

## Development of Highly Sensitive and Quick Determination Using Fluorescent Immunoassay – Examination of Inhibitors

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### Objective

For assuring the safety of foods, it is absolutely necessary to search foods for food poisoning microbes. However, the conventional food poisoning microbe inspection performed based on culture requires enormous time and skill till the final result can be obtained. So, it is desired to develop and establish a quick and simple inspection method. We are examining methods for quickly inspecting Staphylococcal enterotoxins A, B and C2, enterohemorrhagic *E. coli* O157 and verotoxin using fluorescent immunoassay. Recently we examined the possibility of establishing a highly accurate inspection method capable of identifying the enterohemorrhagic *E. coli* O157 (hereinafter called *E. coli* O157) among food poisoning microbes in one day using a fluorescent immunoassay instrument. The results are reported below.

### Materials and method

As the anti-*E. coli* O157 antibody, a commercially available antibody (produced by KPL) was used. The microbes used as specimens were 16 strains of *E. coli* O157 and 6 strains of *Citrobacter. sp* preserved in Tokyo Kenbikyoin Foundation.

#### Experiment 1: Detection Sensitivity Test

The microbes used as specimens were subcultured in TSB twice and diluted by PBT (obtained by adding 0.5% of Tween 20 to 0.01M PB) to  $10^2$  to  $10^7$  cfu/mL, for obtaining the detection sensitivity.

#### Experiment 2: Food Inoculation Tests

$1.1 \times 10^3$  cfu/25 g of *E. coli* O157 was inoculated into commercially available 14 items of food, and proliferated at 42°C for 18 hours. Then, the present instrument and an existing quick determination instrument were used to detect *E. coli* O157. Furthermore, *E. coli* O157 was inoculated into 10 g each of 15 items of fishes, shellfishes and household dishes, to achieve a concentration of 10 cfu/mL or 10 to 30 cfu/mL, and 90 ml of TSB was added. The mixtures were cultured at 42°C for 6 hours, to obtain specimens. They were assayed using the present instrument.

#### Experiment 3: Examination of Inhibitors

The removal of inhibitors such as fats affecting the test results was examined. Furthermore, since some food items showed false positive results, the causes were clarified.

### Results and discussion

With regard to the detection sensitivity of the present instrument for *E. coli* O157, when the bacterial number was larger, the detected signal was higher. Viable cells were detected 92% at  $10^4$  cfu/mL and 100% at  $10^5$  cfu/mL, and dead cells were detected 98% at  $10^4$  cfu/mL and 100% at  $10^5$  cfu/mL. Furthermore, among the six strains of *Citrobacter. sp* binding to O157 serum, four strains were judged to be positive by the present instrument. In the inoculation tests using food by both the detection instruments, *E. coli* O157 was detected from all the specimens after

proliferation for 18 hours. Moreover, when the present instrument was used for assaying after culturing for 6 hours, 13 out of 15 items of food showed positive results after both reaction time periods of 5 minutes and 10 minutes, in the case where the inoculated bacterial number was 10 cfu/mL or less. Furthermore, in the case where it was from 10 to 30 cfu/mL, 12 out of 14 items of food showed positive results after a reaction time period of 5 minutes, and 13 out of 14 items of food showed positive results after a reaction time period of 10 minutes. In the detection after culturing for 6 hours, foods containing much fat such as minced beef and vegetables showed a phenomenon in which the detection was inhibited. However, when an adsorbent and other additives were added to the food items containing much lipids such as minced beef or when skim milk was added to vegetables, *E. coli* O157 could be detected. This suggests that some food items can show false positive results, depending on the strains existing in the food.

As described above, if the present instrument is used, *E. coli* O157 can be detected in one day, and furthermore, if a probe is applied to an isolation medium such as CT-SMAC and cultured, whether or not *E. coli* O157 grows can be confirmed. So, this method is considered to be a quick and highly accurate detection method.



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