Comparison of LFA with PCR and RPLA in detecting SEB from isolated clinical strains of *Staphylococcus aureus* and its application in food samples

Der-Jiang Chiao, Jiunn-Jye Wey, Pei-Yi Tsui, Fu-Gong Lin, Rong-Hwa Shyu

**A R T I C L E   I N F O**

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**A B S T R A C T**

Three sensitive and specific assays, the lateral flow assay (LFA), polymerase chain reaction assay (PCR) and reversed passive latex agglutination assay (RPLA), were selected for detection of staphylococcal enterotoxin B (SEB) from 77 clinical *Staphylococcus aureus* strains isolated from humans. Analytical results revealed that the LFA has almost the same detection sensitivity as that of PCR and RPLA. The concordances between the 3 assays were as follows: LFA–PCR, 92.2%; LFA–RPLA, 94.8%; and PCR–RPLA, 97.4%. For further evaluation, the LFA was used for the detection of SEB in different food matrices. The assay was able to successfully identify SEB in a wide variety of food samples at levels as low as 10 ng/mL in less than 10 min. This study proved that the LFA is an excellent tool for detection of SEB both in isolated clinical *S. aureus* strains and in food specimens and may prove particularly important as an early warning tool to prevent food poisoning in consumers.

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1. Introduction

The heat-stable staphylococcal enterotoxins (SEs) excreted by *Staphylococcus aureus* are thought to be the major cause of food-borne illnesses (Johnson et al., 1991). Recently, various types of staphylococcal enterotoxins have been identified elsewhere. Among these, 5 major antigenic types (SEA, SEB, SEC, SED, and SEE) are categorized as classical types (Klotz, Oppe, Heeg, & Zimmerman, 2003), whereas the other types (SEG–SEU) are grouped as newly developed (Letertre, Perelle, Dilasser, & Fach, 2003; Zouharova & Rysanek, 2008). Ingestion of any one of these enterotoxins may induce emesis, abdominal cramps, and diarrhoea. Although the illness is typically mild (nausea, vomiting, and diarrhoea lasting 12–24 h), fatalities may occasionally occur in weakened patients, especially the elderly (50% of the lethal dose was calculated to be 0.02 µg/kg by both inhalation and intravenous routes) (LeLor, Baron, & Gautier, 2003). Previous studies also reported that in the food industry, clinical illness may occur within a few hours when the manufactured food products contained SE levels above 125 ng per 100 g of food (Reiser, Conaway, & Bergdoll, 1974).

Among the *S. aureus* enterotoxins, SEB has proven to be extremely toxic as an aerosol incapacitant and can be easily synthesized in large quantities (Holecková et al., 2002). Inhalation of SEB could result in a high percentage of clinical illness within a few hours of exposure. In addition, SEB can not only function as a super-antigen that has the potential to be used as a biological warfare agent (Le, Lee, & Gopalakrishnakone, 2006) but also as a prevalent cause of food poisoning in the United States and other countries (Olsen, MacKinnon, Goulding, Bean, & Slutsker, 2000). Therefore, SEB is considered one of the most common causes of food-borne diseases in the world (LeLor et al., 2003), and detection of SEB at extremely low concentrations (i.e., 10–20 ng) in a variety of food samples is required.

Although most of the *S. aureus* strains are capable of producing enterotoxin, this does not mean that the strains can do so in food; the evident proof depends on detection of the enterotoxin in the food itself. However, identification of staphylococcal enterotoxins involved in food poisoning cases is time consuming. Numerous immunological and biochemical assays, such as enzyme-linked immunosorbent assay (ELISA) (Sotte, Langfeldt, Peruski, & Meyer, 2002), radioimmunoassay (RIA) (Holecková et al., 2002), polymerase chain reaction (PCR) (Letertre et al., 2003; Loncarevic, Jorgensen, Lawseth, Mathisen, & Rørvik, 2005), reversed passive latex agglutination (RPLA) (Wieneke, 1988; Boyynkara, Gulhan, Alisarli, Gurturk, & Solmaz, 2008), and even the chromogenic macroarray system (Lin, Chiang, & Tsen, 2009), have been em-
ployed for detecting SEB. These techniques are all sensitive and specific; however, the high equipment cost, requirement of highly trained personnel, relatively longer time required to perform these techniques, and the complex analytical operations always limit their use in the field. To overcome these drawbacks and expand the limits of detection, an ideal method is urgently warranted for rapid, sensitive, accessible, and low-cost detection of low levels of the toxin in routine clinical practice and in foods; the lateral flow assay (LFA) seems to be a good candidate to meet these requirements.

