Novel Single-Tube Agar-Based Test System for Motility Enhancement and Immunocapture of *Escherichia coli* O157:H7 by H7 Flagellar Antigen-Specific Antibodies

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is the most commonly identified member of the Shiga-toxin-producing *E. coli* family involved in human food-borne illnesses (2, 12). EHEC O157:H7 was first implicated in human diarrheal disease in 1982 (7). EHEC O157:H7 has immense clinical, public health (9), and economic significance, as indicated by the USDA Economic Research Service (http://www.ers.usda.gov/emphases/safefood/features.htm).

The spectrum of diseases or conditions caused by EHEC O157:H7 includes asymptomatic infection, mild uncomplicated diarrhea, hemolytic colitis, diarrhea-associated hemolytic-uremic syndrome, diarrhea-associated thrombotic thrombocytopenic purpura, and, in some cases, death (2, 7, 12).

Positive identification of EHEC O157:H7 is commonly made by biochemical confirmation of *E. coli* isolates, O157 serology, and detection of the H7 flagellar antigen by agglutination with H7-specific antiserum. Presumptively positive isolates are further confirmed by genetic methods, such as PCR and pulsed-field gel electrophoresis. Fields et al. (3) reported that clinical isolates may be motile and may react with H7 antisera or they may not react with antisera because they are either nonmotile (NM or H−) or motile but do not react with typing sera (H undetermined).

H7 is one of 53 flagellar antigen groups described for *E. coli* (3, 13). In conventional detection methods, determination of the H7 flagellar antigen phenotype is usually performed with presumptively positive *E. coli* O157 colonies isolated from selective or differential medium to confirm that an isolate is of the H7 serogroup (6). With some isolates, confirmation of H7 serology may be delayed because multiple passages in motility enhancement medium may be required before the flagellar antigen is expressed sufficiently to be detectable in O157 colonies isolated from selective or differential medium, as described by the Centers for Disease Control (http://wonder.cdc.gov/wonder/PrevGuid/p0000445/p0000445.asp). For example, the *E. coli* Reference Center (The Pennsylvania State University, State College, Pa. [C. DebRoy, personal communication]) incubates colonies for 10 days before strains are designated as nonmotile. Ware et al. (14) recently reported a preponderance of aberrant isolates of *E. coli* O157:H7, demonstrating unusual biochemical reactions, that required 4 to 17 passages in vitro before the presence of H7 antigen could be demonstrated serologically.

The detection of O157 antigens independent of H7 antigens is insufficient for the identification of EHEC O157:H7. Critical detection of EHEC O157:H7 relies on demonstration of both somatic (O157) and flagellar (H7) antigens. These diagnostic steps are necessary to avoid false-positive misidentification of other enteric bacteria as EHEC O157:H7, such as other *E. coli* serotypes, *Escherichia hermanii*, *Yersinia enterocolitica*, *Salmonella* group N spp., *Brucella abortus*, *Vibrio cholerae* O1, and some *Citrobacter* spp. (11, 14). Misdiagnosis can be very costly in terms of the loss of recalled or withdrawn perishable food products, the death of patients, and litigation.

While determination of O157 serology by using commercially available antisera is a simple and straightforward process, many laboratories cannot easily perform H7 serology because...
of the relative complexity of the test; thus, they rely on reference laboratories to perform the costly tests (3).

The objective of this study was to develop a simple, inexpensive, reliable, and specific method that allows both enhancement of motility and detection of H7 flagellar antigen and that is amenable to routine use in a small clinical or food microbiology laboratory. The test was designed to provide serological confirmation of the identity of E. coli O157:H7 isolates. The method described herein does not require great technical expertise or elaborate equipment. The long-term goal is to apply the method to the development of pathogen reduction programs for rapid identification of E. coli O157:H7 and possibly for adaptation to other EHEC serotypes of interest.

MATERIALS AND METHODS

Maintenance of bacterial cultures. The test bacterial strains (Table 1) evaluated in this study consisted of O157:H7 strains (n = 19), an O157:H^- strain (n = 1), generic E. coli strains (n = 6), and Salmonella enterica serovars (n = 4) were used as negative controls for reaction with H7 antiserum and as positive motility controls. Working stock cultures were maintained as slants on tryptic soy agar containing (0.6% [wt/vol] yeast extract at 5°C. For long-term storage, Escherichia and Salmonella strains were grown in tryptic soy broth and lactose broth, respectively, for 24 h at 37°C, after which the cultures were mixed with 25% glycerol (1:1, vol/vol) and stored at −80°C.

