A dextran slide test for blood grouping

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Dextran has been used for the detection of incomplete Rhesus antibodies (Grubb, 1949; Richardson-Jones, 1950) and immune anti-A and anti-B (Munk-Andersen, 1956). Other high molecular weight compounds such as gum acacia (Sturgeon, Cedergren, and McQuiston, 1964), polyvinyl pyrrolidone (Szymanski, Valeri, McCallum, Emerson, and Rosenfield, 1968), and polybrene (Lalezari, 1968) have been used in automatic apparatus for blood grouping and antibody detection. In this rapid slide test, dextran is used to induce rouleaux in the presence of antibody: when rouleaux are dispersed by the addition of saline, the cells sensitized by antibody remain bound together, and may be clearly distinguished. The method works well with a wide range of antibodies. A similar principle has been utilized in an AutoAnalyzer method, but with polybrene- and enzyme-treated cells (Lalezari, 1968).

Method

1 To each half of a slide is added one drop of high molecular weight (Mw 150,000) dextran in 3% solution.
2 To one side is added a drop of antiserum: to the other side is added a drop of AB serum.
3 One drop of a 10% suspension of cells is added to each side of the slide.
4 The antiserum/dextran/red cell suspensions are mixed.
5 For warm-acting antibodies the slide is placed in an incubator: cold antibodies may be left at room temperature.
6 After three minutes the slide is rocked for a minute at room temperature to mix the cells. Two drops of saline are added to each half of the slide which is rocked to disperse any rouleaux. The result is read with the naked eye.

The method has given good results with four anti-Kell, one anti-k, three anti-Le^a and three anti-Le^b, two anti-C, one anti-c, three anti-D, three anti-E, and one anti-M sera. Two anti-S and two anti-P sera gave only moderately good results, and there was no enhancement of agglutination with one anti-P and one anti-Lu^a serum. With one anti-Fy^a, one anti-S, and one anti-e the result was poor. When

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tested against a panel of D-positive cells, including D^u cells, satisfactory results were obtained in all cases. The test works particularly well with anti-Kell.

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A quick method for reading the indirect haemagglutination test

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Indirect haemagglutination tests are usually performed in plastic plates with U-shaped wells. The test sera and the antigen (tanned and sensitized red cells) are mixed in the wells, the plates are sealed and left for the cells to settle. The results are read on the pattern of cell sedimentation. In our experience with this technique the end point is difficult to read, and the results are inconsistent unless a considerable amount of time and care is given to washing the plates. The method described here using glass well slides and a rotator in place of the plastic plates is simple, it is quicker and easier to read than the standard method, and it gives highly reproducible results with a clear-cut end point.

Method

The cells used were human O cells as supplied by

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the National Blood Transfusion Service for blood group serology. The method for tanning and sensitizing the cells was that of Kessel, Lewis, Pasquel, and Turner (1965) with one modification, viz, the stabilization of the sensitized cells was effected with phosphate buffer containing 3% of bovine albumin. The test was performed in the following way. Doubling dilutions of sera were made from 1/8 upwards in standard plastic plates. One standard drop (0·035 ml) of the serum dilution being tested was placed on a glass well slide. One standard drop of a 2% suspension of tanned sensitized cells was added. These were mixed gently with a glass rod, the slide was put on a mechanical horizontal rotator for 10 minutes, at about 70 rpm, and the result read immediately.

Positive reactions showed distinct clumping of the cells as seen in the classical ABO grouping tile technique. Agglutination could be graded from 1+ to 3+ on the size of the clumps. To standardize the system it was important to rotate for a fixed time as longer rotating times resulted in higher titres, which would have necessitated an adjustment of the borderline titre indicative of positivity.

Result

We studied this method of reading indirect haemagglutination using filaria antigen to sensitize the cells. Seventy sera from individuals who had never left Britain were all negative at a dilution of 1:32. Eight hundred sera from patients who had resided in areas of endemic filariasis showed 65% of microfilaria carriers and 13% of non-microfilaria carriers to be positive at dilutions of 1:64 or higher. Occasional sera gave high titres of the order of 1:1,000 or higher. We could not detect any particular cross-reactions with any specific helminths, and in contrast to the complement-fixation test for filariasis cross reactions in *Strongyloides* infections were a rarity; but there were some unexplained positives. After treatment for filariasis the haemagglutination test remained positive for much longer than the complement-fixation test. These results are in line with those obtained by other workers using filaria haemagglutination (see Kagan and Norman, 1970, for a review).

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Present day practice

Antibiotics in mycoplasma media and the temporary storage of specimens containing mycoplasmas of the genital tract

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Colour changes in liquid media are used to detect the presence of mycoplasmas, including *Mycoplasma pneumoniae* (Smith, Chanock, Friedewalde, and Alford, 1967) and T-mycoplasmas (Taylor-Robinson, Addey, and Goodwin, 1969). However, these colour changes can also be produced by contaminating bacteria and yeasts, and the growth of these organisms may not be inhibited by the antibacterial agents, penicillin and thallium acetate, commonly used in selective media (Andrews, 1969). Ampicillin, 1 mg/ml, was reported by Hutchinson (1969) to suppress contaminating bacteria, and is recommended by Fallon (1969). Nystatin, 50 units/ml, is included in selective media for the isolation of some animal mycoplasmas (Whittlestone, 1969). Polymyxin B, 50 μg/ml, and amphotericin B, 5 μg/ml, are preferred by some workers (Braun, Klein, Lee, and Kass, 1970).

In our laboratory in 1967, in all selective media, penicillin was replaced by ampicillin (reported by Taylor-Robinson, 1968); in media for the isolation of *M. pneumoniae* we also added methicillin, 1 mg/ml, and nystatin, 57 μg/ml (200 units/ml). When sputum was used as the inoculum, the proportion of false colour changes due to contaminating bacteria was reduced from 53 out of 117 (45%) to two out of 54 (4%) by these additions. The medium still fully supported the growth of *M. pneumoniae*. In media for the isolation of T-mycoplasmas when penicillin was replaced by ampicillin a few false colour changes due to bacteria continued to occur; these were found to be due to *Pseudomonas aeruginosa* and ampicillin-resistant *Proteus* organisms. To inhibit these organisms we have now replaced ampicillin with carbenicillin, 1 mg/ml, and among 150 subsequent specimens from the genital tract no false colour changes have occurred. Nystatin, 57 μg/ml, is also included in the selective medium.

The effect of different temperatures for overnight

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