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A Rapid Precipitation Test for Syphilis.

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Ingredients: This test unlike the other existing precipitation tests for syphilis requires no dilution of the serum and antigen either before or after mixing them. Therefore the test is dealing with only two ingredients: serum and antigen.

Serum: The serum is obtained and inactivated in the usual way. As only small amounts of serum are needed it may be sufficient to secure the blood from the finger.

Antigen: Since the work of Sachs¹ in 1911, it has been a well established fact that the addition of cholesterin to the alcoholic beef heart antigen increases the sensitiveness of the latter in complement fixation and precipitation tests for syphilis. The solubility of cholesterin in alcohol is limited and therefore the cholesterin content in the alcoholic antigen cannot go beyond a certain degree (about 0.8%). It was thought that by using better solvents it would be possible to increase the cholesterin content of antigen. After several experiments acetone (at 37°) was found to be satisfactory for that purpose and the antigen was prepared by adding 2% solution of cholesterin in acetone to an equal volume of alcoholic beef heart extract. This extract is obtained by adding 5 cc. of alcohol (95%) for every gram of beef heart muscle powder from which the ether soluble substances were previously removed by ether extraction. The principle of preliminary ether extraction introduced by Neyman and Gager² and adopted by Kahn,³ Meinicke,⁴ *et al.*, was found to be of advantage also in this test. It is advisable to keep in stock separately the alcoholic extract and cholesterin solution and to prepare mixtures sufficient for only one week's need. If cholesterin crystals precipitate out the solution is placed in an incubator at 37° in order to dissolve them. If a turbidity occurs during the mixing of the cholesterin solution and the alcoholic extract, it is necessary to centrifugalize the mixture and use the supernatant clear fluid. In order to make the final results more conspicuous for reading 0.05 methylene-blue powder is added to 10 cc. of the cholesterinized antigen.

¹ Sachs, *Berl. klin. Wchn.*, 1911, xlviii, 2066.

² Neyman and Gager, *J. Immunol.*, 1917, ii, 573.

³ Kahn, "Serum Diagnosis of Syphilis by Precipitation," Baltimore, 1925.

⁴ Meinicke, *Deut. med. Woch.*, 1922, xlviii, 384.

Glassware: For every test are needed: 1. A hollow ground slide; 2. A set of two capillary pipettes: one for the serum and the other for the antigen. In order to have the same caliber of the capillary stem for serum and for antigen both pipettes are drawn from the one piece of glass tubing. As a standard, we are using pipettes which contain 8 drops to 0.1 cc. of serum. 3. A glass rod.

Performance of the test: Four drops of serum are placed in the cavity of the hollow ground slide, and one drop of antigen is floated on the surface of the serum and allowed to stay for 2 minutes. Then the serum and antigen are mixed thoroughly with a glass rod, the slide is gently tilted and rocked for one-half minute and then examined. If the room temperature is low it is recommended to use serum and antigen which have been warmed in the incubator at 37° for 15 minutes. It must be borne in mind that the ratio of 4 drops serum to 1 drop antigen in reality constitutes a volumetric ratio of about 8:1, inasmuch as the surface tension of the antigen is only one-half of that of the serum.

Examination: The slide is examined under the low power microscope (magnification 1:80), the diaphragm being sufficiently narrowed. The reaction is clear cut. In negative sera the whole field is uniformly bluish and has a fine granular appearance without any clumping. This appearance becomes particularly evident when the lens is focused upon the surface layer. In positive sera a definitely marked clumping is observed. The clumps are stained more intensely than the surrounding fluid. Their size varies. Big clumps indicate a *strongly positive* reaction, clumps of medium size are reported as a *positive* reaction, and fine delicate clumping is reported as a *dubious* reaction (\pm). The clumping is very characteristic and can easily be distinguished from other particles which may be due to the impurities of the serum, to the incomplete dispersion of the antigen in the serum, or to the presence of precipitated cholesterol crystals.

Specificity: The test was performed on 1066 sera and checked by the Wasserman reaction. The following table gives a comparison of the results obtained.

TABLE I.

	Wasserman	Rosenthal
negative	739	735; 4 posit. with a history of syphilis
++++, +++, ++	228	228
+	58	55 posit.; 3 dubious
(dubious) \pm	38	19 posit.; 17 dubious; 2 negative
anticomplementary	3	2 negative; 1 positive

The chart shows almost a perfect agreement of the two tests. It seems that in weak positive and dubious Wasserman sera this test gives a more clear cut reaction. Thus the test combines reliability with technical simplicity.

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Possible Water Balance; Effects of Alkaline Anterior Pituitary Extracts.

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In these studies an inbred strain of well-standardized mice were used as experimental animals. Feeding and environmental conditions were made as nearly identical throughout as possible. The experiments continued over a period of 8 months, and included many series of animals injected and handled under a wide variety of conditions, but with each experiment carefully controlled. In each case the group was divided into animals receiving the alkaline extract of the anterior lobe, a group receiving the ammonium sulphate extractive, and a group of controls. In some of the experiments, the controls received an alkaline liver extract, on others, normal saline, while in a few cases no injections were given the controls. In some cases the dosages were very minute and given only once a day; from this, they varied to 3 times a day and very large dosages. In some series, all of the animals received unlimited quantities of fluid—water or milk, or both; in others, the fluid intake was sharply curtailed. This series of studies reveals the following facts:

Immature animals injected with the alkaline extract, as outlined by Evans,¹ gain weight at a more rapid rate than do the ammonium sulphate extractive injected animals or the controls, provided the allowance of fluid is unlimited or large. The animals receiving the ammonium sulphate extractive gain weight slightly more rapidly than do the control animals. If the fluid intake be sharply curtailed, the animals receiving the alkaline extract do not gain weight as rapidly as do the control animals, or the ammonium sulphate animals. Under these conditions, however, the ammonium sulphate injected

¹ Evans, H. M., Harvey Lectures, 1923-24, 212.