Evaluation of motility test media. Four media (see Table 2), motility indole ornithine (MIO) medium and agar (0.4%, wt/vol) media containing either nutrient broth (NBA), tryptic broth (TBA), or tryptic soy broth (TSBA) were evaluated for their ability to enhance motility of inoculated test bacteria. Six milliliters of the respective motility medium was dispensed into test tubes. Cultures propagated in broth at 37°C for 18 h (about 10^8 CFU/ml) were stab inoculated into the center of the agar. Tubes were incubated at 37°C for 12, 18, 24, 48, and 96 h to establish optimum incubation times. Sixteen E. coli cultures grown in NBA and MIO media at 42°C were used to assess the effect of elevated incubation temperature on motility enhancement. Motility was manifested as clouding of the growth medium that progressed laterally and downward from the stab inoculation line. Salmonella enterica serovars were used as positive motility controls, whereas nonmotile (H^-) E. coli strains (n = 4) were employed as negative controls (Table 1). Growth was scored visually and coded (score = 0 to 3) according to the degree of clouding. The presence of H7-encoded genes was determined by use of PCR techniques (4).

Preparation of single-tube motility and H7 flagellar immunocapture test media. TSBA was the best motility enhancement medium in terms of level of bacterial growth, ease of reading results, and lack of false-positive motile strains. It was therefore used in subsequent studies. Three-milliliter aliquots of agar were dispensed into test tubes that were then autoclaved (121°C for 15 min). A portion of the tube contents was allowed to gel at room temperature, while other tubes were tempered in a water bath at 50°C. The tubes were withdrawn from the water bath, and 30 µl of polyclonal, monovalent, H7 flagellar antigen-specific Denka Seiken E. coli antiserum (Oxoid Inc., Nepean, Ontario, Canada; catalog no. 211057, lot no. 26-111A) was added to 1 ml of TSBA medium. In a parallel experiment, the effect of using 60 µl of antiserum was evaluated. The contents of the tubes were vortexed for about 2 s, after which 1-ml aliquots were dispensed into tubes containing 3 ml of gelled bottom agar, as illustrated in Fig. 1. For controls, the antiserum solution was replaced by 30 or 60 µl of sterile saline solution (0.85% [wt/vol] NaCl). After the middle agar layer gelled (~15 min), 3 ml of tempered TSBA medium (50°C) was gently added along the side of the tube. The tubes were allowed to gel and cool at room temperature before they were used.

Motility and flagellar immunocapture tests. As before, motility-positive and nonmotile controls were employed. Actively growing test cultures (Table 1) in broth (37°C) were stab inoculated into two tubes each, i.e., without antiserum (saline control) and with antiserum. The inoculating needle was stabbed straight down the center of the TSBA column, extending two-thirds down the upper agar layer as illustrated in Fig. 1. The tubes were incubated at 37°C, which had been established as the optimum temperature for motility of the test strains. After 12 to 18 h of incubation, the tubes were scored for development of cloudiness (indicating growth) in the top, middle, and bottom agar layers.

Interpretation of motility and immunocapture tests. Growth in the top, middle, and bottom agar layers was scored independently by using the same criteria employed for the motility media described above. For documentation, the tubes were photographed with a digital camera (model CoolPix 990, 3.34 Megapixel; Nikon, Tokyo, Japan); images were transferred to a computer and stored as JPEG (joint photographers expert group) files that were imported into PowerPoint 2000 (Microsoft Corp., Redmond, Wash.).

RESULTS

Preliminary motility evaluation studies. Ten of 16 E. coli strains that were evaluated for motility at 42°C for 48 h were motile in MIO medium, whereas only one strain was motile in NBA medium. After incubation was extended to 5 days, 14 and

