Detection of Enteroaggregative Escherichia coli with Formalin-Preserved HEp-2 Cells

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Formalin-stored HEp-2 cells were used to assay Escherichia coli for adherence. Cells refrigerated in formalin for up to 28 days and used in a wet assay format demonstrated an assay sensitivity ranging from 94 to 98% to detect enteroaggregative E. coli (EAEC). HEp-2 cells first fixed and stored with formalin and then stored dry in ambient conditions for 6 weeks demonstrated an assay sensitivity of 92% to detect EAEC. Using formalin-fixed HEp-2 cells will improve the efficiency of EAEC identification.

Enterogaergative Escherichia coli (EAEC) has been identified as a cause of bacterial gastroenteritis in developed and developing countries (1, 2, 3, 11, 13).

The true incidence of EAEC infection is underestimated because the current assay is performed only in research laboratories. The standard assay for EAEC, demonstration of aggregative attachment of organisms to human epithelial (HEp-2) cells, is time-consuming to set up and prone to contamination.

The use of formalin-fixed HEp-2 cells offers a means of studying EAEC in a time-efficient way, eliminating the risk of contamination.

The organisms were retrieved from stool samples of U.S. college students during their short-term stays in Guadalajara, Mexico, in the summer of 1999 (1). Consent was obtained and approved by University of Texas Committee for the Protection of Human Subjects. In Mexico, E. coli cells were identified as lactose-fermenting gram-negative organisms on a MacConkey plate and by biochemical testing. Five colonies of E. coli were preserved in peptone stabs, shipped to Houston, Tex., and stored at room temperature. In Houston, E. coli cells were retrieved from the peptone stabs, grown on MacConkey agar plates, and then incubated overnight at 37°C in tryptic soy broth (TSB) with 1% mannose. Then, E. coli isolates were tested for aggregative adherence by using standard HEp-2 cell assay (1, 2): HEp-2 cells monolayers were added to a 24-well plate, and minimal essential medium (MEM) (0.5 ml) with 1% mannose was added to each well along with 25 μl of bacterial culture. The plates were incubated for 3 h at 37°C in room air. Afterwards, the plates were exposed to cold methanol for 10 min for fixation, followed by exposure to 10% crystal violet for staining. Finally, the slide covers were placed over glass slides by using Permount and viewed under light microscopy. EAEC strain JM 221 was used as a positive control, and EAEC strain HS was used as a negative control. Selected samples represented all HEp-2-cell-positive and -negative E. coli isolates from our studies carried out in 1999 (1).
The adherence of EAEC to intestinal mucosa is thought to be through fimbrial structures designated aggregative adherence fimbriae I and II (AAF I and II) (10). Currently, the only assay used to detect EAEC strains is the HEp-2 cell assay, which is time-consuming and requires that a trained laboratory technician perform the assay only when cells are available. Furthermore, the cells are prone to contamination which may further delay results.

The results from our experiment using HEp-2 cells maintained in formalin for up to 28 days and previously fixed HEp-2 cells left in the atmosphere at room temperature for 6 weeks showed a high degree of sensitivity and specificity compared with the standard assay.

Since formalin is bactericidal, it is advisable to thoroughly wash the formalin off the HEp-2 cell-coated coverslips and transfer them to a new 24-well plate. Traces of formalin on the coverslip can potentially cause false negative results and might explain the small number of discordant results found between duplicates. Formalin eliminates the risk of contamination of HEp-2 cells, an additional advantage to this assay. One potential cause of false negative results is that EAEC strains may lose their aggregative adherence ability, presumably via spontaneous plasmid loss. To decrease this possibility, we tested the bacterial strains within 1 week of culturing the bacteria.

Although there was a concern about formalin causing false results by clumping fimbriated organisms, this was not observed in our study since all strains negative by the standard assay were also negative in the formalin assays.

The use of formalin did not alter the aggregative adherence ability of HEp-2 cells nor render nonadherent strains positive in the assay. Formalin fixation is achieved by the cross-linking of neighboring protein groups, and surface carbohydrate receptors remain intact. Hicks et al. (6) demonstrated that EAEC adheres in an aggregative pattern to formalin-fixed pediatric intestinal tissue. Furthermore, Spencer et al. suggested that fixing HEp-2 cell monolayers with formalin prevented cell detachment and improved detection of EAEC (12).

Our data suggest that formalin-fixed HEp-2 cells either maintained in formalin or kept at ambient conditions may be used in the assay for at least 6 weeks. We plan to refine this assay further and anticipate that formalin-fixed cells will perform well after much longer fixation periods.

REFERENCES

TABLE 1. Identification of EAEC

<table>
<thead>
<tr>
<th>Result of formalin-fixed HEp-2 cell assay</th>
<th>No. (%) of isolates after formalin fixation (wet assay)</th>
<th>No. (%) of isolates 6 weeks after removing formalin (dry assay)</th>
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*In an examination of 62 EAEC strains, formalinized HEp-2 cells maintained in formalin (wet assay) or under ambient conditions (dry assay) were identified by a standard HEp-2 cell assay.*