Antibody-Direct Epifluorescent Filter Technique for Rapid, Direct Enumeration of Escherichia coli O157:H7 in Beef

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Artificially inoculated Escherichia coli O157:H7 was directly enumerated in ground beef and beef exudate, without enrichment or selection, by the antibody-direct epifluorescent filter technique (Ab-DEFT). The total assay time of the Ab-DEFT was less than 1 h. The beef was homogenized, treated for 15 min with trypsin and Triton X-100, and passed through a 5-μm-pore-size prefILTER and then through a 0.2-μm-pore-size black polycarbonate filter. The final filter was stained directly with fluorescein-labeled anti-O157 polyclonal antibody, rinsed, and examined by epifluorescence microscopy. The sensitivity of the Ab-DEFT was compared with that of a standard enrichment culture technique. Both methods reliably determined the presence of the pathogen in beef at 16 CFU/g. The Ab-DEFT was also useful for quantifying the pathogen and monitoring its growth in beef.

The direct epifluorescent filter technique (DEFT), an extremely rapid microbiological analytical method, has been used for many years for direct quantitation of microbial load in a variety of applications (22), e.g., environmental testing (11), pharmaceutical manufacturing (9), and predicting the shelf life of food (29). The DEFT requires only 20 min of assay time for determination of microbial numbers in raw milk (5, 23); elimination of overnight incubation is a major advantage. Rapid microbiological testing is valuable in food manufacturing because the quality of materials coming into the production line is often a critical control point, and assay speed is an important economic consideration.

In the DEFT, microorganisms are entrapped on a membrane filter, which is stained with a fluorescent dye, e.g., acridine orange or acridine orange-gold, and examined by epifluorescence microscopy. The total microbial population is then rapidly enumerated. For specific rather than general staining of bacteria, fluorescent antibodies are used. Indirect fluorescent-antibody staining has been adapted to the DEFT for specific enumeration of Salmonella (28) and Listeria (31) organisms in raw meats. A direct (one-step) fluorescent-antibody modification, the antibody-DEFT (Ab-DEFT), was developed to enumerate Escherichia coli O157:H7 in milk and juice (34).

The low sensitivity of traditional microscopic methods is greatly improved in both the DEFT and Ab-DEFT by filter concentration of the material to be analyzed and elimination of irrelevant substances by passage through the filter. The presence of these substances, which often interfere with the outcome of the application of analytical techniques, is an important reason for requiring enrichment and isolation steps before the use of highly specific assays, such as the enzyme-linked immunosorbent assay and PCR.

Food-borne illnesses associated with E. coli O157:H7 have been traced to consumption of undercooked ground beef (8). This report compares the sensitivity of the Ab-DEFT with that of a conventional enrichment culture (EC) procedure for identifying E. coli O157:H7 in ground beef. The Ab-DEFT has also been useful in determining cell numbers of the microorganism in beef exudate and for monitoring growth and survival in beef.

MATERIALS AND METHODS

Bacteria and media. E. coli O157:H7 (ATCC 35150) as well as 17 clinical isolates (University of Iowa Hygienic Laboratory) were cultured at 37°C in Luria-Bertani broth supplemented with 0.1% glucose. A dihydrostreptomycin-resistant variant of the ATCC 35150 strain, E. coli DHS-1, was isolated by successive culture in increasing concentrations of dihydrostreptomycin (Sigma Chemical Co., St. Louis, Mo.) by the gradient plate technique (7) and cultured at 37°C in Luria-Bertani broth supplemented with 0.1% glucose and 1,000 μg of dihydrostreptomycin per ml. All microbiological culture media were purchased from Difco Laboratories (Detroit, Mich.).

Preparation of ground beef and beef serum for microbiological analysis. Ground beef, 80% lean, was purchased from a local retail store. Log-phase bacterial cells, diluted appropriately in 0.01 M phosphate-buffered saline (PBS) (13), pH 7.2, were used in all artificial inoculations of beef and beef exudate. Before inoculation, the log-phase cells were plated onto MacConkey sorbitol agar (MSA) (Difco) and incubated at 37°C for 18 to 24 h to confirm the population size. Cells (1 to 10 ml) were mixed directly into the ground beef. The inoculated beef was placed within the inner mesh lining of a plastic stomacher bag (model 400 filter bags; Seward Medical, London, United Kingdom), and diluent was added for homogenization. For DEFT and Ab-DEFT analyses, 10 g of the inoculated beef was added to 90 ml of PBS; for the EC method, 25 g was added to 225 ml of enrichment broth (17). The beef was then homogenized for 2 min in a stomacher model 400 (Tekmar Co., Cincinnati, Ohio), and the slurry that passed through the mesh lining was harvested. Beef exudate, i.e., the liquid that accumulates during shipment of unprocessed raw beef, was obtained from a local meat processor, and after inoculation with bacterial cells was treated for analysis.