<table>
<thead>
<tr>
<th>E. coli O157 strains</th>
<th>Serotypea</th>
<th>Sourceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground beef/hamburger</td>
<td>O157:H7</td>
<td>DG</td>
</tr>
<tr>
<td>E0019</td>
<td>O157:H7</td>
<td>DG</td>
</tr>
<tr>
<td>Cider</td>
<td>O157:H7</td>
<td>DG</td>
</tr>
<tr>
<td>H1730</td>
<td>O157:H7</td>
<td>DG</td>
</tr>
<tr>
<td>Salami</td>
<td>O157:H7</td>
<td>DG</td>
</tr>
<tr>
<td>994</td>
<td>O157:H7</td>
<td>DG</td>
</tr>
<tr>
<td>4546</td>
<td>O157:H7</td>
<td>DG</td>
</tr>
<tr>
<td>ATCC 43888</td>
<td>O157:H7</td>
<td>ATCC</td>
</tr>
<tr>
<td>ATCC 43889</td>
<td>O157:H7</td>
<td>ATCC</td>
</tr>
<tr>
<td>ATCC 43894</td>
<td>O157:H7</td>
<td>ATCC</td>
</tr>
<tr>
<td>ATCC 700375</td>
<td>O157:H^-</td>
<td>ATCC</td>
</tr>
<tr>
<td>36E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>43E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>142E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>161E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>309E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>384E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>386E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>427E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
<tr>
<td>436E1</td>
<td>O157:H7</td>
<td>SPO</td>
</tr>
</tbody>
</table>

a H^- nonmotile; X, motile, unknown (non-H7) flagellar serotype; Y, unknown (non-O157) somatic serotype.

Non-O157 E. coli strains

| ATCC 4350          | O140-X   | ATCC   |
| ATCC 12014         | O555^-   | ATCC   |
| ATCC 23513         | O18-H^-  | ATCC   |
| ATCC 23514         | O19-H^-  | ATCC   |
| ATCC 23545         | O138-H^- | ATCC   |
| ATCC 31619         | O555^-   | ATCC   |

S. enterica serovar (serogroup)

| Anatum ATCC 9270 (E1) | 3,10:e,h:1,6 | ATCC |
| Muenchenc ATCC 8388 (C2) | 6,8:1,2 | ATCC |
| Newport ATCC 6962 (C2) | 6,8:e,h:1,2 | ATCC |
| Typhimurium ATCC 14028 (B) | 4,5,12,1,2 | ATCC |

<table>
<thead>
<tr>
<th>Source</th>
<th>Y:X</th>
<th>SPO</th>
</tr>
</thead>
</table>

Fl. salmonella serovar (serogroup)

**S. enterica serovar (serogroup)**

| Anatum ATCC 9270 (E1) | 3,10:e,h:1,6 | ATCC |
| Muenchenc ATCC 8388 (C2) | 6,8:1,2 | ATCC |
| Newport ATCC 6962 (C2) | 6,8:e,h:1,2 | ATCC |
| Typhimurium ATCC 14028 (B) | 4,5,12,1,2 | ATCC |

<table>
<thead>
<tr>
<th>Source</th>
<th>Y:X</th>
<th>SPO</th>
</tr>
</thead>
</table>

Antigenic formula representing somatic antigens: flagellar phase 1 antigens; flagellar phase 2 antigens (Difco manual, 11th ed., p. 862; Difco Laboratories, Sparks, Md.).
5 strains were motile in MIO and NBA media, respectively, and 2 of the test strains were nonmotile (data not shown). Bacterial cultures demonstrated a greater number of motile strains and more extensive growth at 37°C than at 42°C; 13 of 16 strains cultured in NBA media and 14 of 16 strains cultured in MIO medium demonstrated motility (data not shown).

Motility evaluation at 37°C. Results of motility tests conducted at 37°C in NBA, TBA, TSBA, and MIO media were compared after the strains were incubated for 18, 24, 48, and 96 h. A greater number of test strains (Tables 2 and 3) demonstrated motility in TSBA and MIO media (20 of 26 and 24 of 26, respectively) than in TBA and NBA media (19 of 26 and 21 of 26, respectively). The strains incubated for 96 h showed improved motility in NBA and TBA media but only slight improvement in TSBA and MIO media. Salmonella serovars grew luxuriantly in the four media evaluated (data not shown). Greater growth vigor was demonstrated in TSBA and MIO media, with MIO medium yielding four more strains that were scored as motile than TSBA medium did (Tables 2 and 3). MIO medium encouraged motility of E. coli strain ATCC 23513 and apparent motility of nonmotile E. coli strains ATCC 12014, ATCC 23545, and ATCC 31619; these isolates were nonmotile in NBA, TBA, and TSBA media. The E. coli O157:H7 ground beef isolate did not exhibit motility in NBA, TBA, or TSBA medium after 18 h of incubation. The longer incubation time of 96 h encouraged motility of the ground beef isolate in the three media and yielded more strains that were scored as motile in MIO and NBA media. However, strains demonstrating new motility at 96 h were not numerous. The results indicate that longer incubation times may increase the number of motile strains. None of the media evaluated enhanced the motility of the E. coli O157:H7 isolate from cider or the nonmotile E. coli O157:H7 strain ATCC 700375 even after incubation was extended to 96 h.