The LFA, also named the immunochromatographic assay or the strip assay, has been well developed for the diagnostic application in different fields for several years, such as in tests for drugs (Wong, 2002), hormones (Henderson & Stewart, 2000), allergens (Tsai, Williams, Mitchell, & Chapman, 2002), antibodies (Peng et al., 2007) and toxins (Chiao, Shyu, Hu, Chiang, & Tang, 2004; Diederen & Peeters, 2006; Shyu, Shyu, Liu, & Tang, 2002). This technique is predominantly based on an immunochromatographic procedure that utilizes the double-antibody sandwich format on a porous nitrocellulose membrane, specified by a red colour band formed by attached colloidal gold beads, to detect the analyte. Although the sensitivity of the LFA (10–20 ng/mL) (Shyu, Tang, Chiao, & Hung, 2010) was found to be much less than that of ELISA assays (100–1000 pg/mL) (Shotte et al., 2002), the strip assays have several benefits, such as a user-friendly format, quick and straightforward results, a long shelf life under room temperature, and relatively inexpensive in manufacture. More importantly, strip assays are also based on visual detection by the naked eye without requirements for other instrumentation. These characteristics make the LFA a good candidate for on-site investigation by untrained users.

Recently, PCR and RPLA assays have been reported for identification of*S. aureus* enterotoxigenic strains (Boynukara et al., 2008; Letertre et al., 2003). Simultaneously, a gold-immunochromatographic assay was developed in our laboratory for detection of SEB (patent pending). In the present study, SEB strips were used to detect SEB toxin from 77 isolated clinical specimens. The results were compared with the results of 2 other biological assays, PCR and RPLA, to determine assay sensitivity and specificity. In addition, to detect the existence of SEB in food, we evaluated the SEB strips as a tool for SEB detection by investigating the presence of SEB in various types of food samples. The studies described here provide evidence that the LFA is an excellent tool for detection of SEB in food and clinical samples.

2. Materials and methods

2.1. Materials

Seventy-seven isolated clinical*S. aureus* strains and ATCC strain 29247 (SEB production strain) were supplied by Tri-Service General Hospital, Taipei, Taiwan. The additional 17 ATCC bacterial strains for cross-reactivity analysis were obtained from IPM, NDMC, Taiwan. Anti-SEB IgG was purified from anti-SEB sera by thiolphilic gel (T-gel; Pierce, Rockford, IL). The anti-SEB sera were obtained from SEB-immunized rabbits (New Zealand white), and the sera titers were determined by ELISA. High-flow nitrocellulose (NC) membranes (AE 98), glass fibre conjugated pads (AccuFlowTM G), sample application pads (#12-S), and reagent adsorption pads (470 Zuschmitte/Cuts) were all purchased from Schleicher & Schuell GmbH (Dassel, Germany). These 4 components were adhered together to create the LFA devices (Fig. 1). The reversed passive latex agglutination kit (SET-RPLA toxin detection kit) used for detection of SEB was purchased from Denka Seiken Co. Ltd., Tokyo, Japan. All food samples used for SEB detection were purchased from local traditional markets or grocery stores, whereas ice cream and yogurt were obtained from the local supermarket. Only ice cream was kept at −20 °C; other food samples were kept at 4 °C until use.

2.2. Preparation of crude SEB toxin

The 77*S. aureus* strains and ATCC strain 29247 were grown in 5 mL Luria broth (LB; Sigma–Aldrich, St. Louis, MO) at 37 °C with shaking. After overnight culture, 0.5 mL of each culture supernatant was centrifuged for 20 min (4 °C, 3000 rpm; 12148-H rotor, sigma 2K15), and supernatants from individual strains were transferred to fresh aseptic micro-tubes and stored at 4 °C until use. The SEB toxin would be contained in the supernatant. Here, ATCC strain 29247 was utilized as a positive control for the production of SEB.

2.3. Method performance in comparison

Three specific assays, PCR, RPLA, and LFA, were selected for detection of SEB.