DEFT and Ab-DEFT. For analysis by DEFT and Ab-DEFT, ground beef slurry and beef exudate were treated by modifications of previously described protocols (6, 24, 34). Filters housed in Swinnex-type filter holders, syringes, and reagents

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were prewarmed by incubation at 50°C to facilitate filtration. To 4 ml of beef slurry or beef extract, 4 ml of 0.5% (wt/vol) Triton X-100 and 1 ml of trypsin (Sigma) prepared in PBS at 10 mg/ml were added. The ground beef mixture was incubated at 50°C for 15 min before filtration. The beef extract mixture was incubated at 37°C for 15 min before filtration. Plastic 10-ml syringes with Luer-Lok tips were used in the filtrations. The homogenized beef was passed through 5-µm-pore-size nylon filters (Micon Separations, Inc., Westboro, Mass.). For beef extract, 5-µm-pore-size polycarbonate filters (Poretics Corp., Livermore, Calif.) were used. After prefiltration, portions (2 to 100 ml) of the beef or beef extract filtrates were passed through 0.2-µm-pore-size black polycarbonate filters (Poretics). If necessary, the mixture was diluted appropriately in PBS after prefiltration to obtain 10 to 100 cells per microscope field for ease in counting. The polycarbonate filters were rinsed by passing them through 5 ml of 0.1% (wt/vol) Triton X-100 and 5 ml of PBS before staining with acridine orange (DEFT) or fluorescent antibody (Ab-DEFT).

The DEFT and Ab-DEFT were performed as described previously (34), with the following modifications. P5 filter paper discs (Fisher Scientific, Pittsburgh, Pa.) were placed under the nylon filters and black polycarbonate filters to provide support in Swinnex-type filter holders. For the DEFT, after acridine orange staining, the black polycarbonate filters were washed with 5 ml of PBS, air dried, and mounted on glass slides in low-fluorescence immersion oil. For the Ab-DEFT, fluorescein-labeled, affinity-purified polyclonal antibody to E. coli O157:H7 (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) was diluted 1:2,000 in 1% (wt/vol) bovine serum albumin in PBS and layered on top of the black polycarbonate filters for 15 min; this was followed by rinses of 5 ml of PBS-0.05% Tween 20 and 5 ml of PBS. The fluorescein-antibody-stained filters were mounted on glass slides in Vectashield mounting medium (Vector Laboratories, Burlingame, Calif.).

EC. EC was performed as recommended for the isolation and identification of E. coli O157:H7 in ground beef (18). Briefly, the EC procedure involved 24 h of enrichment in modified EC-novobiocin broth (17) followed by selective isolation of E. coli colonies from the enrichment on MSA supplemented with 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (BCIG) (Biodynamics International, Skokie, Ill.). A modification involved use of the sodium salt of BCIG instead of the cyclohexylaminon salt in MSA for selective isolation of E. coli colonies from the enrichment broth. The substitution allowed the BCIG reagent to be solubilized directly in the aqueous MSA (MSA-BCIG) (15) instead of in alkaline ethanol. The Minitek System (Becton Dickinson, Cockeysville, Md.) was used to characterize presumptively positive colonies by indole production, H₂S production, colibactin utilization, Voges-Proskauer reaction, citrate utilization, lysine decarboxylase, and ornithine decarboxylase. Serological identification was performed with the Oxoid E. coli O157 Test Kit (Unipath Ltd., Hampshire, United Kingdom).

**Bacterial cell counting.** Bacterial cells were counted by the DEFT and Ab-DEFT as described previously (34). Membrane filter microscope factors (MMFM) were used to calculate cell concentrations and depended on the microscope objective lens used: 1,670 for the 40× lens, 3,959 for the 63× lens, and 9,440 for the 100× lens. The bacterial cell concentration was calculated as follows: number of cells per milliliter = (average cell count/microscope field) (MMFM) (dilution factor)/volume filtered (milliliters).

