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# Rapid Latex Agglutination Test for Detection of Staphylococcal Enterotoxins A to E That Uses High-Density Latex Particles

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**A rapid reversed passive latex agglutination method that uses high-density latex particles for the detection of staphylococcal enterotoxins (SE) A to E was developed. It took 3 h for incubation, much less than the 16 h needed with a customary latex agglutination test for SE detection such as a commercial test kit (SET-RPLA; Denka Seiken Co. Ltd., Tokyo, Japan). The rapid test was shown to be highly specific and sensitive for SE detection (detection limit, about 0.5 ng of SE per ml), comparable to the SET-RPLA test. The rapid test was also efficient in SE detection in foods and culture supernatants of staphylococcal strains, similar to the SET-RPLA test. This showed that a rapid test with high-density latex particles is fully reliable for use.**

Staphylococcal food poisoning outbreaks are characterized by vomiting and diarrhea and occur quite frequently worldwide. In such outbreaks, the detection of staphylococcal enterotoxin (SE) is epidemiologically essential. Various methods for SE detection have been developed and used, including gel diffusion tube or plate assays (2, 12), a reversed passive hemagglutination method (6, 17), reversed passive latex agglutination (RPLA) (13), radioimmunoassay (10), and enzyme-linked immunosorbent assay (14). Of these, the RPLA method was simple and sensitive, but not as specific, because the latex particles were sensitized with nonpurified antiserum (13). The method was improved for specificity by sensitizing latex particles with purified anti-SE immunoglobulin (4, 9, 15). A commercial RPLA test kit, SET-RPLA, is now available from Denka Seiken Co. Ltd., Tokyo, Japan, and Oxoid Ltd., Basingstoke, England. The test presently takes 16 h for incubation. Therefore, we sought to develop a more rapid RPLA test, using high-density latex particles. Several brands of heavy particles were tested as candidates for the method. A rapid RPLA test for SE detection was successfully developed, using a 3-h incubation time. In our study, this rapid test for SE detection in foods and culture supernatants of staphylococcal strains was compared with the commercial RPLA test, SET-RPLA.

## MATERIALS AND METHODS

**Bacterial strains.** *Staphylococcus aureus* strains associated with food poisoning outbreaks were collected in this laboratory in 1987 and were used in this study.

**SEs and their specific immunoglobulins.** SEs (SEA, SEB, SEC, SED, and SEE) were purified chromatographically by the method of Oda (7, 8) from culture supernatants of strains that produced each toxin. Antisera were prepared by injecting the toxins into rabbits subcutaneously (4). The specific immunoglobulins were purified by the affinity chromatography method of Yamada et al. (17). Antisera to SEA or SEE are known to have a common antibody to both toxins and the specific one to the corresponding toxin (5). Thus, the specific immunoglobulin to SEA was purified from anti-SEA serum by passing first through a SEA-coupled column and then through a SEE-coupled one. As for the specific immunoglobulin to SEE, anti-SEE serum was applied first to the SEE-coupled column and then to the SEA-coupled one.

**Sensitizing of latex particles.** High-density latex particles (Immutex H1011CR, HD-PS) with a density of 1.5 g/cm$^3$ and 1.29 μm in diameter were purchased from Japan Synthetic Rubber Co. Ltd., Tokyo. A 10% suspension of latex particles was diluted 1:20 with phosphate-buffered saline (PBS) consisting of 1 volume of 67 mM phosphate buffer, pH 7.2, and 3 volumes of saline with 0.05% $NaN_3$. Equal volumes of a diluted latex particle suspension and 4 μg of each specific immunoglobulin per ml (concentration determined by $A_{280}$) were mixed thoroughly, using an IKA-Vibrax-VXR shaker (Janke & Kunkel GmbH & Co. GK, Staufen Imbreisgau, Federal Republic of Germany) at 600 strokes per min, for 1 h at room temperature. After centrifugation at 2,800 × *g* for 10 min, the latex particles were suspended in a diluent consisting of 1% bovine serum albumin in PBS and then mixed thoroughly for 30 min as described above. The sensitized latex particles were washed twice with PBS and suspended in the diluent at a concentration of 0.05% for use. Control latex particles were sensitized with normal rabbit globulin in the same manner as anti-SE-coupled particles. Normal rabbit globulin was purified from normal rabbit sera with protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden). The sensitized latex particles were stored at 4°C before use.