2.3.1. PCR protocol

Oligonucleotide primers for seb detection were designed based on published gene sequences (Johnson et al., 1991) and synthesized by Genomics Bioscience and Technology, Taiwan. The primers were targeted to internal regions of the toxin genes. After PCR, the amplified nucleic acid fragments were examined by agarose gel electrophoresis. All the PCR reactions were performed in a GeneAmp T3000 thermocycler (Biometra, Germany), and completed in a 50-μL reaction mixture as previously described (Pollard, Johnson, Lior, Tyler, & Rozee, 1990). In brief, 10 ng of nucleic acids (as templates) from each of the 77 *S. aureus* strains were amplified with the following amplification cycles: 2 min at 94 °C for denaturation, 2 min at 55 °C for annealing, and 1 min at 72 °C for extension. Finally, the reactions were terminated by a 5-min final extension at 72 °C. Each sample was subjected to 40 PCR cycles.

Base sequences and locations of the staphylococcal toxin-specific oligonucleotide primers:

\[
\text{SEB1: 634-5}^{\text{tcgcatcaaa}}-\text{gacaaccg}-3^\prime \text{)} -653 \text{(20-mer); SEB2: 1091-5}^{\text{cagagactctataagtgcc}}-3^\prime \text{)} -1110 \text{ (20-mer) (Johnson et al., 1991).}
\]

The amplification product had a predicted size of 478 nucleotides.

2.3.2. Detection of SEB by RPLA

SEB toxin was also detected by a commercially available standardized test kit, the SET-RPLA toxin detection kit. This kit is based on the reverse passive latex agglutination test, which enables the detection of 5 staphylococcal enterotoxins (SEA–SEE) in*S. aureus* culture medium. In brief, 5 mL culture supernatant of each*S. aureus* strain was centrifuged at 3000 rpm for 20 min at 4 °C (sigma 2K15, rotor 12148-H). Serial dilutions (2×, 25 μL) were prepared (sample concentrations, 100–0.8%) and applied to the RPLA kit to detect SEB. The aforementioned*S. aureus* strain 29247 was used as a positive control strain for each test.

2.3.3. Lateral flow assay

The supernatants of*S. aureus* strains and ATCC strain 29247 were grown in 5 mL Luria broth (LB; Sigma–Aldrich, St. Louis, MO) at 37 °C with shaking. After overnight culture, 0.5 mL of each culture supernatant was centrifuged for 20 min (4 °C, 3000 rpm; 12148-H rotor, sigma 2K15), and supernatants from individual strains were transferred to fresh aseptic micro-tubes and stored at 4 °C until use. The SEB toxin would be contained in the supernatant. Here, ATCC strain 29247 was utilized as a positive control for the production of SEB.
was accomplished in less than 10 min after sample addition, and false-positive results did not occur in any of the assays performed.

2.4. Statistical analysis

The chi-square test was used to examine the positive ratio in the 3 analyzed methods. They are LFA, PCR, and RPLA. Cohen’s kappa test was used to evaluate the agreement between results obtained from the above 3 tests. Landis and Koch (1977) proposed the standard $\kappa$ to describe the degree of concordance: below 0.2, “Poor”; 0.21–0.40, “Fair”; 0.41–0.60, “Moderate”; 0.61–0.80, “Substantial”; and 0.81–1.00, “Almost perfect.”

2.5. Cross-reactivity of SEB test strip to various enteropathogens

Various bacterial agents associated with different food-borne microbial diseases were applied onto SEB test strips to evaluate the cross reactivity of the strips. The analysis was focused on certain widespread bacterial enteropathogens found in developed countries, such as Staphylococcus spp., the Enterobacteriaceae group, vibrios pathogens, and some anaerobic bacteria. Most of the bacteria were grown in brain–heart infusion (BHI) medium except Listeria monocytogenes (tryptic soy broth) and 4 anaerobic Clostridium spp. (cooked meat medium). Only the anaerobic strains were grown at 28 °C in an anaerobic jar (Mart Microbiology B.V., Drachten, The Netherlands) for 7 days; the others were grown overnight at 37 °C with shaking. After the growth period, the bacterial agents were centrifuged, and the supernatants were transferred to fresh aseptic micro-tubes, stored at 4 °C until use.

2.6. Food sample preparation

A variety of food samples were divided into liquid, solid, and semi-solid categories. Each component was tested separately.

