

# Comparison of Y1 mouse adrenal cell and coagglutination assays for detection of *Escherichia coli* heat labile enterotoxin

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**SUMMARY** A commercial coagglutination assay (COA; Phadebact LT-ETEC) was compared with a Y1 mouse adrenal cell assay for detecting the heat labile enterotoxin of *Escherichia coli*. Of four different media evaluated for use with the COA, only one (modified blood agar) gave a positive result with all strains known to produce heat labile enterotoxin. With modified blood agar, the COA detected 74 (85%) of 87 such strains. Eighty six strains negative by cell culture assay were also negative by COA, and one strain positive by COA could not be confirmed by cell culture. The Phadebact LT-ETEC kit provides a simple, sensitive, and economical method for detecting *E coli* heat labile enterotoxin.

Heat labile enterotoxin-producing strains of *Escherichia coli* (LT<sup>+</sup>ETEC) are an important cause of diarrhoea in developing countries, and in those who travel from developed to less developed countries.<sup>1,2</sup> Though an uncommon cause of diarrhoea in some developed countries,<sup>3-5</sup> LT<sup>+</sup>ETEC have recently been reported as a common finding in patients with diarrhoea returning to the United Kingdom from popular mediterranean holiday resorts.<sup>2</sup>

The aim of this study was to compare a commercial coagglutination assay (COA) kit (Phadebact ETEC-LT, Pharmacia LKB Diagnostics) with a simplified Y1 mouse adrenal cell assay<sup>6</sup> for detecting LT<sup>+</sup>ETEC.

## Material and methods

*E coli* test strains were those isolated in previous studies<sup>2,6</sup> from patients with diarrhoea returning to Sheffield from abroad. Strains had been stored in nutrient broth with 15% glycerol at -70°C since first isolation. All were retested in the Y1 mouse adrenal cell assay in parallel with the COA. Eighty seven LT positive and 87 LT negative strains were used to compare the two methods.

The simplified Y1 mouse adrenal cell assay was performed as described earlier,<sup>6</sup> but with substitution

of clindamycin 45 µg/ml for lincomycin 90 µg/ml in the medium used to enhance toxin production.

The initial evaluation of the coagglutination assay was performed using 10 LT<sup>+</sup>ETEC strains isolated from travellers returning from diverse locations; strains produced widely differing amounts of heat labile enterotoxin as determined by cell culture assay. Each strain was grown for 24 hours on four different media: MacConkey agar (Oxoid CM7b), xylose lysine desoxycholate agar (Oxoid CM469), blood agar (Oxoid columbia agar base with horse blood 5%), and modified blood agar (blood agar with added clindamycin 45 µg/ml and a final pH of 8.0). Five colonies were then suspended in 100 µl of extraction fluid supplied with the kit, vortex mixed, and incubated at 37°C for one hour. Cells and cell debris were then removed by centrifugation at 3000 g for two minutes. The clear supernate was then used to prepare a series of doubling dilutions from 1/2 to 1/32 in phosphate buffered saline. The neat supernate and dilutions were then used in the test procedure according to the manufacturer's instructions. Positive results were scored as follows: +++ = strong agglutination occurring within one minute; ++ = moderate agglutination occurring within one minute; and + = weak agglutination occurring within two minutes.

The COA was compared with the Y1 mouse adrenal cell assay using *E coli* strains grown on modified blood agar and processed as above; only the neat supernate was tested.

## Results

Table 1 shows the results of the initial evaluation of the COA. Because the only medium to give a positive result on all 10 strains was modified blood agar, this was used in the comparison of the assay with the Y1 mouse adrenal cell assay. Table 2 summarises the results obtained. The COA detected 74 (85%) of 87 strains shown to produce heat labile enterotoxin by cell culture assay. Thirteen strains positive by cell culture were negative by the COA. One strain positive by the COA could not be confirmed by cell culture; 86 strains were negative in both assays.

## Discussion

Despite the importance of LT<sup>+</sup>ETEC as a cause of diarrhoea,<sup>12</sup> their recognition is usually accomplished in only a few diagnostic microbiology laboratories. This may be partly due to difficulties in previously recommended detection methods.<sup>67</sup>

Cell culture assays for heat labile enterotoxin can be simple, economical, and sensitive, but need to be performed in laboratories with specialist cell culture facilities. While the Y1 cell assay is a reliable and sensitive assay,<sup>8</sup> other cell culture assays, such as the Chinese hamster ovary (CHO) cell assay<sup>9</sup> may be