**Extraction of SE from foods.** The 50% food homogenates were prepared by homogenating food (30 g) and 0.01 M phosphate buffer (PB), pH 7.3 (30 ml), with a stomacher 400 (Seward Medical, London, England) for 1 min. If a 50% homogenate was too viscous, a 33% homogenate consisting of 30 g of food and 60 ml of PB was used. Each 0.1 ml of SE was added at a certain concentration to 10 ml of the homogenate and mixed. In the control, PB (10 ml) was substituted for each food homogenate. Then the homogenate was centrifuged at 25,000 × *g* and 4°C for 20 min. The supernatant fluid was gently taken up with a capillary tube.

**Ether treatment of food extract.** With cheese, if a supernatant fluid prepared as above was turbid, the fluid was mixed with an equal volume of diethyl ether in a glass tube with a stopper, using the IKA-Vibrax shaker at 600 strokes per min for 10 min at room temperature. After centrifugation at 1,600 × *g* for 10 min, the water layer was carefully separated.

**Preparation of culture supernatants.** Culture supernatants

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TABLE 1. Detection of SE added in food homogenates by RPLA methods[a]

| Food homogenate (50%) | SE added | | Rapid RPLA test | | | SET-RPLA | | |
|---|---|---|---|---|---|---|---|---|
| | | | Concn detected (ng/ml) | | Ratio (%)[c] | Concn detected (ng/ml) | | Ratio (%) |
| | Type | ng/ml | Sample | Control[b] | | Sample | Control | |
| Bacon | A | 4 | 4 | 4 | 100 | 4 | 4 | 100 |
| | A | 2 | 2 | 2 | 100 | 2 | 2 | 100 |
| Cooked rice | A | 10 | 16 | 16 | 100 | 16 | 16 | 100 |
| | B | 2 | 2 | 2 | 100 | 2 | 2 | 100 |
| Omelette | A | 10 | 16 | 16 | 100 | 16 | 16 | 100 |
| | C | 2 | 2 | 2 | 100 | 2 | 2 | 100 |
| Cake[d] | A | 10 | 16 | 16 | 100 | 16 | 16 | 100 |
| | C | 4 | 4 | 4 | 100 | 4 | 4 | 100 |
| Cheese | | | | | | | | |
| Sample 1[d,e] | A | 2 | 2 | 2 | 100 | 2 | 2 | 100 |
| | A | 1 | 1 | 1 | 100 | 1 | 1 | 100 |
| Sample 2[d,e] | A | 2 | 2 | 2 | 100 | 2 | 2 | 100 |
| | B | 4 | 4 | 4 | 100 | 4 | 4 | 100 |
| Sample 3[d] | B | 2 | 1.5 | 2 | 75 | 2 | 2 | 100 |
| | B | 1 | 1 | 1 | 100 | 1 | 1 | 100 |
| Sample 4[d,e] | B | 2 | 2 | 2 | 100 | 2 | 2 | 100 |
| Cream puff[d] | C | 1 | 0.75 | 1 | 75 | 0.75 | 1 | 75 |
| | D | 1 | 1 | 1 | 100 | 0.75 | 1 | 75 |
| | E | 1 | 0.75 | 1 | 75 | NT[f] | NT | |
| Yokan (sweet bean jelly) | A | 10 | 16 | 16 | 100 | 16 | 16 | 100 |
| An (bean jam) | A | 2 | ND[g] | 2 | | ND | 2 | |
| | D | 2 | ND | 2 | | ND | 2 | |
| | E | 2 | ND | 2 | | NT | NT | |
| Pork and beans | B | 2 | ND | 2 | | ND | 2 | |
| Amanatto (sugared beans) | B | 2 | ND | 2 | | ND | 2 | |

[a] Data are averages of duplicate tests.
[b] SE dissolved in PB to the same concentration as the food homogenate was assayed.
[c] Concentration of toxin detected in the sample/concentration of the control.
[d] 33% food homogenate.
[e] Treated with diethylether.
[f] NT, Not tested because anti-SEE-sensitized latex has not yet been produced.
[g] ND, Not detected due to nonspecific agglutination.

of staphylococcal strains with 0.1% $NaN_3$ were prepared as described before (3).