For counting bacteria by viable plate counts (VPC), dilutions were made in PBS and then spread plated on agar media and incubated at 37°C for 18 to 24 h. For total aerobic plate counts, colonies cultured on standard plate counts agar were counted. For E. coli O157:H7, white colonies cultured on MSA, indicative of sorbitol nonfermenters, were counted. For E. coli DHS-1, white colonies cultured on MSA supplemented with 1,000 mg of dihydrostreptomycin per ml were counted. All VPC data were expressed in CFU.

### Experimental design for comparison of the sensitivities of Ab-DEFT and EC

Ground beef was artificially inoculated with E. coli O157:H7 at concentrations of approximately 10², 10³, 10⁴, 10⁵, and 1 CFU/g. For each inoculation level, log-phase cells were diluted appropriately in PBS, and portions (1 to 10 ml) were mixed into 125 g of ground beef, which was then divided into triplicate 10-g and 25-g portions. The 10-g portions were analyzed by the Ab-DEFT, and the 25-g portions were analyzed by the EC method.

### RESULTS

**Comparison of the sensitivities of Ab-DEFT and EC.** The abilities of the Ab-DEFT and EC methods to determine the presence of E. coli O157:H7 artificially inoculated into ground beef were compared (Table 1). Both methods identified all three replicates of the inoculated beef at cell concentrations of 2.4 × 10⁴ to 16 CFU/g of beef as positive. However, at 2 CFU/g of beef, neither method was reliable. The Ab-DEFT lacked sensitivity at this level: no positive identifications were made for the three replicate beef portions. The EC method, which identified one of the three replicates as contaminated, was more sensitive than the Ab-DEFT. This result was supported by a second trial of triplicate beef test portions inoculated at 2 CFU/g, in which the EC again identified one of three inoculated beef replicates as positive.

In the description of the EC method, positive identifications were reported for 11 of 12 beef test portions artificially inoculated at 0.6 to 0.7 CFU/g (18). Because our results did not

<table>
<thead>
<tr>
<th>CFU inoculated per g of beef</th>
<th>Replicate</th>
<th>Ab-DEFT</th>
<th>EC</th>
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<tbody>
<tr>
<td>240,000</td>
<td>1</td>
<td>+</td>
<td>320,000</td>
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<tr>
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<td>2</td>
<td>+</td>
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<td>3</td>
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* Calculated from VPC of log-phase culture on MSA.

ND, not determined.
agreed with this reported sensitivity, we tested additional inoculation levels of 0.6 and 0.1 CFU/g by EC. (The Ab-DEFT was not tested at these inoculation levels.) At 0.6 CFU/g, the EC method identified one of three inoculated beef replicates as positive; at 0.1 CFU/g, none of the three replicates was identified as positive.

The detectability of *E. coli* O157:H7 by the Ab-DEFT may be assessed for each inoculation level by considering the number of microscope fields examined, the number of positive microscope fields, and the volume of beef filtered (Table 2). At 240,000 CFU/g, with only a 2-ml test portion filtered, every field examined contained fluorescent cells. At lower levels of inoculation, larger test portions were necessary and more fields had to be examined for identification. At the lowest inoculation level of 2 CFU/g of beef and a filtration volume of 100 ml, the Ab-DEFT result was recorded as negative after 500 fields per filter were examined for each replicate. If it is assumed that none of the 500 fields overlapped, then the area examined in each case represented approximately 30% of the total filter area.

**Table 2. Detectability of artificially inoculated *E. coli* O157:H7 by Ab-DEFT**

<table>
<thead>
<tr>
<th>CFU inoculateda per g of beef</th>
<th>Filtered (ml)</th>
<th>Replicate</th>
<th>No. of fieldsb examined</th>
<th>No. of positivec fields</th>
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<tbody>
<tr>
<td>240,000</td>
<td>2</td>
<td>1</td>
<td>20</td>
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<tr>
<td></td>
<td></td>
<td>3</td>
<td>500</td>
<td>0</td>
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</table>

a) Calculated from VPC of log-phase culture on MSA.

b) Homogenized beef treated with trypsin and Triton X-100 for 15 min at 50°C (see Materials and Methods).

c) Number of microscope fields examined with 40× objective lens.

d) Number of microscope fields that showed the presence of at least one fluorescent cell.