2.6.1. Liquid foods

For liquid foods, 5 mL of each sample was fully mixed with SEB to 100 ng/mL, then incubated at room temperature (25 °C) for 30 min. Samples were subsequently centrifuged at 3000 g (8178 swing-out rotor, Labofuge 400R; Heraeus Instruments) for 30 min at 4 °C, to remove any solid particles. Afterward, 500 µL of the supernatant was carefully mixed with 500 µL of 0.01 M phosphate-buffered saline (PBS; pH 7.4) and stored at 4 °C until use.

2.6.2. Semi-solid and viscous foods

For semi-solid and viscous foods (e.g., honey and ice cream), 10 g of each sample was first diluted with the same volume of 0.01 M PBS (wt./vol.); 5 mL of the mixture was then spiked with SEB (final concentration, 100 ng/mL) and homogenized with a blender, then incubated at room temperature for 30 min. After incubation, the samples were centrifuged to remove solid particles and/or the lipid layer. Then, 500 µL of the supernatant was thoroughly mixed with 500 µL of 0.01 M PBS, and stored at 4 °C.

2.6.3. Solid food samples

For solid food samples, 10 g of each sample was chopped into pieces, diluted with 0.01 M PBS (1:1, wt/vol), and homogenized with a blender. Afterwards, 5 mL of the slurry was fully spiked with SEB (final concentration, 100 ng/mL), followed by incubation, dilution, centrifugation and mixed with PBS as previously described, the mixtures were stored at 4 °C before use.

Before analysis, all samples were left at room temperature for 1 h.

3. Results

3.1. Sensitivity of SEB test strips

In our previous study (Shyu et al., 2010), we successfully developed an SEB test strip kit, which could rapidly detect SEB at concentrations as low as 10 ng/mL without cross-reactivity against
other tested SEs. Results were determined by the appearance (positive result) or absence (negative result) of a red line in the test area, under conditions in which a red line could be visualized in the control area (Fig. 2). Analyses were completed in less than 10 min, and all the results were highly reproducible throughout the assay. The intensity of the red colour (in the test area) is proportional to the toxin concentration. However, in the absence of SEB (buffer only), no immunogold is bound to the solid-phase test antibody; hence, no coloured lines develop in the test region.

3.2. Analysis of consistency among LFA, PCR, and RPLA results

Seventy-seven isolated clinical S. aureus strains were simultaneously analyzed by the LFA, PCR, and RPLA assays for SEB detection. The results, shown in Table 1, revealed that S. aureus strains isolated from various clinical human specimens were highly SEB enterotoxigenic. In a total of 77 samples, 48 (62.34%) were determined to be SEB positive by LFA. This positive rate was comparable to the result of 42 (54.55%) analyzed by PCR assays and 44 (57.14%) analyzed by RPLA. The chi-square test for positive ratio revealed no significant difference (p > 0.05) between the results of these assays. Further analyses demonstrated that 42 of 48 positive samples analyzed by LFA were positively verified by PCR, and the remaining 29 negative samples determined by LFA were all confirmed negative by PCR. The ratio of positive and negative consistency for the 2 methods was 87.50% and 100%, respectively (Table 2-1). The Cohen’s kappa coefficient (κ) of these 2 methods was 0.841, suggesting equal agreement between the 2 methods (p < 0.001). Compared with RPLA, 44 of 48 positive samples determined by LFA were positively verified by RPLA. Again, the 29 negative samples analyzed by LFA were also fully confirmed by RPLA assays (κ = 0.892, p < 0.001; Table 2-2). Comparing PCR and RPLA, all of the PCR positive samples had been recognized by RPLA, and 33 of 35 PCR negative samples were negatively identified by RPLA tests (κ = 0.947, p < 0.001; Table 2-3). These results suggest that, among the 3 methods, there is no significant difference in detecting SEB from isolated clinical S. aureus strains.

3.3. Cross-reactivity of SEB test strips to enteropathogens

The specificity of SEB test strips among different SEs has previously been verified (Shyu et al., 2010), and the strips were now tested to examine cross reactivity to different food-borne enteropathogens. Various ATCC enteropathogens were divided into 4 groups (Table 3). For each individual sample, 80 μl of culture supernatant was analyzed using SEB test strips. As shown in Table 3, only the strain ATCC 14458 (SEB producing strain) showed a positive result, whereas all other bacterial strains, such as Salmonella, Vibrio and Clostridium spp., did not show red signals in the “test” region (negative results). This means that, although a polyclonal antibody was used in the SEB strip kit for the assays, no cross reaction occurred when the kits were tested with other selected bacteria. All the tests were accomplished in approximately 10 min.