**Procedure for RPLA test.** For comparison, a commercial RPLA test kit (SET-RPLA) for detection of SEA through SED was obtained from Denka Seiken. The test procedure was carried out as described by Igarashi et al. (3) and Park and Szabo (11), using type U microdilution plates (N-1182; Nunc, Roskilde, Denmark). Samples of food extracts and culture supernatants were diluted serially two- and tenfold, respectively. For high-density latex particles, agglutination was observed against a white background after a 3-h incubation. In the commercial test kit, agglutination was observed with transmitted light through the bottom of the plate after about 16 h of incubation. The titer of the sample was determined as described before (3). The concentration of toxin detected in the sample was calculated by multiplying the toxin titer of the sample by the detection limit of each toxin (0.5 ng/ml). The recovery of toxin from the sample was determined as a ratio of the concentration of toxin detected in the sample (10 ml of food homogenate) to that in the control (10 ml of PB).

## RESULTS

The rapid RPLA test was evaluated for SE detection, compared with the commercial test kit (SET-RPLA).

**Type specificity and sensitivity for SE detection of the rapid RPLA test.** Each purified SE (SEA through SEE) dissolved in PBS was analyzed for specificity and sensitivity of the RPLA method with high-density particles. Each sensitized latex particle was specific for the corresponding toxin type only, similar to SET-RPLA. The sensitivity for detection of each toxin was about 0.5 ng/ml, equal to that of SET-RPLA. These data showed that the test was highly specific and sensitive for the detection of SEs, similar to the commercial test kit.

**Detection of SE in foods by the rapid RPLA test.** SEs added to food homogenates were assayed by the rapid method (Table 1). In many kinds of food, SE was mostly recovered by the rapid test, similar to the commercial test kit. The sensitivity of detection of each SE in food homogenates by the rapid test was about 0.5 ng/ml (1 or 1.5 ng/g of food). With most cooked beans, both RPLA methods showed nonspecific reactions. In all food homogenates shown in Table 1, SEs adjusted to a concentration of <1 ng/ml could not be detected by either test.

**Detection of SE in culture supernatants by the rapid method.** SE production of strains associated with staphylococcal food poisoning outbreaks was analyzed by the rapid test. Some of the results are shown in Table 2. With all strains tested, the rapid RPLA test corresponded to the SET-RPLA in terms of specificity and sensitivity for SE detection. No strain tested had a nonspecific reaction by either method.

These results showed that the rapid RPLA test was fully reliable for detecting SE in foods and culture supernatants, similar to the commercial test kit.

**Protein A interference.** Purified protein A (200 μg/ml) dissolved in PBS did not react with any anti-SE-sensitized

TABLE 2. Production of SE by staphylococcal strains by using RPLA methods

| Strain | Rapid RPLA test | | SET-RPLA | |
|---|---|---|---|---|
| | SE type | Concn detected (μg/ml) | SE type | Concn detected (μg/ml) |
| 9823 | C | 50 | C | 50 |
| 9824 | A | 0.5 | A | 0.5 |
| | B | 5 | B | 5 |
| 9825 | A | 0.5 | A | 0.5 |
| 9833 | | 0 | | 0 |
| 9851 | A | 0.5 | A | 0.5 |
| | B | 50 | B | 50 |
| 9856 | | 0 | | 0 |
| 9863 | A | 0.5 | A | 0.5 |
| | B | 5 | B | 5 |
| 9880 | A | 0.5 | A | 0.5 |
| | B | 5 | B | 5 |
| 9881 | A | 0.5 | A | 0.5 |
| | B | 5 | B | 5 |

high-density particles except those sensitized with anti-SEB; the sample had a weak reaction to a 10-fold dilution (i.e., 20 μg/ml) of protein A with anti-SEB-sensitized particles only. The same phenomenon was observed with anti-SEB-sensitized particles of the commercial test kit. However, no culture supernatant of staphylococcal strains, including Cowan I, had a nonspecific reaction by either RPLA methods.