Escherichia vulneris and S. enterica serovars. S. enterica serovar Typhimurium ATCC 14028, serovar Anatum ATCC 43894, and apparent motility of nonmotile E. coli strains ATCC 12014, ATCC 23545, and ATCC 31619; these isolates were nonmotile in NBA, TBA, and TSBA media. The E. coli O157:H7 ground beef isolate did not exhibit motility in NBA, TBA, or TSBA medium after 18 h of incubation. The longer incubation time of 96 h encouraged motility of the ground beef isolate in the three media and yielded more strains that were scored as motile in MIO and NBA media. However, strains demonstrating new motility at 96 h were not numerous. The results indicate that longer incubation times may increase the number of motile strains. None of the media evaluated enhanced the motility of the E. coli O157:H7 isolate from cider or the nonmotile E. coli O157:H7 strain ATCC 700375 even after incubation was extended to 96 h.

### TABLE 2. Representative motility scores of E. coli isolates at 37°C after incubation for 18 and 96 h

<table>
<thead>
<tr>
<th>Isolate no. or source</th>
<th>Motility score in indicated medium after indicated incubation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBA</td>
</tr>
<tr>
<td></td>
<td>18</td>
</tr>
<tr>
<td>994</td>
<td>2</td>
</tr>
<tr>
<td>F4546</td>
<td>1</td>
</tr>
<tr>
<td>E0019</td>
<td>1</td>
</tr>
<tr>
<td>Hamburger</td>
<td>0</td>
</tr>
<tr>
<td>H1730</td>
<td>1</td>
</tr>
<tr>
<td>Cider</td>
<td>0</td>
</tr>
<tr>
<td>Salami</td>
<td>2</td>
</tr>
<tr>
<td>ATCC 43894</td>
<td>2</td>
</tr>
<tr>
<td>ATCC 43889</td>
<td>2</td>
</tr>
<tr>
<td>ATCC 43888</td>
<td>3</td>
</tr>
<tr>
<td>ATCC 700375</td>
<td>0</td>
</tr>
<tr>
<td>ATCC 4350</td>
<td>1</td>
</tr>
<tr>
<td>ATCC 31619</td>
<td>0</td>
</tr>
<tr>
<td>ATCC 12014</td>
<td>0</td>
</tr>
<tr>
<td>ATCC 23514</td>
<td>1</td>
</tr>
<tr>
<td>ATCC 23545</td>
<td>0</td>
</tr>
<tr>
<td>ATCC 23513</td>
<td>0</td>
</tr>
<tr>
<td>36E1</td>
<td>1</td>
</tr>
<tr>
<td>43E1</td>
<td>2</td>
</tr>
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<tr>
<td>436E1</td>
<td>2</td>
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</tbody>
</table>

*Salmonella* serovars and *E. vulneris* (data not shown) exhibited luxuriant growth in the four media evaluated.

*n* (data not shown) exhibited luxuriant growth in the four media evaluated. However, *Salmonella* serovar Muenchen ATCC 8388 occasionally did not demonstrate good growth (data not shown). Although the levels of motility enhancement were comparable in all media, TSBA and MIO media showed the best growth promotion. However, motility results were easier to read and interpret in TSBA medium (Fig. 2). MIO

### TABLE 3. Motility test results in different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Incubation time (h)</th>
<th>No. (%) of strains (n = 26)</th>
<th>Motile</th>
<th>Nonmotile</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBA</td>
<td>18</td>
<td>19 (73.1)</td>
<td>7 (26.9)</td>
<td>75</td>
<td>90.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>21 (80.8)</td>
<td>5 (19.2)</td>
<td>75</td>
<td>90.91</td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>18</td>
<td>19 (73.1)</td>
<td>7 (26.9)</td>
<td>100</td>
<td>90.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>20 (76.9)</td>
<td>6 (23.1)</td>
<td>100</td>
<td>90.91</td>
<td></td>
</tr>
<tr>
<td>TSBA</td>
<td>18</td>
<td>19 (73.1)</td>
<td>7 (26.9)</td>
<td>100</td>
<td>90.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>20 (76.9)</td>
<td>6 (23.1)</td>
<td>100</td>
<td>90.91</td>
<td></td>
</tr>
<tr>
<td>MIO</td>
<td>18</td>
<td>20 (76.9)</td>
<td>6 (23.1)</td>
<td>25</td>
<td>95.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>24 (92.3)</td>
<td>2 (7.7)</td>
<td>25</td>
<td>95.45</td>
<td></td>
</tr>
</tbody>
</table>