**Specificity of the Ab-DEFT.** The sensitivity of the Ab-DEFT is due in part to the specificity of the fluorescent antibody used in the method. The photomicrographs in Fig. 1 demonstrate the clarity of staining and high intensity of fluorescence achieved by using this reagent for analysis of ground beef inoculated with *E. coli* O157:H7 at a concentration of 2 × 10⁶ CFU/g of beef. Figure 1A shows acridine orange staining of the inoculated beef; Fig. 1B shows specific antibody staining of the same beef mixture. In Fig. 1A, a dense population of many types of cells is visible because the acridine orange stained the indigenous microbial population as well as the inoculated pathogenic cells. Figure 1B, however, shows only a few fluorescent rod-shaped cells. The dense background population was not stained by the O157:H7-specific antibody. Repeated testing of uninoculated beef samples failed to demonstrate positive Ab-DEFT reactions.

The specificity of the fluorescent antibody was further tested by reactivity to pure cultures of the following bacteria in the Ab-DEFT, and the antibody was found to be nonreactive: 8 non-O157:H7 *E. coli* strains (including *E. coli* O18A:H7), *Escherichia blattae*, *Escherichia hermannii*, 12 *Salmonella* strains, 3 *Staphylococcus aureus* strains, 2 *Vibrio* species, 2 *Listeria* species, *Enterococcus faecalis*, *Serratia marcescens*, *Yersinia enterocolitica*, *Streptococcus agalactiae*, a *Citrobacter* sp., a *Providencia* sp., an *Enterobacter* sp., *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*. In addition, 17 clinical isolates of *E. coli* O157:H7 reacted to the fluorescent antibody in the
Ab-DEFT. However, a false-positive reaction to Salmonella cholerasuis serotype urbana was observed.

Enumeration of E. coli O157:H7 in beef and beef exudate. Quantitation of contamination levels of E. coli O157:H7 in ground beef was possible by the Ab-DEFT but not by the EC method. With the Ab-DEFT, specific microbial cell concentrations were measured in the beef, generally with an accuracy within an order of magnitude of the inoculation levels.

In previous studies there was inconsistent correlation between inoculated cell counts and DEFT filter counts, depending on the food analyzed (24). Poor correlations may be related to the prefiltration step. The 5-μm-pore-size nylon filter is necessary for removal of large particulates and facilitates filtration of the food through the analytical 0.2-μm-pore-size filter. However, the prefilter may be a barrier for microbial cells entrapped within the particulates and may cause lower-than-expected cell counts on the 0.2-μm-pore-size filter. For ground beef, virtually no cells were lost from the treated mixture after prefiltration through 5-μm-pore-size nylon filters (Fig. 2). This finding supports previous data obtained in similar studies in which the DEFT was used for analysis of fresh meat and fish (24).

Bacterial cell enumeration by Ab-DEFT was also demonstrated for beef exudate, which accumulates in containers of packed beef during transport and is a convenient medium for microbiological testing in meat processing plants. Figure 3 shows the results of the Ab-DEFT analysis of beef exudate inoculated with E. coli O157:H7 at levels varying from 10² to 10⁷ CFU/ml of beef exudate. The Ab-DEFT counts were linear and correlated well with numbers of cells inoculated, except at the lowest concentrations (10² to 10⁴ CFU/ml), for which counting accuracy declined. The use of 5-μm-pore-size polycarbonate (instead of nylon) prefilters was necessary to obtain this correlation in the beef exudate experiments. In several trials using nylon prefilters, counts were linear but were approximately 10 times lower than the number of cells inoculated into the serum. This result may have been related to entrapment of cells on the nylon prefilter after the trypsin-Triton X-100 treatment at 50°C.