## DISCUSSION

An RPLA method has several advantages at present, including (i) high specificity and sensitivity, (ii) simplicity (no need for complicated procedures or expensive equipment), and (iii) economy (3, 11). The disadvantage of the test is that it is time-consuming (about 16 h for incubation). Thus, we wanted to develop a rapid RPLA test that uses high-density particles. We successfully developed a rapid RPLA test requiring a 3-h incubation.

In an RPLA test, a sample and the reagent react in one compartment throughout the procedure. Some materials tend to undergo nonspecific reactions with sensitized latex particles. A nonspecific reaction is determined as agglutination of all types of sensitized latex particles as well as control particles with some materials. The sample fluid should thus be prepared to be as clear as possible, removing from the sample any possible interfering materials such as starch and lipid particles.

Cooled centrifugation is a great help for this. In almost all food samples, a supernatant fluid from cooled centrifugation was usable in the test. With cheese, however, the supernatant fluid, which was still turbid, caused a nonspecific reaction in the RPLA test. Ether treatment of the fluid suppressed the nonspecific agglutination successfully (Table 1). Some fluids from bean products and gelled starch also showed nonspecific reactions with the latex particles. Naturally, ether treatment was unsuccessful with these fluids. In some foods other than these, a weak nonspecific reaction was also observed. In some, substitution of a diluent of higher pH, e.g., 0.01 M Tris hydrochloride, pH 8.0, for PB was effective in suppressing the reaction.

Park and Szabo (11) calculated the recovery of toxin from foods by the concentration of toxin detected by the RPLA method and the volume of the supernatant fluid. However, the RPLA method is semiquantitative. Concentration of toxin by this method is calculated discontinuously by the toxin titer of a sample and the sensitivity of toxin. For this reason, it is thought impossible to evaluate the exact recovery of toxin from a sample by RPLA. In this study, therefore, we compared the concentration of toxin detected in a sample with that of the control, for recovery.

The rapid RPLA test was also developed for detection of toxic shock syndrome toxin 1 (TSST-1) (1). TSST-1 is thought to be the toxin responsible for toxic shock syndrome, an acute, severe, multisystem disease caused by *S. aureus* (16). The rapid RPLA method, using high-density particles sensitized with anti-TSST-1, proved to be specific and sensitive for TSST-1 detection (detection limit, about 0.5 ng of TSST-1 per ml), similar to the RPLA test developed before (3) and another commercial test kit (TST-RPLA; Denka). Thus, the rapid method was also found to be reliable for detecting toxin in culture supernatants.

Only anti-SEB-coupled latex particles reacted with protein A up to about 20 μg/ml in this study. It is not known whether protein A and SEB have a common region in molecular structure. However, no culture supernatant of staphylococcal strains has reacted nonspecifically with the sensitized latex particles so far. Thus, it is thought that the rapid RPLA test and the SET-RPLA may be fully reliable for detecting SE in culture supernatants. If necessary, we recommended adding a small quantity of normal rabbit sera to the diluent to avoid occurrence of a nonspecific reaction with protein A.

In a preliminary study, when culture supernatants were prepared, filtration through a membrane (MILLEX-HV; pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) was substituted for cooled centrifugation. SE in the filtrate was detected by the RPLA test without any trouble. The absorption of SE to the membrane is negligible, because culture fluids contain far more proteins than SE. If it is necessary to hurry, the filtration method is recommended.

Several brands of heavy particles were tested as candidates for the rapid RPLA test. As a result, Immutex was the most suitable for the test. It is possible that some new commercial particles that are heavier than Immutex would make the incubation period even shorter in the RPLA method.

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