A Method for Quickly Detecting Pathogenic Microbes by Fluorescent Immunoassay

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Objective

To assure 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 until the final result can be obtained. So, it is important to develop and establish a quick and simple inspection method. Recently we examined the possibility of establishing an 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.

Assay principle and time

This fluorescent immunoassay instrument is an automatic inspection instrument using evanescent light. As for the assay principle, a probe having a specific antibody solid-phased in a part of the fiber portion of a fiber (made of polystyrene) with a lens is immersed in a test solution, to react with an antigen, and further to react with a fluorescent specific antibody. The antigen-antibody reaction is determined by introducing a semiconductor laser from the lens surface and converting the fluorescence excited by the evanescent light leaking from the fiber surface into an electric signal. The time taken for assaying four specimens is about 1 hour.

Materials and method

The anti-*E. coli* O157 antibody was prepared by using a commercially available antibody (produced by KPL). The microbes used as specimens were 27 strains of *E. coli* O157, 1 strain of *C. fruendii* and 1 strain of *E. coli* respectively preserved in Tokyo Kenbikyoin Foundation.

- Experiment 1: Detection Sensitivity Test
  The microbes used as specimens were subcultured in TSB twice, and diluted by 0.1% peptone physiological salt solution to 10^4, 10^3 and 10^2 cfu/ml, for obtaining the detection sensitivity.

- Experiment 2: Influence of Microbial Impurity
  The microbes used as specimens were subcultured in TSB twice, and 0.1% peptone physiological salt solution was used to adjust *E. coli* O157 to 10^3 cfu/ml and 10^4 cfu/ml. Furthermore, *E. coli* was adjusted to 10^4, 10^5 and 10^6 cfu/ml, and an equal amount of 10^3 cfu/ml *E. coli* O157 solution or 10^4 cfu/ml *E. coli* solution was mixed with each of the *E. coli* solutions, to prepare test solutions.

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- Experiment 3: Food Inoculation Tests
  E. coli O157 was added to a solution obtained by adding 225 ml of sterilized phosphate buffer to 25 g of commercially available boiled scallop, minced beef, cow's milk or apple juice to prepare specimens respectively containing 10^3 or 10^4 cfu/ml of *E. coli* O157. Furthermore, *E. coli* O 157 was inoculated into 25 g of boiled scallop, minced beef, cow's milk or apple juice to achieve an *E. coli* O157 concentration of 3 to 12 cfu/g, and 225 ml of TSB was added. The respective mixtures were cultured at 42°C for 6 to 7 hours, to prepare specimens.

Results and Discussion

With regard to the detection sensitivity of this instrument, when the bacterial number of *E. coli* O157 was larger, the detected signal was higher. At 10^2 cfu/ml, 4 strains out of 27 strains were positive, but at 10^3 cfu/ml, 17 strains were positive, while at 10^4 cfu/ml, 21 strains were positive. As for the detection of *E. coli* O157 in the presence of microbial impurity, *E. coli* O157 could be detected even when the bacterial number of *E. coli* was 100 times that of *E. coli* O157. The test solution with *E. coli* concentration kept at 10^6 cfu/mu was negative. In the inoculation tests using food, good results could be obtained with boiled scallop, but detection results were irregular depending on the kinds of food. As a feature of this instrument, when a probe obtained by reaction with a culture solution was applied to an isolation medium such as CT-SMAC, and cultured for a day and night, the growth of *E. coli* O157 could be observed.

The above results show that with this instrument, *E. coli* O157 can be detected in a day and that colonies can be confirmed on the next day. Therefore, this method is considered to be a quick and highly accurate detection method.

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