloring matter adapted, in a positive reaction, to produce among the red corpuscles of the blood being tested clumps which are bluish in color in contrast to the red color of said corpuscles.

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