Micromethod for Serogrouping Escherichia coli

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A micromethod for serogrouping strains of Escherichia coli is described. The method is accurate, rapid, and economical in terms of time and reagents utilized to perform the test.

The study of patients with infections due to Escherichia coli was facilitated greatly by the development of a serologic scheme for subclassification by Kauffman (3). The method depended on the presence of heat-stable, O-specific, cell wall antigens and led to the recognition of some 148 different O groups. The initial application of this scheme resulted in the identification of strains of a few serogroups (enteropathogenic) which were commonly associated with infantile diarrhea (1). More recently, the technique allowed the identification of six to eight serogroups of E. coli which were commonly associated with extraintestinal infections and provided a means of improving our understanding of the implications of recurrent disease in such patients (6-8).

The conventional method for serogrouping strains of E. coli by tube agglutination is not practical for a more routine application of this technique since it is time consuming, cumbersome, and requires large amounts of difficult-to-acquire O-specific antisera (2). This report describes the standardization of a micromethod which allows for the rapid and accurate serogrouping of strains of E. coli with a limited expenditure of time and reagents.

MATERIALS AND METHODS

Bacteria. Most strains of E. coli produced acid on a slant of triple sugar iron agar and acid and gas in the butt, did not utilize citrate as a sole source of carbon, and produced indol from tryptophan. A few late lactose-fermenting strains which otherwise resembled E. coli were also included. These strains were from three sources: isolates from fresh specimens of urine obtained from patients with infections of the urinary tract and of feces obtained from healthy primates; isolates of known serogroups which were stored for 1 to 10 years in paraffin-sealed, nutrient agar slants and were initially recovered from specimens of urine, feces, and blood of human origin; and 23 standard strains of E. coli of known serogroups which were obtained originally from the Center for Disease Control (CDC), Atlanta, Ga.

Antigens. Each of the individual strains of E. coli was grown in 40 ml of tryptose broth for 16 to 18 hr at 37 C and heated at 100 C for 2 hr in an Arnold sterilizer. The final suspensions were used as the test antigens.

Antisera. Standard unabsorbed rabbit antisera for the different serogroups of E. coli were obtained as 1:1 dilutions in glycerol from the Reagents Section of the CDC.

Macroagglutination method. The tests were performed as described previously (9).

Microagglutination method. The tests were performed in microtiter plates with U-shaped bottoms (Cooke Engineering Co.). The test volumes of antisera and bacteria were added to the wells with calibrated pipette droppers. The known standard antigens were included as positive controls for each antisera on each day the test was performed. After mixing the reagents by tapping, the plates were incubated for 16 to 18 hr at 4 C.

Patterns of agglutination were read by tipping the microtiter plate at an angle over a dark-field illuminator. The patterns ranged from negative to 4+ as seen in Fig. 1. A matt with or without folded edges was read as 4+, a matt with a small central button as 3+, a plain button which did not run as 2+, a button which ran slowly as 1+, and a button which ran quickly leaving a tail as negative.

RESULTS

Optimum concentration reagents. The grouping antisera were tested at dilutions of 1:500, 1:1,000, 1:2,000, and 1:4,000 for their ability to agglutinate homologous and heterologous standard strains of known serogroups. As seen in Table 1, antisera diluted 1:500 gave strong homologous reactions but also significant cross-reactions with 6 of the 12 sera tested. Optimum homologous reactions of 3+ or greater and negligible (2+ or less) or absent cross-reactions were attained by 1:1,000 or 1:2,000 dilutions of each antiserum (Table 2). A 0.025-ml volume of the test antigen gave the sharpest and easiest to read end point even
though it was more commonly associated with heterologous cross-reactions.

In addition to the above strains, standard strains of O groups 12, 13, 14, 51, 73, 86, 102, 117, 123, and 137 gave negligible or absent reactions with the 12 standard antisera although some of the sera were known to contain low levels of cross-reactive antibodies for these strains. The standard strain of O group 2 gave a reaction with the 050 antiserum which could not be differentiated by dilution of the antiserum from that with the standard strain of 050.

