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Comparison of an HEp-2 Tissue Culture Test with the Serény Test for Detection of Enteroinvasiveness in *Shigella* spp. and *Escherichia coli*

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A good correlation was observed between the Serény test and an HEp-2 tissue culture test for the detection of enteroinvasiveness in *Shigella* spp. and *Escherichia coli*.

The ability to invade intestinal epithelial cells is important in the pathogenesis of acute diarrhea caused by *Shigella* spp. and enteroinvasive *Escherichia coli*. In the laboratory, this property is usually investigated with the Serény test, which assesses the ability of a living culture to cause an ulcerative keratoconjunctivitis after instillation onto the cornea of a guinea pig (9). This assay has disadvantages because the animals are costly, the technique may be painful, and objections are often made on humane grounds.

In this study, we compared the Serény test with a method which utilizes HEp-2 tissue culture cells.

All of the test strains were isolated from the feces of patients with diarrhea and maintained at room temperature on Dorset egg medium. *Shigella* spp. and *E. coli* were identified biochemically and serologically (3). A total of 30 *Shigella* strains were examined; 2 *S. dysenteriae*, 23 *S. flexneri*, and 5 *S. boydii*. Fifteen enteroinvasive *E. coli* were selected; they belonged to O serogroups which have been frequently associated with dysentery-like diseases (8). The control groups of *E. coli* comprised 10 strains belonging to the enteropathogenic O serogroups (8) and 8 strains belonging to O serogroups frequently found in the feces of healthy subjects.

HEp-2 cells were grown in Eagle IX basal medium supplemented with 15% fetal bovine serum, glutamine (1 mM), penicillin (100 μg/ml), and streptomycin (100 μg/ml). HEp-2 cells in Eagle IX basal medium at a concentration of 5×10^5 cells per ml were seeded in a 40-mm plastic petri dish (Nunc, Denmark) containing a glass cover slip. The seeded petri dishes were incubated at 37°C for 24 to 48 h, and the monolayers obtained were used for the experiments.

The HEp-2 cell invasiveness test used in this study was a modification of the Mehlman invasiveness test (6). Strains were grown overnight in 10 ml of Hedley-Wright broth (1) without aeration, spun down, and suspended in 10 ml of brain heart infusion broth. This bacterial suspension was diluted 1:50 with infection medium (70 ml of Earle salts, 10 ml of brain heart infusion broth, and 20 ml of heat-inactivated newborn calf serum). The HEp-2 cell monolayer was washed with Earle salts and overlaid with 2 ml of the diluted bacterial suspension containing 10^7 bacteria. After incubation at 37°C for 2.5 h, the monolayer was washed three times with Earle salts and covered with 2 ml of Eagle IX basal medium supplemented with 15% fetal bovine serum, glutamine (1 mM), gentamicin (20 μg/ml), and lysozyme (300 μg/ml) and incubated for 3 h more at 37°C. The monolayer was then washed twice with Earle salts, fixed in 100% methanol for 10 min, and stained for 30 min with 10% Giemsa. After staining, the cover slips were cleared by passage through acetone, acetone-xylene (50:50), and xylene and mounted on glass slides. The cover slips were examined under oil immersion at a magnification of ×1,000 by light microscopy, and 300 cells were counted for the presence of intracellular bacteria.

For the Serény test, strains were grown overnight at 37°C on Hartley digest agar slopes (1) and suspended in 1 ml of phosphate-buffered saline (pH 7.4) to give a concentration of 10^{10} to 10^{11} bacteria per ml. A 0.02-ml amount of the bacterial suspension was instilled into the left conjunctival sac of an adult Duncan-Hartley guinea pig. The animal was examined daily for the development of keratoconjunctivitis over a 3-day period.

As determined by the Serény test, 29 *Shigella* and 8 *E. coli* strains caused a keratoconjunctivitis within 48 h and were considered to be invasive. No strains became positive after 48 h. The 18 control *E. coli* strains were all negative by the Serény test even at 72 h.

As determined by the HEp-2 test, the 29 Serény-positive *Shigella* strains invaded between 1.7 and 38% of the cells, with a mean of 9.6%.

TABLE 1. *Comparison of the Serény test with an HEp-2 tissue culture test*

Strains	No. tested	No. Serény positive and:		No. Serény negative and:	
		HEp-2 positive	HEp-2 negative	HEp-2 positive	HEp-2 negative
Shigella spp.	30	29	0	0	1
Invasive serogroups of *E. coli*	15	8	0	1	6
Control *E. coli*	18	0	0	0	18

For the Serény-positive *E. coli* strains, the percentage of cells invaded was between 1.5 and 36.3, with a mean percentage of 15.3. The number of bacteria seen in each cell varied considerably between 2 and 100, with 20 to 30 being the most frequent. In the HEp-2 test, no bacteria were seen in the cells exposed to the control group of *E. coli*. No bacteria were seen adhering to the cells or to the glass cover slip despite the fact that 12 of these 18 control strains were highly motile and that at least 6 (33%) possessed type 1 pili (as determined by mannose-sensitive haemagglutination of guinea pig erythrocytes).

Of the 30 *Shigella* spp. tested, 29 were positive in both tests, and 1 (*S. flexneri* 6) was negative in both. Only 8 of the 33 *E. coli* strains were positive in both tests; 24 were negative in both, and 1 was positive only in the HEp-2 test (Table 1). All eight *E. coli* strains which were positive in both tests belonged to enteroinvasive serogroups, six belonged to serogroup O124, one belonged to serogroup O143, and one belonged to serogroup O164. The strain which was positive only in tissue culture was of serogroup O124.

Of the 63 strains tested by the two methods, 37 were positive in both, 25 were negative in both, and 1 was positive only in the HEp-2 test. The tests of this strain were repeated three times with freshly prepared suspensions, different guinea pigs, and fresh tissue cultures. In the HEp-2 test, a positive result was seen on each occasion, whereas results of the Serény test were consistently negative.

Mehlman (6) reported that the HEp-2 and the HeLa cell tests were suitable alternatives to the Serény test; however, he did not give details of experimental comparisons. Dupont et al. (2) examined a small number of strains of *E. coli* in the Serény and HeLa cell tests, finding three strains positive in both and four strains negative in both. One *Shigella* strain was tested and was positive in both tests. The three invasive *E. coli* strains were tested in human volunteers, and, at a challenge dose of 10^8 organisms, two of the strains caused dysentery. In the present study of 63 *Shigella* and *E. coli* strains, the HEp-2 test correlated well with the Serény test and did not possess the disadvantages of the latter animal model. Additionally, the HEp-2 tissue culture test offers the possibility for more detailed study of the invasive mechanism; such studies have been done with other tissue culture tests (4, 5, 7). Because a strain gives a positive result in an in vitro test for enteroinvasiveness it does not, of necessity, mean that the strain will produce diarrhea in humans. With this reservation, we suggest that the HEp-2 test is suitable for screening *Shigella* and *E. coli* strains for enteroinvasiveness and for the more detailed study of the invasive mechanism.

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