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A USEFUL APPLICATION OF THE AGAR DOUBLE-DIFFUSION TECHNIQUE IN THE PRECIPITIN TEST

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The necessity of using well clarified bloodstain and seminal stain solutions in the precipitin test has been emphasised by Nickolls (1) who describes some of the methods employed to obtain suitable extracts. In some cases, however, it is impossible to obtain a suitably clear extract by filtration, centrifugation, or by extraction with organic solvents. This difficulty can arise when bloodstains and seminal stains are mixed with mucus or faeces or when it is necessary to extract a piece of decayed flesh. Frequently, in these cases an opalescent colloidal solution is obtained which obscures any ring formation at the interface.

In an effort to overcome this difficulty, we tried an agar double-diffusion method (2). This method, as finally elaborated, required only minor alteration to our normal precipitin test and, with sufficient soluble antigen present, gives an unequivocal result even when the stain extract is virtually opaque. Positive reactions are shown by a band or bands of precipitation in the clear agar.

METHOD

Our normal method is that described by Nickolls (1) and is modified in the following way. Columns of antisera about 1 cm. long are run into the bottoms of precipitin tubes of 2 mm. internal diameter and 3 cm. length using a fine bore Pasteur pipette.

The tubes are next plugged with agar at the surface of the antiserum by the following procedure. 1.5% w/v agar in McIlvaine buffer of pH 7.4 is clarified by repeated filtration at the pump through hot filter paper pulp. Ten mg. Thiomer-salate (sodium ethyl-mercuri-thiosalicylate) is added as a preservative to each 100 ml. of the preparation. The agar can be stored and repeatedly melted as and when necessary.

When required for use the agar is melted in a boiling water bath. Using a fine bore Pasteur pipette with a rubber teat the molten agar is drawn up and rejected back into the hot bulk three times to heat the pipette. On the fourth occasion an approximately 0.5 cm. column of agar is run onto the surface of the antiserum in the first precipitin tube and allowed to solidify. The remaining agar in the pipette is rejected back into the hot bulk, and the process repeated for the next and subsequent precipitin tubes. Gelling of the agar on reaching the liquid antiserum surface is virtually instantaneous, and any heat denaturation of the antiserum proteins is negligible.

When all the tubes are plugged the stain extracts are run onto the solid agar surfaces in the tube. Details of the basic technique, before modification, can be found in Nickolls's book.

RESULTS

The method has proved satisfactory as exemplified by the following case of alleged bestiality with a hen.

A dead Light Sussex hen was submitted to the laboratory with a request for examination of the cloaca for human semen. The cloaca contained a mixture of blood, faeces, and mucus, the extract from which could not be clarified by the usual methods. Agar double-diffusion tests were run as described above with the results shown in Table 1.

The only reacting tubes were those for anti-hen and anti-human. Figure 1 shows the appearance of the anti-human/cloacal extract and anti-hen/cloacal extract tubes after 24 hours. The cloacal contents also yielded a good acid phosphatase reaction and spermatozoa morphologically similar to human spermatozoa.

Table 1

	Saline Control	Hen Egg Albumen	Human Serum	Cloacal swab.
Rabbit Antisera				
Anti Human.....	-	-	+	+
Anti Sheep/Goat.....	-	-	-	-
Anti Cow.....	-	-	-	-
Anti Hen.....	-	+	-	+

The band(s) of precipitate usually appear in from two to twenty-four hours depending on the influence of certain factors, of which extract strength, antiserum strength, length of agar column, and temperature seem to be the most important.

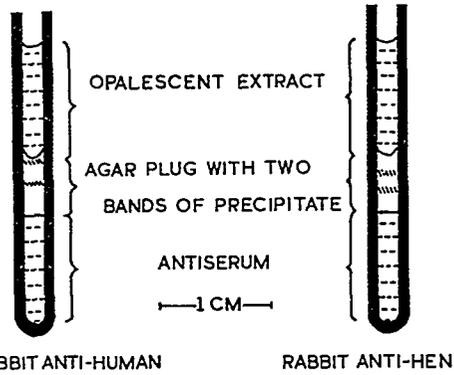


Figure 1

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2. OUCHTERLONY, O., *ARK. KEMI. MINERAL. GEOL. B*, 26, 1, (1948).