Detection of *Escherichia coli* Antigens by a Latex Agglutination Test

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A latex particle agglutination technique to detect ethylenediaminetetraacetate-solubilized extracts from *Escherichia coli* and whole *E. coli* cells is described. The sensitivity of the serological test was found to be 0.5 to 2.5 ng for the solubilized antigens and $1.5 \times 10^4$ to $5.7 \times 10^4$ ng per ml for the particulate antigens. The test was 100 to 1,000 times more sensitive than the standard bacterial agglutination test. Furthermore, it detected *E. coli* antigens during all phases of bacterial growth, whereas the bacterial test detected the antigens only after the mid-log phase. No significant cross-reactivity was observed between latex-anti-*E. coli* preparations and heterologous bacterial strains used in the experimental procedure. A buffer formula containing fatty acid-free bovine albumin prevented nonspecific aggregation of the latex particles.

Standard test procedures for the identification of *Escherichia coli* serotypes involve agglutination (3) or immunofluorescence (2) with whole cells, or gel precipitation (5) with soluble antigens. The relative insensitivity or complexity of these tests is a major disadvantage when the quantity of antigen or the time available is limited. This led us to study the application of the latex particle technique to *E. coli*. Latex particles have been used as inert carriers of antigens for detecting the corresponding serum antibodies (9, 12, 13) and, conversely, as vehicles for antibodies for detecting antigen in biological fluids (1, 10).

This paper reports the development and evaluation of a test using anti-*E. coli* globulin adsorbed to latex, which makes possible the rapid as well as sensitive detection of both *E. coli* cells and ethylenediaminetetraacetate-solubilized extracts from those cells.

**MATERIALS AND METHODS**

**Buffers.** Four buffers were used: 0.12 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 8.0; 0.1 M glycine-buffered saline (GBS), pH 8.2; and GBS containing 1 or 0.1% bovine albumin, type F (BAF; Sigma Chemical Co., St. Louis, Mo.).

**Cultures.** Stock strains from the Division's culture collection were maintained as stab cultures in nutrient agar at room temperature.

**Antigen preparation.** A 0.1-ml volume of an 18-h broth culture was inoculated into 600 ml of beef heart infusion broth containing 1% glucose and incubated on a rotary shaker for 16 h at 37 °C. The cells were harvested and washed three times by centrifugation at $10,000 \times g$ with the tris(hydroxymethyl)aminomethane buffer.

Soluble antigens (SAg) were prepared essentially as described by Leive and Shovlin (8). Stock SAg solutions (10 mg/ml) were prepared by dissolving lyophilized material in GBS with 0.1% BAF. These were stored at -20°C.

Particulate antigens (PAg) were suspensions of resting cells diluted in membrane-filtered (Millipore Corp.) saline. The maximum dilution that reacted (+) in the latex agglutination test was determined, and the cells were counted with a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.).

**Globulins.** Suspensions of various serotypes of *E. coli* cells from 18- to 24-h cultures were adjusted to an optical density (at 540 nm) of 350 Klett units in 0.3% formalin solution in saline. Rabbits were immunized (6), and the antisera obtained were tested for bacterial agglutination with the homologous culture. Globulins from preimmune and immunized rabbits were precipitated by ammonium sulfate and separated further on a diethylaminoethyl-cellulose column (7). The purity of the fraction obtained was determined by immunoelectrophoresis, and the protein was adjusted to 1 mg/ml with saline by the biuret method (4).

**Latex-IgG suspension.** Stock suspensions of latex particles (Difeo Laboratories, Detroit, Mich.) were first adjusted for adsorption of a single globulin as described by Newman et al. (10). The latex-single immunoglobulin G (IgG) suspensions were prepared by mixing the reagents in the following order: latex, IgG, GBS, and GBS with 1% BAF. After each addition the mixture was shaken for 30 s. For tests of SAg and PAg, the ratios by volume of the reagents were: for *E. coli* serotype O127:B8, 3:1:1:2:0.8; for serotype O128:B12, 3:0.5:1.5:1; for serotype O124:B17, 4:2:1:1; and for the six remaining sero-
types, 3:1:1:1. For tests of all whole cultures, the latex-IgG composition was latex-IgG-GBS, 3:1:2.