3.4. Evaluation of SEB test strips for food analysis

A wide variety of food samples, including liquid, solid, and semi-solid foods, were applied to SEB strips for SEB detection (Table 4). If the samples were too viscous or the slurry was too thick, samples were diluted in 0.01 M PBS. Table 4 shows that after 10 min, all diluted food samples spiked with SEB toxin showed positive results, whereas samples without SEB showed negative results.

In the 26 food samples tested (with SEB), only 6 liquid samples which showed positive results within 10 min in undiluted forms. They are fruit–vegetable juice, cola, black tea, soybean milk, bottled water and energy drink. However, for apple juice, orange juice, and grape juice, although these samples were filtered through a Whatman No. 4 filter paper (Whatman Laboratory Division, Springfield Mill, Kent, UK), the strip membrane still turned considerably red, olive yellow, and purple, making it difficult to read the results during test times.

Most viscous samples or semi-solid samples like ice cream or honey did not show detectable results in their undiluted form (with/without SEB) because these samples were too viscous to reach the detection window. These results remained unchanged even after 30 min (except for yogurt). However, the assays were accomplished within 10 min after the samples were diluted with PBS, and the results were a clear positive (with SEB) or negative (without SEB). Undiluted solid food samples also failed to show detectable results in the first 10 min, but specific results were
The consistency ratios between LFA and RPLA.

<table>
<thead>
<tr>
<th></th>
<th>PCR positive</th>
<th>PCR negative</th>
<th>RPLA total</th>
<th>RPLA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPLA positive</td>
<td>42</td>
<td>2</td>
<td>44</td>
<td>57.14</td>
</tr>
<tr>
<td>RPLA negative</td>
<td>0</td>
<td>33</td>
<td>33</td>
<td>42.86</td>
</tr>
<tr>
<td>PCR total</td>
<td>42</td>
<td>35</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>PCR (%)</td>
<td>54.55</td>
<td>45.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The ratio of positive and negative consistency for these 2 methods is 91.67% and 100%, respectively. The kappa coefficient of these 2 methods was 0.892, p < 0.001.

The consistency ratios between PCR and RPLA.

<table>
<thead>
<tr>
<th></th>
<th>LFA positive</th>
<th>LFA negative</th>
<th>RPLA total</th>
<th>RPLA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPLA positive</td>
<td>44</td>
<td>0</td>
<td>44</td>
<td>57.14</td>
</tr>
<tr>
<td>RPLA negative</td>
<td>4</td>
<td>29</td>
<td>33</td>
<td>42.86</td>
</tr>
<tr>
<td>LFA total</td>
<td>48</td>
<td>29</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>LFA (%)</td>
<td>62.34</td>
<td>37.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The ratio of positive and negative consistency for these 2 methods is 100% and 94.29%, respectively. The kappa coefficient of these 2 methods was 0.947, p < 0.001.

Table 3

Application of SEB strip in analysis of enteropathogen.

<table>
<thead>
<tr>
<th>Species</th>
<th>ATCC</th>
<th>Strip results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staph. aureus (SEB)</td>
<td>14458</td>
<td>+</td>
</tr>
<tr>
<td>Staph. epidermidis</td>
<td>14990</td>
<td></td>
</tr>
<tr>
<td>Staph. haemolyticus</td>
<td>29968</td>
<td></td>
</tr>
<tr>
<td>Staph. hominis</td>
<td>29885</td>
<td></td>
</tr>
<tr>
<td>BHI medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>14028</td>
<td></td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>11835</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli O157H7</td>
<td>43895</td>
<td></td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>23715</td>
<td></td>
</tr>
<tr>
<td>Vibrioaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio cholera O1</td>
<td>9458</td>
<td></td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>17802</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>9818</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>7644</td>
<td></td>
</tr>
<tr>
<td>Tryptic soy broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium botulinum A</td>
<td>7948</td>
<td></td>
</tr>
<tr>
<td>Clostridium botulinum B</td>
<td>7949</td>
<td></td>
</tr>
<tr>
<td>Clostridium botulinum E</td>
<td>17852</td>
<td></td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>3624</td>
<td></td>
</tr>
<tr>
<td>Cooked meat medium</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The analysis was divided in four groups: (A) some Staphylococcus spp.; (B) enterobacteriaceae group; (C) several vibrio pathogens and D, 4 anaerobic bacteria. All the bacteria were grown in BHI (brain–heart infusion) medium, except L. monocytogenes (tryptic soy broth) and 4-anaerobic Clostridium spp. (cooked-meat medium). The results shown in this table suggest that no cross reaction occurred in the SEB strip analysis.

