Techniques Used in the Routine Examination of Specimens for the Specific Serological Types of *Escherichia coli*

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In this routine laboratory every "coliform" colony from the cultures of any specimen, except colonies on desoxycholate citrate plates, is examined by the following methods to identify specific serotypes of the *Escherichia coli*. The techniques employed are designed to provide reliable results in the shortest possible time.

Rapid Tube Agglutination Using a Centrifuge

Each serotype, from a new patient or specimen, which is identified by slide agglutination must have its identity confirmed by a tube agglutination test carried out to the titre of the specific antiserum (Taylor, 1956). It is the practice here to perform slide agglutinations between 9 and 10.30 in the morning.

A portion of a colony which has given a positive slide agglutination is subcultured into a tube of warm infusion broth, a batch of which is always kept in the 37° C. incubator. After four hours' incubation there is sufficient growth available for confirmatory tube agglutinations to be carried out. Some of the broth culture is used to set up the confirmatory tests that the laboratory employs for the identification of the members of the *Enterobacteriaceae*, and a drop is spread on a MacConkey plate, which is incubated, and single colonies examined the next day to ensure that only one serotype is present. The remainder of the broth culture is employed for the confirmatory tube agglutination tests. One portion is boiled for 15 minutes and is then centrifuged at 3,000 revolutions per minute for five minutes. The deposited organisms are re-suspended in a suitable quantity of saline to give a density of about 250 by 10⁶ organisms per millilitre and they are used as the O antigen for agglutinations in round-bottomed 2 in. by ½ in. test tubes. The tubes are placed in a water-bath at 50° C. A control tube, in which the antiserum is replaced by normal saline, must be incubated at the same time. After 15 minutes' incubation the tubes are numbered, and then centrifuged for one or two minutes at about 1,000 revolutions per minute. After centrifuging the tubes are placed in a rack and each is rotated rapidly within the rack by a series of stroking movements made with the index finger on the side of the tube. The mixing is continued until the organisms in the control tube have made an even suspension: if specific agglutination has taken place the aggregates can then be seen as coarse clumps. It

sometimes helps to dislodge the deposited organisms if the tube is gently tilted. The supernatant fluid runs down the tube and forms a new meniscus, behind which there is an air bubble, which runs up the inverted tube and displaces the clumped organisms from whence they are deposited on the rounded bottom (Fleming, 1928).

Braun, Seeliger, and Wagner (1954) used a centrifuge technique for the demonstration of O agglutinins in the sera of babies who had had *E. coli* gastro-enteritis. They employed this method because conventional incubation of the agglutination reaction failed to show any antibodies in the same sera.

It is really unnecessary to examine each strain for both K and O antigens, but if both are required some of the broth culture is centrifuged and the centrifuged deposit killed by the addition of three drops of 1:1,000 mercuric iodide in saline: this suspension is employed as the antigen for a K agglutination test. The K agglutinations are made, as is usual, in 4 in. by ½ in. test tubes. The tubes are incubated for 15 minutes at 37° C., then centrifuged for one minute at 1,000 revolutions per minute, and after this the bottom of each tube is flicked in the routine that is used to demonstrate the presence of a K agglutination (Kauffmann, 1951).

Using these techniques it is possible to provide the results of the confirmatory tube agglutinations in the late afternoon the day after a specimen has been received, but, as the faeces of a baby with enteritis due to one of these specific serotypes contain vast numbers of that serotype in a nearly pure culture (Rogers, 1951; Thomson, 1955), it is possible to make a saline suspension from the thickly inoculated portion of a plate and to provide a tentative diagnosis four hours after planting a specimen. A rapid diagnosis of a specific serotype infection permits chemoprophylaxis to be given early both to the affected baby and to any other infants in the ward.

When a great number of culture plates have to be examined, rapid screening can be effected if each culture is examined by slide agglutination of a suspension made from the confluent part of the growth. For two years investigations have been begun with this technique, a note has been made of the results, and then at least four individual colonies have been tested from that portion of the plate on which differentiation has provided separate discrete colonies. It is usual for this preliminary screening to demonstrate a specific serotype if any is present on the plate.

Saline Pencil Technique

In her description of the preliminary slide agglutinations, Taylor (1956) briefly described a technique using ordinary clean 3 in. by 1 in. microscope slides on which colonies are suspended in drops of normal physiological saline. This saline is dispensed from a pencil and a thin wire loop is used to remove a small portion of the colony.

A reservoir type Pasteur pipette is cut off squarely near to where the actual pipette is formed from the main butt, and this square-cut opening closed by melting the glass in a flame until it forms a strong thickened glass end with a pinpoint-sized hole in the centre. As it is narrowed by the heating the size of the aperture can be checked with a hand-lens, or, when the pipette is cool, by filling it with water and seeing how much comes out, when the pipette is touched on to a glass slide. If the hole is too large and fluid comes out too quickly, the water is shaken out and the glass re-melted to make the hole smaller. Before the saline pencil is put into use, the end opposite to that with the pinhole opening is heated in the flame until it is soft enough to be bent, so that the pipette becomes eccentric: this prevents the pencil from rolling when it is dropped back or placed on the bench.

When it is used the pencil is filled with 0.85% saline and the thickened end is used to tap a slide, on which it then leaves a small drop of saline.

The small wire loop is of 33 S.W.G. (0.01 in. in diameter) nichrome or platinum wire made into a 2 mm. loop. This thin wire is used because it cools very quickly after it has been sterilized and, also, its small size allows several portions of any colony to be removed.

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BOOK REVIEWS

Chromatographic Techniques. Clinical and Biochemical Applications. By Ivor Smith; Foreword by C. E. Dent. (Pp. xiii+309; illustrated. 45s.) London: Heinemann. 1958.

Chromatography has not been altogether neglected in the ever-increasing flow of new publications in biochemistry, but there has been a real need for a good practical book on filter paper chromatography to which the clinical biochemist can refer for guidance. Dr. Smith, with the assistance of several colleagues, has successfully remedied this deficiency and presents here a mine of information for the identification of many substances of medical importance. Much of it is new and hitherto unpublished material.

The first chapters are given to general principles of technique, and the author describes a simple "universal" apparatus for ascending and descending chromatography using 10 in. square papers. There is a good account of preparatory methods including the desalting techniques, viz., electrolytic desalting, ion exchange separations, and organic solvent extraction. The electrolytic desalter perfected by Dr. Smith and his associates is discussed in detail. Those who do not possess a desalter will find the alternative methods helpful, but the author has not applied them so much to body fluids so that a certain amount of preliminary testing is still required to secure optimum conditions before putting these into routine use.

Following chapters deal specifically with the analysis of amino-acids, indoles, imidazoles, guanidines, purines and pyrimidines, sugars, ketoacids, phenols, barbiturates, steroids, etc. In each case clear instructions are given regarding the choice of solvent and location agents, a general procedure is laid down, location maps are usually provided, and the clinical applications discussed. The sections on amino-acids, indoles, and steroids are particularly helpful. Some of the other sections are of necessity short, and their relation to routine clinical biochemistry is at the moment limited.

If there is any weakness in the book, it is in the clinical notes, which contain some unusual statements, e.g., glycosuria is suggested as a possible guide to treatment and progress of renal tubular necrosis, and a distinction is drawn between cystinosis and the Fanconi syndrome. Crystals of cystine are said to have a "characteristic octagonal shape."

These sections will require revision in any future edition, but the real value of the book lies in its wealth of technical detail not readily available elsewhere, and for this reason it is likely to become a standard manual in its field.

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