Validity of Ab-DEFT for measuring growth of E. coli O157:H7 in beef. The Ab-DEFT was compared with standard plate counting for its ability to monitor the increase in cell number of E. coli O157:H7 during growth at 37°C in ground beef. MSA has been recommended as a differential and selective medium for isolation and identification of E. coli O157:H7 (16). However, other microorganisms present in ground beef can also grow and have a colony morphology resembling that of E. coli O157:H7 on this medium; i.e., MSA is not absolutely selective for this pathogen. Therefore, to perform this test with an unambiguously selectable microorganism, we isolated a dihydrostreptomycin-resistant variant of E. coli O157:H7, strain DHS-1, and incorporated the antibiotic into MSA (at 1,000 μg/ml) to provide selective conditions. The microscopic counts in this growth experiment at 37°C were compared with VPC (Fig. 4) and included total microbial counts obtained by Ab-DEFT and specific E. coli DHS-1 counts obtained by Ab-DEFT. The VPC included total viable counts, obtained by plating on standard plate count agar, and specific E. coli DHS-1 counts, obtained by plating on MSA plus 1,000 μg of dihydrostreptomycin per ml.

The Ab-DEFT counts were virtually identical to specific E. coli DHS-1 VPC from the start time (0 h) through 6 h of growth at 37°C (Fig. 4). The Ab-DEFT specifically enumerated the pathogen in the presence of approximately a 100-fold excess of background indigenous microbial species, which were measured by DEFT and by total VPC. Enumeration by DEFT compared with total VPC is also demonstrated in Fig. 4. The DEFT counts and total VPC varied only at the starting point (0 h), when the DEFT count was higher than the total VPC. This result may be explained by the inclusion in the DEFT count of
the population as microbial growth occurred and as nonviable cells diminished to negligible levels.

**DISCUSSION**

The Ab-DEFT and EC methods were compared for analysis of ground beef inoculated with *E. coli* O157:H7. The sensitivity of the Ab-DEFT compared favorably with that of the EC procedure, if reliability is considered. Both methods identified the pathogen at a low concentration of 16 CFU/g of beef. The EC method showed better sensitivity than the Ab-DEFT, positively identifying one of the three replicates inoculated at 2 CFU/g of beef. However, at this low inoculation level, two of three test portions would have been incorrectly identified as pathogen free by the EC method. Therefore, considering the reliability of the method, the sensitivity of the Ab-DEFT (16 CFU/g) was similar to that of the EC procedure.

In the initial description of the Ab-DEFT (34), a sensitivity of 10 cells per ml was reported for a pure culture of *E. coli* O157:H7 cells suspended in laboratory buffer. We report a similar sensitivity for cells inoculated into beef. Sensitivity depends to some extent on the quality of the fluorescent antibody. Use of a purified reagent that exhibited little non-specific binding resulted in very little background fluorescence, so that bacterial cells were recognized when the 40× objective lens was used. Even with a complex substrate such as ground beef, the background of the microscope field remained dark and in sharp contrast to the specific fluorescent staining of the *E. coli* O157:H7 cells (Fig. 1).

Specific enumeration of the pathogen is possible with the Ab-DEFT because of the direct nature of the analysis. In contrast, EC relies on a 24-h growth period; therefore, measurements of original contamination levels in beef are not possible by this method. For many years, total bacteria in foods have been enumerated by using acidine orange stain in the DEFT. However, Pettipher (22) reported that accurate DEFT counts of bacteria in milk were not possible at concentrations below approximately 10³ cells per ml. At and below 820 cells per g, detection is rare (Table 2) because the distribution of cells is not necessarily random or homogeneous. Nevertheless, enumeration by the Ab-DEFT may be considered approximate (i.e., accurate within an order of magnitude), and this level of accuracy may be sufficient for many applications (Table 1). Specific rapid and direct counting is a major advantage of the Ab-DEFT.

The agreement we found between microscope counts and VPC supports previous data on the applicability of filtration and epifluorescence microscopy to the microbiological analysis of particulate foods such as meat (6, 24, 26, 30). The use of mesh-lined stomacher bags is particularly helpful for filtering homogenized beef, as Qvist and Jakobsen (26) also noted in their use of a gauze lining to contain large particulate matter within the bags.

Both the sensitivity and accuracy of enumeration by the Ab-DEFT may be improved by the development of automatic scanning and image analysis technologies. With automation, the entire membrane surface and the multiple membranes prepared from a single test sample could be scanned, and the amount of sample examined could be increased without incurring operator fatigue, which is a limiting factor in the manual performance of the Ab-DEFT. An instrument which has been described as providing full automation of the DEFT includes filtration, staining, rinsing and drying of filters, and automated cell counting (25) and will most likely be applicable to the Ab-DEFT as well.