For adsorption of several globulins to a single latex preparation, 0.3 ml volumes of stock latex were centrifuged at 4,000 \( \times g \) and all the supernatants were decanted and pooled. For each suspension, the sediment from 0.3 ml of latex was mixed with 0.3 ml of an IgG mixture (0.1 ml from each of three IgG solutions) and shaken for 2 min. To restore the smooth and even suspension, 0.4 ml of the original latex supernatant was returned to the mixture, followed by 0.1 ml of GBS with 1% BAF. The final suspension was shaken for an additional 2 min.

**Latex test.** Forty microliters of an SAg solution or PAg suspension was mixed with 20 \( \mu l \) of latex globulin suspension on a glass slide. The slide was hand-tilted for 2 min. The degree of agglutination was read macroscopically and recorded as 4+, 3+, 2+, or 1+. The specificity of the test with the soluble antigen(s) was determined after finding the concentration of SAg which, when mixed with homologous IgG, resulted in particle agglutination. Antigen at this concentration was then mixed with each of eight remaining IgG preparations. Cross-reactivity was recorded if agglutination was 2+ or greater.

**Specificity of latex tests.** The following controls for nonspecific aggregation were included with all tests: (i) latex-IgG plus GBS with 0.1% BAF; (ii) latex-normal globulin plus GBS with 0.1% BAF; (iii) latex-normal globulin plus antigen; and (iv) latex plus antigen buffer.

The specificity of the test with the soluble antigen(s) was determined after finding the concentration of SAg which, when mixed with homologous IgG, resulted in particle agglutination. Antigen at this concentration was then mixed with each of eight remaining IgG preparations. Cross-reactivity was recorded if agglutination was 2+ or greater.

Test specificity with whole cultures was also studied. A number of organisms (see Table 1) were grown with shaking at 37 C for 16 h in beef heart infusion-glucose. The cultures were then mixed with latex-anti- \( E. coli \) IgG suspensions and with anti- \( E. coli \) IgG solutions (1 mg of protein/ml). At the end of the incubation period, the degree of specific and heterologous reactivity was recorded as described above.

**Sensitivity of latex and bacterial tests.** SAg solutions were diluted and tested by the particle agglutination test. In addition, latex and bacterial agglutination tests of resting cells and growing cultures were performed. Resting \( E. coli \) O55:B5 cells (10-fold dilutions of 5.57 \( \times 10^6 \) PAg/ml) were mixed with latex-IgG suspensions or with IgG solutions (1 mg of protein per ml) for the bacterial agglutination test. The growing culture was initially prepared from a 16-h beef heart infusion-glucose culture. The cells were harvested by centrifugation and suspended in 10 ml of beef heart infusion-glucose, and 1 ml of this suspension was inoculated into 5 ml of the same medium. The culture was then incubated with shaking at 37 C, and samples were withdrawn at intervals and examined by latex and bacterial agglutination tests. The test mixtures in both systems were incubated for 2 min at room temperature; the results were read macroscopically.

**RESULTS**

Ethylendiaminetetraacetate-solubilized antigen(s). The ethylendiaminetetraacetate extraction procedure yielded 35 to 50 mg of SAg per gram (dry weight) of \( E. coli \) cells. The lyophilized extracts were fluffy white powders with a faint greenish tinge. They were soluble in water to a concentration of 10 mg/ml at 25 C.

**Specificity of the latex test.** The appearance of test and control mixtures is illustrated in Fig. 1. Aggregation was not observed with control mixtures. Moreover, latex buffer suspension alone with either PAg suspension or SAg solution did not cause clumping (not illustrated). These results indicate that the agglutination shown is an immunological reaction.

Reactions obtained with nine globulin-coated particle preparations and the corresponding \( E. coli \) extracts were strain specific. In addition, tests of anti- \( E. coli \) and latex-anti- \( E. coli \) suspensions against a number of pathogenic organisms indicated the specificity of this procedure (Table 1). With the exception of \( Staphylococcus aureus \), cross-reactivity in both the bacterial and particle agglutination tests was minimal (≤1+) and similar.