Comparison of micro- and macromethods. Each of the 289 wild strains of E. coli was tested with both the micro- and macromethods. In the micromethod the optimum 1:1,000 or 1:2,000 dilution of antiserum and the 0.025 ml volume of the test antigens were used. In Table 3, the number of strains tested for each serogroup and the agreement of the micro- with the macromethod are shown. Twelve (4%) among the 289 strains failed to give the same result with both methods. Six of these errors were contributed by strains from two patients: the three strains of O7 which were classified as rough (autoagglutinable) in the micromethod and the three strains of another serogroup (O12) which were classified as 025. All but 1 of these 12 strains were groupable with the macromethod. However, in the microtest, 3 failed to react with any of the 12 antisera, 3 were rough, and 6 gave a different reaction. Strains which were rough with the macromethod gave identical reactions in the microtest.

If only smooth strains were considered, 12 (5%) among 238 strains failed to give the same reaction in both tests. When only strains that were groupable by the macromethod were considered, 11 (7%) among 163 strains did not give the same result. As mentioned previously, three strains from each of the two patients accounted for six of these differences. If only one of each of these strains was considered in assessing agreement, then the percent disagreement would be three, three, and four, respectively, for total, only smooth, and only groupable strains classified by the macromethod.

DISCUSSION

A micromethod for serogrouping E. coli is described. The results of serogrouping strains with this technique compare favorably with those obtained with the more commonly used macromethod. In addition, the micromethod possesses several technical advantages as it is extremely economical in terms of the amount of time utilized and reagents consumed in its performance. Instead of using many test tubes, racks, and pipettes, only microtiter plates and the specially calibrated pipette droppers are required. With a single plate, eight samples may be tested against 12 different O antisera. The amount of diluted antiserum required to test one strain in the macromethod will allow the testing of 60 to 80 strains in the micromethod. Because of the small volumes of antisera used, it is practical to screen directly with the individual antisera rather than utilize pools as described for the macromethod (2). The use of individual sera has the added advantage of providing an answer within 24 hr, thus avoiding the two-step procedure of the macromethod which delays the final result for 48 hr or more. The classification of a high proportion of the strains can be controlled by the inclusion of sera for those common serogroups which have already been identified as having a broad geographic distribution (6).

The serologic classification of only 60% of the strains with either the macro- or micromethod may not reflect the true potential of this technique since the high proportion of rough and non-typable smooth strains included in this study reflects, primarily, the inclusion of a large number of strains isolated from the feces of primates. Past experience in our laboratory with the macromethod and isolates obtained from specimens of urine and blood obtained from patients suggests that, when these methods are applied to the study of strains from
TABLE 1. Microagglutination patterns of 12 strains of standard O groups of E. coli when reacted with 1:500 dilutions of homologous and heterologous O antisera

| Antigen | O1 | O4 | O6 | O7 | O16 | O17 | O18ac | O50 | O25 | O50 | O62 | O75 |
|---------|----|----|----|----|-----|-----|-------|-----|-----|-----|-----|-----|
|         | A+ | B+ | A+ | B+ | A+ | B+ | A+    | B+  | A+  | B+  | A+  | B+  |
| O1      | +4 | +4 | -  | -  | -   | -   | -     | -   | -   | -   | -   | -   |
| O4      | -  | +4 | +4 | -  | -   | -   | -     | +3  | +2  | -   | +3  | +2  |
| O6      | -  | -  | +4 | +4 | -   | -   | -     | -   | -   | -   | -   | -   |
| O7      | -  | -  | -  | +4 | +4  | -   | -     | -   | -   | -   | -   | -   |
| O16     | -  | -  | -  | -  | -   | +4  | +4    | -   | +1  | +1  | -   | -   |
| O17     | -  | -  | -  | -  | +4  | +4  | -     | -   | -   | -   | -   | -   |
| O18ac   | -  | -  | -  | -  | -   | -   | -     | -   | -   | -   | -   | +4  |
| O22     | -  | -  | -  | -  | -   | -   | +4    | +3  | -   | -   | -   | -   |
| O25     | -  | +3 | +1 | +3 | +4  | -   | +2    | +4  | +4  | -   | -   | -   |
| O50     | +3 | +2 | -  | +3 | +4  | -   | -     | +4  | +4  | -   | -   | -   |
| O62     | -  | -  | -  | -  | -   | -   | -     | +4  | +3  | -   | -   | -   |
| O75     | -  | -  | -  | -  | -   | -   | -     | +4  | +4  | -   | -   | -   |

* A 0.025-ml volume test antigen.
* A 0.050-ml volume test antigen.

