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Rapid ID of Diarrhea Causing K88 E. coli

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Summary

The efficiency of the standard agglutination test, Y-1 mouse adrenal-cell test, and infant-mouse gastric test was compared with a recently developed enzyme-linked immunosorbent assay (ELISA) for detecting the K88 pilus antigen and enterotoxin-producing E. coli. Isolates from clinical samples were identified on suspensions of bacteria. The sensitivity of the assay was in the nanogram-per-ml range as determined by measuring purified pili.

Introduction

In man and animals, enterotoxigenic Escherichia coli (ETEC) are associated with acute diarrhea, caused by two different toxins: a high molecular-weight, choleragen-like, heat-labile enterotoxin (LT); and/or a low molecular-weight, heat-stable enterotoxin (ST). These ETEC do not invade the intestinal epithelial cells but adhere to the surface of the mucosa. Adherence is mediated by specific heat-labile surface antigens, which are pilus-like, fine, filamentous structures. Three types of pilus antigens have been characterized in animal ETEC: the K88 and 987P antigens, found principally in swine; and the K(antigen, most often found in the bovine and ovine species).

Several laboratory methods are used to identify ETEC in animals with diahrrhea. The infant-mouse test is used to detect ST; the Y-1 adrenal cell culture test, to detect LT; and the agglutination and fluorescent antibody test, to identify the pilus antigens. Tests are also used to detect ETEC in the gut loops of pigs, calves, and rabbits.

We developed an enzyme-linked immunosorbent assay (ELISA) for detecting K88 antigen and compared the sensitivity of that new test for detecting ETEC with the standard agglutination test, the Y-1 mouse adrenal cell-culture test, and the infant-mouse gastric assay.

The ELISA test has been used to diagnose a variety of bacterial, viral, and fungal diseases. Its application for diagnosing E. coli infections in man by detecting LT and ST and in calves by detecting K99 protein has been reported. Used in the diagnostic laboratory, the sensitive ELISA test is rapid, quantitative, and inexpensive; and it does not produce radioactive waste. We report here on an ELISA test, which can accurately identify E. coli possessing the K88 antigen.

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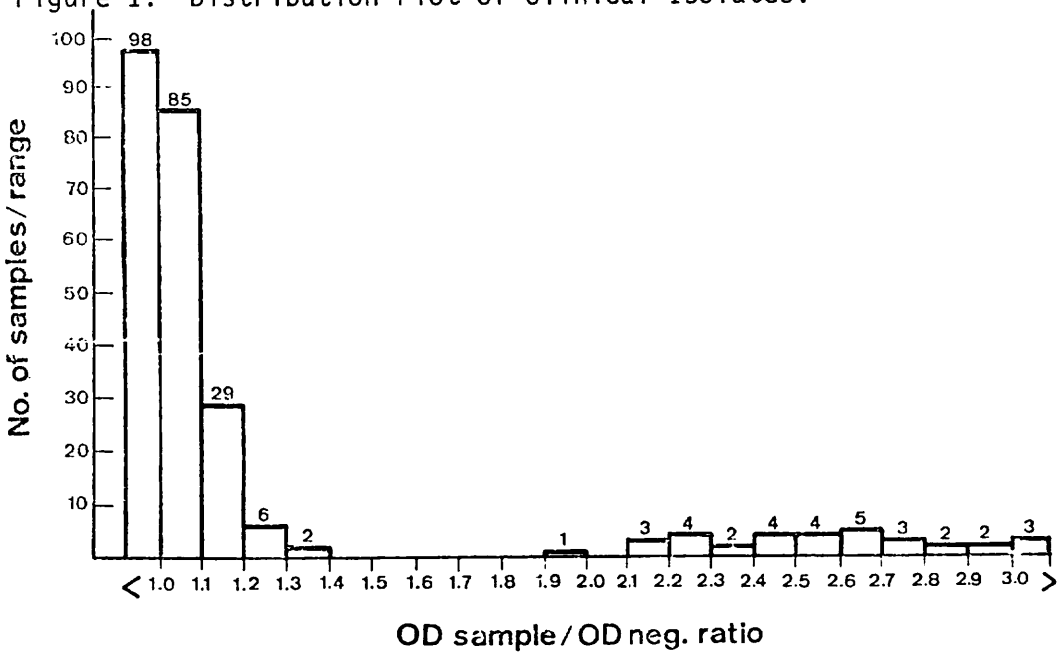
Procedures

Two hundred fifty-three clinical isolates of E. coli were grown and tested by standard agglutination and by the Y-1 mouse adrenal-cell and infant-mouse gastric tests. Results were compared with those obtained by using a recently developed ELISA test. The ELISA tests were performed on polystyrene microtiter plates, always by using 0.1-ml volumes, and quantitative results were read on a spectrophotometer.

Results and Discussion

The positive-negative cut-off point, determined by preliminary experimentation, was set at an OD-sample/OD-known negative of 1.5. That arbitrary point later was supported by the distribution plot (Figure 1) of 253 clinical isolates.

Figure 1. Distribution Plot of Clinical Isolates.



ELISA , agglutination, and Y-1 results (Table 41) agreed well.