Aggregation of \( S. aureus \) cultures by latex-IgG was noted with all the antibody particle preparations available but not with any of the globulins in solution. Cultures of \( S. aureus \) were tested against latex-anti- \( E. coli \) O111:B4, latex-normal globulin, and latex-GBS with 0.1% BAF. Maximum reactivity (Table 2) was observed with both particle-globulins and undiluted cultures, although no agglutination was noted with the particle-buffer preparation. When \( S. aureus \) culture was diluted 1:8, no reaction was observed. Cultures of \( E. coli \) O111:B4, however, were reactive in a dilution of 1:1,000.

**Sensitivity of the latex test.** The optimum concentration of the IgG prepared for this study was 0.17 mg of protein per ml in latex-single IgG suspensions, except for IgG against serotypes O124: B17 and O128: B12, which were optimum at 0.125 and 0.08 mg/ml, respectively. The limit of detection of antigen(s) by the particle agglutination test is summarized in Table 3. With the latex-single IgG preparation, the minimum detectable concentration of SAg varied between 0.5 and 2.5 ng; with the latex-multiple IgG, it varied between 1 and 5 ng. The number of cells that could be detected with either preparation ranged between 1.5 and 5.7 \( \times 10^4 \) per ml.

The comparative sensitivity of the latex and bacterial agglutination tests was shown by two experiments. The first indicated that a suspension of PAg (5.57 \( \times 10^4 \) cells/ml) was visibly aggregated when tested with either latex-IgG or IgG in solution. However, agglutination of PAg
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**FIG. 1.** Latex agglutination. (A, B) Tests with soluble (SAg) and particulate (PAg) antigens, respectively. The rest are control mixtures: (C) latex IgG and buffer, (D) latex-normal globulin and PAg, (E) latex-normal globulin and SAg and (F) latex-normal globulin and buffer.

| Culture* | Degree of agglutination of immune globulins from *E. coli* serotypes |
|----------|---------------------------------------------------------------------|
|          | O26: B6          | O111: B4         | O119: B14        | O126: B16        | O124: B17        |
| L        | B                | L                | B                | L                | B                |
| *Proteus rettgeri* | ± 1+             | -                | -                | -                | -                |
| *Enterobacter hafniae* | - 1+              | -                | -                | -                | 1+                |
| *Pseudomonas aeruginosa* | - 1+              | -                | -                | -                | -                |
| *Staphylococcus aureus* | 4+  -  4+      | 4+              | 4+              | -                | 4+                |
| *Enterobacter cloacae* | -                | -                | -                | -                | 1+                |
| *Arizona henshawii* | -                | ±  1+             | 1+              | ±                | ±  1+             |
| *Neisseria meningitidis* | ±                | -                | -                | -                | -                |
| *Salmonella cubana* | -                | ±  1+             | 1+              | ±                | -                |
| *E. coli* O26: B6 | 4+                | 4+              | 4+              | 4+              | -                |
| *E. coli* O111: B4 | -                | 4+              | 4+              | 4+              | -                |
| *E. coli* O119: B14 | -                | 4+              | 4+              | 4+              | -                |
| *E. coli* O126: B16 | -                | -                | -                | -                | -                |
| *E. coli* O124: B17 | -                | -                | -                | -                | -                |

* No reactions occurred with the following cultures: *Yersinia enterolitica*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Provenicia alcalificiens*, *Shigella dysenteriae*, *Proteus morganii*, *Edwardsiella tarda*, *Alkalescens dispar*, *E. coli* O55: B5, *E. coli* O127: B8, and *E. coli* O128: B12.

* L, Latex agglutination test; B, bacterial agglutination test.