* Specification and sensitivity were determined as described in the text. The values shown in the table were determined using the motile and nonmotile *E. coli* strains listed in Table 2.
medium yields growth in variegated colors, i.e., mixtures of purple, white, and yellow, depending on the test bacterial strain. This could confound interpretation of motility with pH or other changes, leading to false-positive motility results. Although MIO medium had higher sensitivity for motility enhancement than TSBA, TBA, and NBA media (95.45 versus 90.91%), its specificity was 50 to 75% lower than those of the other media (Table 3). TSBA medium was thus selected for use in further studies of the development of a single-tube agar-based test for both motility enhancement and H7 flagellar immunocapture.

**H7 flagellar immunocapture tests.** *Salmonella* strains grew luxuriantly in both test and saline control TSBA tubes (data not shown). Figure 3 shows representative motility and immunocapture reactions for *E. coli* O157:H7 strains 36E1 and ATCC 43894. Both strains grew throughout the saline control tube; however, growth was retarded in the tubes containing H7 antiserum in the middle agar layer. At times, slight growth was found in the middle agar layer but the specific reaction of H7 bacteria and antibodies was evident. A sharply defined, and usually convex-concave interphase, appeared between the leading edge of advancing bacterial growth and the H7 antiserum-containing middle agar layer.

Gas bubbles were present at times in the motility media (Fig. 3); these could confound interpretation of motility and binding results or cause breakage at the interfaces of agar layers. However, refrigeration of tubes at 5°C (~30 min) and/or gentle tapping of tubes on the bench top improved the reading of results since dissipation of the formed bubbles enabled visualization of bacterial growth boundaries.

**Specificity and sensitivity of immunocapture tests.** The specificities and sensitivities of the tests were determined by using *E. coli* O157, H7, and H- variants, generic *E. coli*, *E. vulneris*, and *S. enterica* serovars. Specificity was determined by dividing the number of true negatives by the number of true negatives plus the number of false positives and multiplying the result by 100. Sensitivity was determined by dividing the number of true positives by the number of true positives plus the...
The use of 60 μl of antiserum in place of 30 μl per ml of TSBA middle agar layer improved both sensitivity and specificity measures for validating immunocapture of E. coli O157:H7 by adsorbed H7 flagellar antibodies (specificities, 55.5 and 75%; sensitivities, 92 and 100% [with 30 and 60 μl, respectively]). The specificity and sensitivity improved by about 19.5 and 7.7%, respectively.

**DISCUSSION**

The development of methods for accurate and rapid detection of E. coli O157:H7 is an important area of research internationally. The present study evaluated motility enhancement media and H7 flagellar immunocapture by using a single-tube agar-based test system. The principle of this assay is that motile bacteria inoculated at the top of a semisolid agar column grow and migrate downwards towards a zone that contains adsorbed H7 flagellated antigens, and captured bacteria display a visible zone demarcating stoppage or retardation of growth. The adsorbed H7 antibodies retained antigen recognition properties as indicated by test strains used as positive and negative controls.

The described test system was used to confirm pure cultures of H7 flagellar E. coli O157 strains within 12 to 18 h. Longer incubation times might be required to encourage the motility of some strains. Bacteria vary in their expression of flagellar antigens, and therefore it is sometimes necessary to serially passage them through motility agar media (14) or to extend incubation times in order to enhance motility (11, 14). Ware et al. (14) reported a large number of E. coli O157:H7 isolates that remained nonmotile even after 96 h of incubation at 37°C. The E. coli Reference Center (The Pennsylvania State University) protocol uses an extended incubation of up to 10 days to enhance motility. The media evaluated in the present study failed to enhance the motilities of E. coli O157:H7 isolated from cider and E. coli O157:H7 strain ATCC 700375, even after incubation was extended to 96 h. The latter strain is nonmotile, whereas the isolate from cider was demonstrated to be H7 positive by use of latex agglutination tests and tests for the presence of H7 flagellar gene-encoding sequences (10). Other nonmotile E. coli strains, ATCC 31619, ATCC 12014, and ATCC 23545, demonstrated apparent motility in MIO medium but were nonmotile in TSBA, NBA, and TBA media.