TABLE 2. Microagglutination patterns of 12 strains of standard O groups of E. coli when reacted with 1:2,000 dilutions of homologous and heterologous O antisera

| Antigen | O1 | O4 | O6 | O7 | O16 | O17 | O18ac | O22 | O25 | O50 | O62 | O75 |
|---------|----|----|----|----|-----|-----|-------|-----|-----|-----|-----|-----|
|         | A+ | B+ | A+ | B+ | A+  | B+  | A+    | B+  | A+  | B+  | A+  | B+  |
| O1      | +4 | +4 | -  | -  | -   | -   | -     | -   | -   | -   | -   | -   |
| O4      | -  | +3 | +3 | -  | -   | -   | -     | +2  | +1  | -   | +1  | -   |
| O6      | -  | +3 | +3 | -  | +4  | +4  | -     | -   | -   | -   | -   | -   |
| O7      | -  | -  | +4 | +4 | -   | -   | -     | -   | -   | -   | -   | -   |
| O16     | -  | -  | +3 | +2 | +2  | -   | +1    | -   | -   | -   | -   | -   |
| O17     | -  | -  | -  | +3 | +2  | -   | -     | +1  | -   | -   | -   | -   |
| O18ac   | -  | -  | -  | -  | +2  | +2  | +1    | -   | -   | -   | -   | -   |
| O22     | -  | -  | -  | +3 | +1  | -   | -     | +4  | +3  | -   | -   | -   |
| O25     | -  | -  | -  | +2 | +2  | -   | +4    | +3  | +3  | -   | -   | -   |
| O50     | -  | -  | -  | -  | -   | +3  | +3    | -   | -   | -   | -   | -   |
| O62     | -  | -  | -  | -  | -   | -   | +3    | +2  | -   | -   | -   | -   |
| O75     | -  | -  | -  | -  | -   | -   | -     | +4  | +3  | -   | -   | -   |

* A 0.025-ml volume test antigen.
* A 0.050-ml volume test antigen.

these sources, one can expect to classify nearly 70% of strains with the 12 antisera included in the present study.

The availability of a practical test for serogrouping E. coli makes it feasible to consider the routine use of this technique in the diagnostic laboratory rather than limiting its use to a few research laboratories. Such a step would require the availability of commercial sources of antisera similar to those used to identify the enteropathogenic strains of E. coli. The important information gained through the ability to serogroup E. coli in studies of patients with recurrent infections of the urinary tract and in
TABLE 3. Comparison of the results of serogrouping strains of E. coli by the micro- and macroagglutination methods

| Serogroup | Macro Agreement both tests | Micro result |
|-----------|---------------------------|--------------|
| O1        | 5                         | CT*          |
| O4        | 24                        | R, R, R*     |
| O6        | 60                        | 06           |
| O7        | 7                         | 06           |
| O16       | 1                         |              |
| O17       | 1                         |              |
| O18ac     | 14                        |              |
| O22       | 1                         |              |
| O25       | 5                         | CT           |
| O50       | 4                         |              |
| O62       | 1                         |              |
| O75       | 21                        |              |
| Othersc   | 13                        | 017, 025, 025, 025 |
| Roughh    | 31                        | 06           |
| CT’s      | 75                        | 06           |

*CT, smooth but not groupable with available antisera.

*Rough (R), spontaneous agglutination and therefore not groupable.

c Others: 1-OX2, 1-O21, 1-O113, 1-O101, 1-09, 1-O20ab, 1-O73, 2-OX9, 3-O12, 1-O15.

the definition of problems associated with nosocomial infections with E. coli are only two examples of areas where care of a patient may be improved with such information (6–8). These alone would seem to justify the need for commercial sources of antisera for at least 10 to 20 of the most common serogroups.

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