Observations were made in 30 min. In the diluted form, all samples in this category showed clear results within 10 min of assay time.

4. Discussion

The heat-stable staphylococcal enterotoxins (SEs) produced by S. aureus have proved to be the leading cause of food poisoning in the United States and other countries, and SEB is believed to be the major trigger. Therefore, the development of sensitive detection methods to monitor levels of SEB toxin in food or clinical samples is essential. In our previous report, we described the development of SEB strip kits to detect SEB both in serum and environmental specimens, and now aimed to evaluate the SEB strip as an early warning tool to detect SEB in clinical and food samples. In this study, 77 strains of S. aureus isolated from various clinical wounds were chosen for analysis. The strip kits, accompanied by 2 other diagnostic assays (PCR and RPLA), were employed for the evaluation. Although it is possible to detect the toxin by using other methods such as enzyme-linked immunosorbent assays and radioimmunoassays, the chosen assays in this study are quicker, sensitive, reliable, and reproducible (Boynukara et al., 2008; Letertre et al., 2003; Shyu et al., 2010).

The results presented in Table 1 illustrate the positive rate of SEB detection for each assay: LFA strips, 62.34%; PCR, 54.55%; and RPLA, 57.14%. The chi-square test for positive ratio revealed no obvious difference in these assays ($\chi^2 = 0.99; p > 0.05$); this suggested that the sensitivity of the LFA is comparable to the highly sensitive PCR and RPLA methods. However, the detection time for LFA strips is approximately 10 min, which is much faster than the time required for PCR (2 h) and RPLA assays (more than 10 h). Furthermore, the LFA results can be read directly by the naked eye without any equipment or skilled technicians; hence, LFA strip tests can be easily performed in hospitals by medical care/examination personnel. In other words, the LFA-strip is a powerful tool for detection of SEB, which can be adapted for on-site surveillance and diagnosis of the toxin.

Table 2-2 (2-1, 2-2, and 2-3) show the consistency ratios between selected pairs of methods. The positive consistencies and Kappa tests for the methods indicate that every 2 assays have almost perfect agreement for the same test subject, although the Kappa test does not reveal which is better.

Both RPLA and PCR have proved to be sensitive and specific immunomethods for detection of SEB (Letertre et al., 2003; Panneerselvan & Muriana, 2009). However, the potential for cross contamination, long turnaround times, high cost, and the need for specialized equipment and trained staff may deter some laboratories from using PCR and RPLA for clinical diagnosis. In addition, using different primer sets for the PCR assay is likely to result in amplification of different products. In our study, the oligonucleotide primers we initially used for the PCR assay were based on published data (Johnson et al., 1991), which gave positive and negative consistency between LFA and PCR of 87.5% and 100%, respectively. However, when we employed another set of primers (sequence not shown), both the positive and negative consistency between the assays was found to be 100%. In other words, these 2 methods provided identical results in detecting SEB from the 77 clinical samples when we used the second set of PCR primers. Previous studies have shown that SEB-producing strains can be classified into a number of subtypes (Goh, Byrne, Zhang, & Chow, 1992; Vimercati et al., 2006); we therefore assume these 2 different PCR results may be due to a limited number of subtypes present in the S. aureus strains.

In the second part of our study, all food samples used were either prepared in the laboratory or purchased from a local market. Solid and semi-solid samples were used as slurries prepared by combining 1 part food with an equal part PBS buffer and homogenizing the mixture in a blender. Since the undiluted high-viscosity content foods were unable to reach the detection window within 10 min, an extra 20 min treatment was allowed for the samples to flow forward to the detection window. However, according to our experience, red lines appearing in the test zone of the strip after 20 min were not meaningful and should be ignored. Hence, the results of solid samples (plus the semi-solid yogurt) in undiluted form, shown in Table 4, are regarded as invalid. Only the diluted samples, in which the red lines appeared in both the test and control areas in less than 10 min, were considered significant.