* Tests were made and gave negative results.
The colloidal protective effect of serum protein on the hydrophobic latex particles was first reported by Singer et al. (11), who found that, of the various proteins tested, Cohn fraction V (albumin) contributed a maximum stability to suspended particles. Bloomfield et al. (1) first used GBS-bovine serum albumin as a stabilizer in the latex test for *Cryptococcus* antigen. In our study, a similar atypical aggregation with latex particles mixed with GBS-bovine serum albumin suggested that the phenomenon is not due to adsorbed globulin. When the nondefatted serum albumin was replaced by BAF in GBS, whether freshly prepared or stored for periods up to 6 months at 5 C, the nonspecific aggregation was eliminated. Whenever whole cultures were tested, however, BAF was omitted. The effect of lipid from culture media on the reaction was minimal, presumably because of the overriding effect of the large amount of protein present in culture media. Therefore, consideration of the protein and lipid content of the reaction mixtures is essential in establishing optimum test reactivity.

### DISCUSSION

The GBS-with-BAF formula provided a uniform, nongranular dispersion of latex in buffer and greatly enhanced the reliability and reproducibility of the test. BAF may also be useful in other latex test preparations where an amount of antigen or antibody sufficient to keep the particles in suspension would interfere with the aggregation of the particles.

A second cause of nonspecific aggregation was a low concentration of anti-*S. aureus* in both immunized and nonimmunized rabbits which was solved by diluting the serum. When the *E. coli* extracts and antisera were tested, type cross-reactivity was not observed, even between the closely related O86 and O127 types (3). Although a large number of *E. coli* serotypes were not studied with the latex test, it is probable that cross-reactivity within the genus is comparable to that obtained by the bacterial test.

To achieve maximum sensitivity, the ratio of
components and the order of addition of reagents in preparing a latex-globulin suspension are important. The IgG solution must first be exposed to the latex, and GBS with 1% BAF must be added last. Adding a pool of three or more globulins to latex in suspension resulted in a reduction in the amount of antigen detected by a factor of 40 to 100. In contrast, there is only a 2- to 10-fold decrease in sensitivity when the IgG pool is mixed with the sedimented latex.

With PAg, a tentative explanation for the identical sensitivity obtained with single and multiple IgG is that visible aggregates form more easily when both reactants are in suspension. Suspended particles, such as latex-IgG and bacteria, are in comparatively unstable suspension; any disturbance, such as antigen-antibody reaction, would cause the rapid formation of visible aggregates.

This simple and rapid serological test permits the detection of antigen at a concentration far less than is observed with the classical bacterial agglutination test. With the particle test, small 6-h colonies of E. coli on nutrient agar or selective media could be serotyped before any bacterial agglutination is observed. The time needed for serotyping is thus decreased by 24 to 48 h. Furthermore, a preliminary study in this laboratory indicates that antigens in E. coli-seeded spinal fluid and buffered urine (pH 8.2) were detected by the test at concentrations similar to the detection limit in a growth culture medium (unpublished data).

The system described in this report may have other applications, since it permits identification of antigens (SAg and PAg) while bacterial growth culture experiments are being performed. Macromolecules released into the medium, e.g., components of the cell envelope, could be identified during such experiments, provided the appropriate immunoglobulins were obtained and adsorbed on latex.

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**TABLE 4. Detection of E. coli O111:B4 cells in culture by latex and bacterial agglutination**

| Sample time (h) | Cells/ml (×10⁹) | Latex agglutination | Bacterial agglutination |
|----------------|----------------|---------------------|------------------------|
|                |                | Culture            | Filtrate               | Culture*                |
| 0              | 0.007          | ±                   | ±                      | -                      |
| 1              | 0.016          | 2+                  | ±                      | -                      |
| 2              | 0.031          | 3+                  | 1+                     | -                      |
| 2.5            | 0.064          | 4+                  | 1+                     | -                      |
| 3              | 0.21           | 4+                  | 1+                     | -                      |
| 3.5            | 2.31           | 4+                  | 2+                     | 2+                     |
| 4              | 3.6            | 4+                  | 4+                     | 4+                     |
| 5              | 6.7            | 4+                  | 4+                     | 4+                     |
| 6              | 6.2            | 4+                  | 3+                     | 3+                     |

* - , Negative results.
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