Specificity and sensitivity validations of H7 flagellar antigen immunocapture were increased by about 19.5 and 7.7%, respectively, by increasing the H7 antiserum content from 30 to 60 μ/ml of TSBA medium. The large increment in specificity seems to indicate that the level of antibodies in the agar matrix was not optimal when 30 μl of antiserum/ml of TSBA medium was used. This may partially explain why in some instances there was limited growth of H7 flagellated bacteria in the antiserum-containing middle agar layer. Growth could have been due to saturation of the available binding sites, resulting in free passage of unadsorbed motile bacteria that grew in the middle agar layer and continued growth in the bottom agar layer. Known positive and negative controls were employed to ascertain the validity of the tests; the absence of spontaneous reaction was tested by using physiological saline in place of H7 flagellar antiserum.

Comparable antigen-antibody procedures that employed agar gel matrices that eliminated the need to establish optimal proportions of antigens and antibodies in immunoprecipitation studies have been reported (8). Farmer and Davis (1) described a single-tube screening medium for detecting E. coli O157:H7. The system was based on sorbitol fermentation and the presence of H7 antiserum throughout the growth medium. Bacterial immobilization by H7 antiserum was indicated by failure of the test strain to migrate from the stab inoculation line. Our test system first enabled luxuriant growth of potentially motile bacterial strains and their uninterrupted downward motility through an antiserum-free agar column; then H7 flagellated E. coli bacteria were immobilized by antibodies in a horizontal layer of soft agar placed in the middle of the agar column. The test described by Farmer and Davis (1) contained antibodies that were uniformly distributed throughout a soft agar (0.4%, wt/vol) medium.

The greater growth and diffusion in MIO medium than in the other media evaluated can be partly explained by the lower concentration of agar present (0.2%, wt/vol), which formed a network of large pores allowing freer movement of bacteria within the gel matrix. NBA, TBA, and TSBA media contained higher agar concentrations (0.4%, wt/vol). Our observations indicate that movement of bacteria within the agar matrix is largely a function of motility of the growing bacteria.

An advantage of the single-tube agar-based test system is that it is a contained test. After culturing for motility, there is no need for secondary handling of bacteria to conduct H7 serology. Additionally, the test avoids the difficulties of reading slide- or test tube-based agglutination reactions which require separate motility enhancement procedures. Bubbles that occasionally formed in motility test media were ascribed to the presence of fermentable sugar, which was metabolized during gas production. It is possible to reduce the bubbling phenomenon by constructing media devoid of a fermentable sugar carbon source.

In many field and clinical studies, the services of a reference laboratory are required for flagellar serotyping since there is currently no easy method to confirm the presence or absence of H7 phenotypes in E. coli isolates (5). Flagellar H7 gene sequences, and polymorphisms thereof, are often found in nonmotile E. coli O157 strains (3, 4, 13) and in about 10% of non-O175 E. coli strains (1). Accurate identification of H7 flagellar antigen is critical for the confirmation of the identity of E. coli O157:H7 strains, in distinguishing them from (benign) nonmotile strains, for determining the specificity of the PCR methods used in detecting the toxin gene-containing sequences, and for correlating the presence of a gene with phenotypic expression. Such information is relevant to detailed taxonomic and epidemiological studies and for purposes of timely and appropriate clinical intervention and release or recall of products from the market.

The single-tube method described can be extended to evaluation of motility and flagellar antigen-specific immunocapture of other serotypes of Shiga-toxin-producing E. coli by using relevant flagellar antisera of strains of interest.

The present study addressed the development of a practical and cost-effective single-tube agar-based test system for en-
enhancement of motility followed by immunoimmobilization of motile E. coli O157:H7 by H7-specific antibodies. There is the potential for routine use of this method in small clinical or food microbiology laboratories. The system developed has high sensitivity (100%) and specificity (75%) and includes, in a single assay, both motility enhancement and subsequent detection of H7-flagellated E. coli O157 isolates.

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