As shown in Table 4, all food samples in diluted form gave obvious positive results within

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The consistency ratios between PCR and RPLA.

<table>
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The ratio of positive and negative consistency for these 2 methods is 91.67% and 100%, respectively. The kappa coefficient of these 2 methods was 0.892, p < 0.001.

The consistency ratios between LFA and RPLA.

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<tbody>
<tr>
<td>RPLA positive</td>
<td>44</td>
<td>0</td>
<td>44</td>
<td>57.14</td>
</tr>
<tr>
<td>RPLA negative</td>
<td>4</td>
<td>29</td>
<td>33</td>
<td>42.86</td>
</tr>
<tr>
<td>LFA total</td>
<td>48</td>
<td>29</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>LFA (%)</td>
<td>62.34</td>
<td>37.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The ratio of positive and negative consistency for these 2 methods is 100% and 94.29%, respectively. The kappa coefficient of these 2 methods was 0.947, p < 0.001.

Table 3

Application of SEB strips in analysis of enteropathogen.
Table 4
Detection of SEB in various food samples.

<table>
<thead>
<tr>
<th>Food matrix</th>
<th>No SEB spiking</th>
<th>Spiked with SEB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undiluted 10 min 30 min</td>
<td>Diluted 10 min 30 min</td>
</tr>
<tr>
<td>Liquid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Apple juice</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Grape juice</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Coffee</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Fruit–vegetable juice</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Cola</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Black tea</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Soybean milk</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Bottled water</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Energy drink</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Semi-solid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honey</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Ice cream</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Yogurt</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Ketchup</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Jelly</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Lemon cream pie</td>
<td>ND* ND*</td>
<td>ND* ND*</td>
</tr>
<tr>
<td>Solid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato chips</td>
<td>ND*</td>
<td>— —</td>
</tr>
<tr>
<td>Broccoli</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Burg–meat</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Fried chicken</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Smoked salmon</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Smoked turkey</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Sausage</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Cured beef</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Carp</td>
<td>ND* —</td>
<td>— —</td>
</tr>
<tr>
<td>Shrimp</td>
<td>ND* —</td>
<td>— —</td>
</tr>
</tbody>
</table>

* Not detectable.

10 min. The assay results were sufficiently robust, indicating that SEB strip kits can detect SEB successfully from different simulated food samples. On the other hand, some purified samples (after 30 min centrifugation) generated a more ambiguous signal than the unpurified samples, suggesting that some cross-reactive materials had been removed from the samples. The detection limits of the SEB strip kits in selected food matrices ranged from 10 to 100 ng (data not shown). Previous studies have reported that as little as 100 ng of SEB may make a person ill with symptoms of classic food poisoning (Asao et al., 2003; Evenson, Hinds, Berstein, & Bergdoll, 1988), and the LFA strip seems to have the ability to meet the requirements for detecting the toxin at the concentration that causes food poisoning in humans.

LFAs are based on the immunochromatographic procedure and capillary forces. In previous study, we found that several parameters, such as the concentration of tested samples, size of colloidal gold particles, amount of colloidal gold–antibody mixture, and types of lateral flow membranes, can affect the sensitivity and reproducibility of the assay (Chiao et al., 2004; Shyu et al., 2002, 2010). In addition, because the assay results are characterized by formation of red lines, the identification of a weak-positive result would depend on the skill of the user. Therefore, the sensitivity of the LFA is limited to what can be seen by the naked eye and is lower than that of sensitive instrumentation such as a spectrophotometer or a fluorescent reader. Nevertheless, LFAs can still be used as an early warning tool for the detection of SEB toxins in various food samples.

5. Conclusion

This study has demonstrated the ability of the SEB strip kits to detect SEB both in isolated clinical S. aureus strains and in complex food matrices. The assays are quick, simple to perform, show no cross-reactivity, and require little or no sample pretreatment. In conclusion, the LFA test strip is not only a highly specific and sensitive method for detecting SEB in patient specimen but also a powerful early warning tool for the on-site surveillance and diagnosis of SEB.

Acknowledgements

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References