Unlike the EC and VPC techniques, which rely on microbial cell growth, microscopy counting techniques identify nonculturable microbial cells, e.g., those that have been injured or killed, and chromogenic electron acceptors have been used to distinguish nonviable cells microscopically (4). MSA, which was used in VPC determinations, is not a good recovery medium for resuscitation of stressed *E. coli* O157:H7 cells (1). The Ab-DEFT should be able to identify these nonculturable cells, regardless of their metabolic state, as long as the cell surface antigens recognized by antibodies remain intact. Thus, the Ab-DEFT may be able to detect nonviable cells when the source of disease outbreaks is being traced, since the pathogen is not always recoverable in culture from the epidemiologically implicated items (3). The data in Fig. 4 imply that the relevant cell surface antigens of *E. coli* O157:H7 are expressed and recognized by antibodies throughout at least nine cell division cycles in beef.

The specificity of the affinity-purified polyclonal antibody was adequate to distinguish *E. coli* O157:H7 from the indigenous microbial species in ground beef. Serological relatedness between *E. coli* O157:H7 and other microbial species, including *E. hermannii*, *Salmonella* O30 strains, *Y. enterocolitica* O9, and *Brucella* species, has been reported (2, 20, 27, 32). The fluorescent antibody used in this study did not cross-react with *E. hermannii*, *Y. enterocolitica*, or *E. coli* O18A:H7. The lack of reactivity to the H7-expressing *E. coli* strain indicated the primary specificity of the antibody reagent to the O157 antigen. This finding was supported by the false-positive reaction of the antibody to the urbana serotype of *Salmonella choleraesuis*, which expresses an O antigen (O30) with a structure identical to that of O157 (32).

Reagent specificity will ultimately influence the usefulness of the Ab-DEFT. Nevertheless, if certain strains cross-react with...
the O157 antibody, a more appropriate antibody can easily be substituted. For example, monoclonal antibodies that recognize E. coli O157:H7 (19, 21) may be fluorochrome labeled and adapted to the Ab-DEFT. A relevant antibody for use in the Ab-DEFT might be one that is directed against the outer membrane protein which is the product of the eae gene. This gene product mediates the attaching and effacing mechanism of host cell invasion, which Gannon et al. (12) suggested may be a good predictor of pathogenicity of enterohemorrhagic E. coli.

Our results may not be directly extrapolated to practical situations, because our experiments involved artificial inoculation of E. coli O157:H7 cells in beef rather than naturally contaminated beef. It is possible that the intense fluorescence exhibited by laboratory-grown cells may not be matched by cells in a natural setting. This uncertainty ultimately depends on conditions governing expression of the O157 and H7 cell surface antigens recognized by fluorescent antibodies. Variation in bacterial cell surface antigen expression is well documented, even for this pathogen. For example, E. coli O157:H7 produced alterations in the O polysaccharide when the growth rate was varied in chemostat studies (10). Exopolysaccharide production by this strain has been reported and shown to be dependent upon culture conditions (14). It is likely that the O157 and H7 antigens exist in the natural state of the organism. However, there is no direct proof of this, because no other methods attempt to identify these antigens directly in the environment; i.e., all methods for identification of this microbe require preliminary laboratory culture. Even in the unlikely case that these antigens are uniquely associated with laboratory culture, the technology of the Ab-DEFT would still be applicable if an antibody that recognizes an expressed antigen is identified.

Todd et al. (33) described a 24-h procedure that involved specific antibody-based enumeration of E. coli O157:H7 in a hemolyzing grid membrane filter format. The method involved no enrichment but only membrane filtration of foods and overnight incubation of the membranes on selective media. Membranes were reacted in a typical enzyme-labeled antibody procedure, and the technique specifically enumerated the pathogen at 10 to 1,000 cells per g in meats associated with food-borne illness outbreaks. The Ab-DEFT is conceptually similar to this technique, with apparently similar sensitivity, but saves time because it eliminates the growth step and the enzymatic color development.

The Ab-DEFT is a sensitive, specific, and direct method of identifying and enumerating E. coli O157:H7. The assay can be completed in less than 1 h, making it feasible for use in Hazard Analysis Critical Control Point programs in the food industry. Although its use has been demonstrated for a particular food-borne pathogen, the method may be generalized to identify other microbes or groups of microbes, provided that appropriate fluorescent-antibody probes are available.

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