Latex Test for Quantitative Determination of *Escherichia coli*
Antibody

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A latex agglutination test was developed for assay of anti-*Escherichia coli* antiserum. The test is simple, specific, sensitive, and reproducible.

Comparative studies (5, 6) on serum titers to *Escherichia coli* antigens indicate that indirect hemagglutination is a more sensitive technique than bacterial cell agglutination. Hemagglutination, however, is elaborate and time consuming. Latex particles have been used instead of erythrocytes (RBC) as antigen carriers (7, 8). This note describes a simple, sensitive, reproducible latex scheme for detecting *E. coli* antibody.

Soluble antigens (SAg) were obtained (4) from *E. coli*, and antisera were prepared by injecting whole cells into rabbits (1). The sera were inactivated at 56°C for 30 min, and dilutions were made with glycine-buffered saline, pH 8.2.

Sensitized sheep RBC (RBC-SAg) were prepared as follows: SAg was treated (4) overnight at 37°C with 0.02 N NaOH at 1 mg/ml and buffered with 1 ml of glycine-buffered saline, and the volume was brought up to 10 ml with saline. To standardize the RBC suspension (2), 0.15-ml portions of a 1% saline suspension of saline-washed cells were lysed with 1.85 ml of water; the optical density at 550 nm was read against a water blank. The cell suspension was then adjusted with more cells or with triethanolamine-buffered saline solution, pH 7.3 to 7.4, so that its lysates read an optical density of 0.130. To sensitize the cells, equal volumes of adjusted cell suspension and treated SAg were mixed and incubated at 37°C for 2 h. The cells were then washed three times in saline, and the sensitized cells were resuspended so that the optical density of lysates was 0.130 for the tube test and 0.260 for the slide test.

Two latex-antigen (latex-SAg) reagents were also prepared. For the slide test (qualitative), a mixture of 0.03 ml of SAg (1 mg/ml) and 0.3 ml of latex (0.81 μm, Difco; 3.7 × 10¹⁸ particles/ml) was shaken for 2 min, and 0.27 ml of buffer was added. For the tube test (quantitative), 0.1 ml of latex and 0.5 ml of varying SAg concentrations were mixed gently, shaken for 30 min at 25°C, and brought to a 10-ml volume with glycine-buffered saline. Unadsorbed SAg was not removed from the latex reagents as in RBC-SAg reagent.

For the slide test, 20 μl of latex-SAg and 40 μl of RBC-SAg were each mixed on slides with 40 μl of serum for 2 and 5 min, respectively. For the tube test, equal volumes (0.2 ml) of each serum dilution and antigen reagent were mixed, incubated for 2 h at 56°C (latex) or 37°C (hemagglutination), left overnight at 5°C, and centrifuged at 700 × g for 3 min. All tests were read macroscopically; the titers were recorded as the reciprocal of the highest serum dilution exhibiting a 2+ agglutination.

The slide test findings paralleled the tube test results. Similar titers were obtained with the latex and hemagglutination tests (Table 1, Fig. 1), but the latex test was more specific (Table 1). Reproducibility of quantitative test results within one dilution was 89% (16 of 18 trials) for latex and 64% (23 of 36 trials) for hemagglutination.

The determination of SAg concentration for optimal latex test reactivity is important. The maximal serum titer determined by block titration is obtained at an antigen concentration of 2 to 10 μg/ml (Fig. 2). Below 2 μg/ml the particles aggregate spontaneously; above 10 μg/ml the SAg in solution apparently competes for antibodies with the SAg adsorbed on latex and partially inhibits the serological reaction. Within the narrow range of optimal concentration, however, SAg in solution stabilizes the suspended particles and appears to facilitate particle aggregation.

The disadvantages of hemagglutination as a routine procedure to detect *E. coli* antibody are numerous. Sheep RBCs are not biologically inert; they contain surface antigens which may react with components of human and animal sera (7, 8). The preparation of RBC-SAg is elaborate and time consuming (24 h), and the
TABLE 1. Homologous and heterologous titers by latex (L) and hemagglutination (H) tests

| E. coli antisera | E. coli antigen* |
|------------------|------------------|
| 0111:B4         | 0111:B4 055:B5 026:B6 087:B7 0127:B8 0119:B14 0125:B15 0126:B16 |
| L               | 2,048 2,048 4 32 2 1,024 2 8 |
| H               | 2 2 1,024 8 |
| 055:B5          | L 2,048 1,024 2 |
| H               | 2 1,024 |
| 026:B6          | L 2,048 2,048 |
| H               | 32 8 |
| 087:B7          | L - 2,048 |
| H               | - 8 |
| 0127:B8         | L - 2,048 |
| H               | - 2 |
| 0119:B14        | L 32 512 |
| H               | 16 |
| 0125:B15        | L 8 |
| H               | |
| 0126:B16        | L - 4 |
| H               | - 1,024 |

* No reactivity was obtained with glycine-buffered saline, normal rabbit serum, or unsensitized cells.

** (-) No reactivity obtained.

**Fig. 1.** Titers of 14 anti-E. coli 0111:B4 antisera by latex and hemagglutination procedures.

**Fig. 2.** Tube test: block titration by latex (□) and hemagglutination (○) techniques. SAg concentration in each test is the amount added to the carriers. Each symbol marks the highest dilution at which a serological reaction (2+) occurs.
sensitized cells are unstable. The titer of a serum, tested repeatedly at daily intervals with different lots of RBC-SAg preparation and even with the same lot, can vary by four twofold dilutions. Latex as a vehicle, on the other hand, is biologically inert. Preparation of latex-SAg is simple and rapid (30 min), and the results are reproducible.

This latex scheme, instead of the elaborate hemagglutination technique, could be used efficiently, to monitor antibody level, e.g., after vaccination to guard against colibacillosis in piglets (3), or as an investigative tool in studying immune responses to E. coli antigens.

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