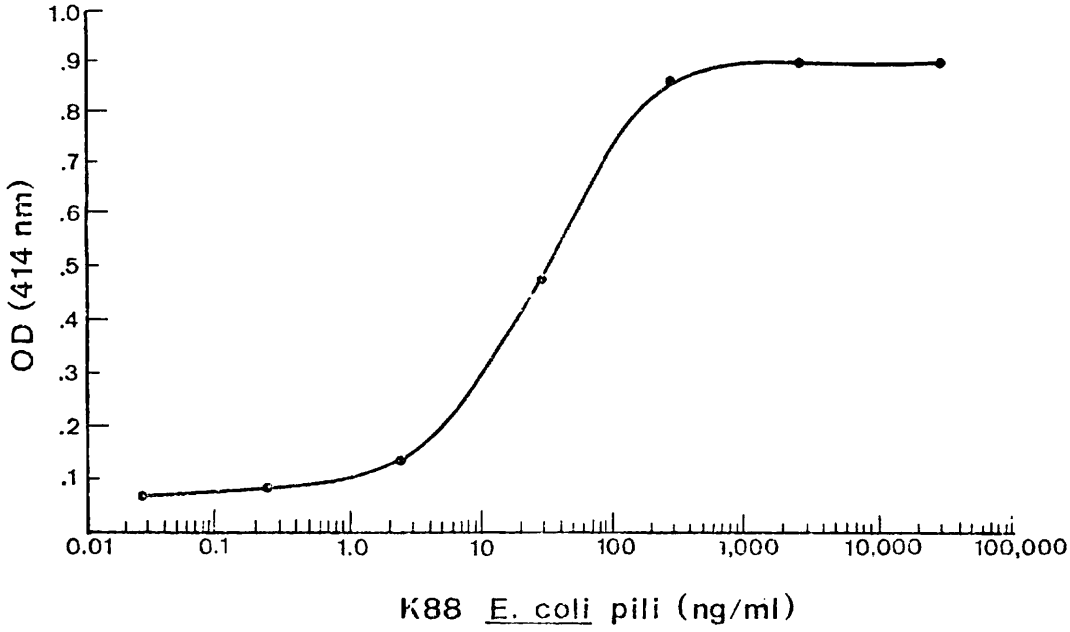
Table 41. ELISA Results Compared with Tests for Labile Toxin, K88 Pili, and Stable Toxin

	ELISA results		
	Positive	Negative	Agreement
Y-1 toxin results			
Positive	33	2	94%
Negative	0	218	100%
Agglutination results			
Positive	32	1	97%
Negative	0	219	100%
Questionable	1	0	0%
ST-mouse test			
Positive	3	33	10%
Negative	0	217	100%

Very little agreement was found or expected between ELISA results and ST mouse assay. Of the two samples that were positive by the Y-1 mouse adrenal test and negative by ELISA, one was determined to be an E. coli possessing the 987 pili. The other sample was determined, by agglutination, to possess K88.

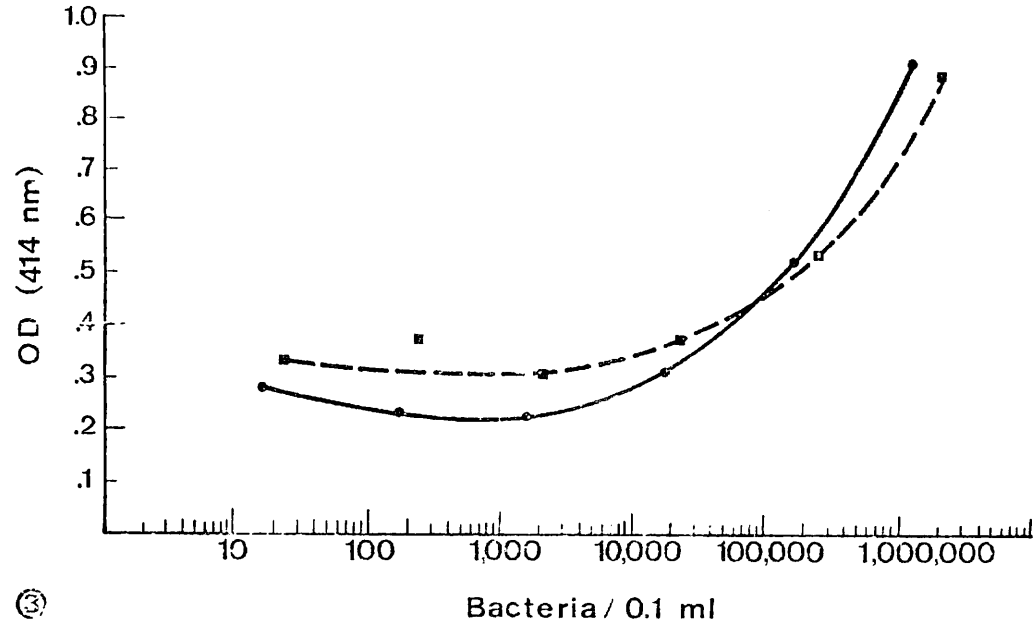
Data presented in Figure 2 indicate that the ELISA test for K88 pili required only the presence of approximately 10 ng/ml to be called positive.

Figure 2. ELISA Test for K88 Pili.



The number of E. coli necessary for detecting K88 pili by ELISA (Figure 3) was determined to be 10^6 bacteria/0.1 ml.

Figure 3. Number of E. coli Necessary for Detecting K88 Pili by ELISA Test.



We demonstrated that the ELISA test is extremely specific, and because there is complete separation of positive and negative populations, no decision nor mathematical determination of positivity is required.

The ELISA test offers a reliable, accurate, inexpensive replacement for existing methods to detect K88 pili. Replacing the Y-1 mouse adrenal test with the ELISA method also would reduce the cost/test for detecting diarrhea-producing E. coli. The assay could also be applied to quantitate K88-containing E. coli in standardizing commercial vaccines.

The Y-1 mouse adrenal test is also subject to the drawbacks of any assay requiring the propagation of living cells, such as variation in cell quality and contamination. Those variables can make that test less sensitive or even unreadable. The ELISA test does not rely on cell culture and can therefore be standardized.