Table 1 Initial evaluation of coagglutination assay

Isolate (source)	Toxin titre	Coagglutination assay						
		Medium	Supernate dilution					
			Neat	2	4	8	16	32
P163 (Brazil)	> = 2560	MA	-	-	-	-	-	-
		XLD	++	-	-	-	-	-
		BA	+++	+++	+++	+	-	-
		MBA	+++	+++	+++	+++	+++	++
P164 (Egypt)	> = 2560	MA	-	-	-	-	-	-
		XLD	-	-	-	-	-	-
		BA	-	-	-	-	-	-
		MBA	++	-	-	-	-	-
P165 (Russia)	2560	MA	-	-	-	-	-	-
		XLD	-	-	-	-	-	-
		BA	-	-	-	-	-	-
		MBA	++	-	-	-	-	-
P166 (Tunisia)	640	MA	-	-	-	-	-	-
		XLD	-	-	-	-	-	-
		BA	-	-	-	-	-	-
		MBA	+++	++	-	-	-	-
P167 (Spain)	2560	MA	-	-	-	-	-	-
		XLD	++	++	+	-	-	-
		BA	+++	+++	+++	+++	+	-
		MBA	+++	+++	+++	+++	+	-
P168 (Bulgaria)	1280	MA	-	-	-	-	-	-
		XLD	++	+	-	-	-	-
		BA	+++	++	-	-	-	-
		MBA	+++	+++	-	-	-	-
P169 (India)	80	MA	-	-	-	-	-	-
		XLD	-	-	-	-	-	-
		BA	-	-	-	-	-	-
		MBA	++	-	-	-	-	-
P171 (Pakistan)	20	MA	-	-	-	-	-	-
		XLD	-	-	-	-	-	-
		BA	-	-	-	-	-	-
		MBA	+	-	-	-	-	-
P174 (Spain)	640	MA	-	-	-	-	-	-
		XLD	-	-	-	-	-	-
		BA	+++	+	-	-	-	-
		MBA	+++	+++	+++	+	-	-
P182 (Portugal)	40	MA	-	-	-	-	-	-
		XLD	-	-	-	-	-	-
		BA	+	-	-	-	-	-
		MBA	+	-	-	-	-	-

MA = MacConkey agar; XLD = xylose lysine desoxycholate agar; BA = blood agar; MBA = modified blood agar.

Table 2 Comparison of Phadebact ETEC-LT coagglutination and Y1 mouse adrenal assay for detecting LT<sup>+</sup> ETEC

Cell culture	Coagglutination	Number
Positive	Positive	74
Positive	Negative	13
Negative	Positive	1
Negative	Negative	86

affected by the cytolethal distending toxin of *E coli*, which may be misinterpreted as having been produced by heat labile enterotoxin.<sup>10</sup> Heat labile enterotoxin is antigenic and various immunoassays for its detection have been described,<sup>11-15</sup> but despite the relative ease with which these tests may be performed, they all have one common limitation—all require a high titre of antitoxin to highly purified heat labile enterotoxin. No such antitoxin is available commercially, and its production would almost certainly be beyond the scope of many diagnostic laboratories.

The introduction of a simple commercial kit for detecting heat labile enterotoxin obviates the need for specialist cell culture facilities and puts testing for LT<sup>+</sup> ETEC within the scope of any diagnostic microbiology laboratory. The kit as it is currently produced by the manufacturer, however, presented several problems. The instructions did not make clear that the whole test procedure should be controlled by inclusion of known LT<sup>+</sup> ETEC strains in each batch of tests. The instructions also implied, wrongly, that various selective media such as MacConkey and xylose lysine desoxycholate agar may be used directly in the assay. LT<sup>+</sup> ETEC produce little or no toxin when grown on most selective media.<sup>12</sup> Indeed, in our initial evaluation of the kit no isolates produced detectable toxin after culture on MacConkey agar, and only two of 10 isolates (both strongly toxigenic) produced detectable toxin after culture on xylose lysine desoxycholate agar. Evaluation with normal blood agar gave disappointing results, detecting only five of 10 strains. This conflicts with a previously published evaluation of the COA<sup>12</sup>; one reason for this discrepancy might be the widely differing amounts of toxin produced by strains of LT<sup>+</sup> ETEC from various geographical locations.<sup>2</sup> Lincomycin, an inhibitor of protein synthesis, may block production of the enzymes which regulate toxin production in *E coli*, and can significantly increase the amount of heat labile enterotoxin produced by strains.<sup>6,16</sup> Clindamycin, a compound closely related to lincomycin, produces the same effect (Sheffield Public Health Laboratory, unpublished data). The pH of culture media is also important if optimal production of heat labile enterotoxin is to be achieved, heat labile enterotoxin being less readily produced or released from cells at pH

values below neutral.<sup>17</sup> Combining clindamycin with a raised pH considerably increased toxin production on blood agar and detected even weakly toxigenic isolates. Using such a modified procedure the kit detected 74 of 87 heat labile enterotoxin plus strains (sensitivity 85%) and gave a positive result in only one of 87 heat labile enterotoxin minus strains (specificity 98.6%).

We conclude that with suitable modifications to the instructions provided with the kit, and to the control and performance of the test, the Phadebact ETEC-LT kit provides a simple, sensitive, and economical method for detecting LT<sup>+</sup> ETEC. The pronounced increase in foreign travel seen over the past decade seems likely to continue,<sup>18,19</sup> and therefore a rapid screening test for one of the major causes of travellers' diarrhoea is a useful addition to laboratory diagnostic procedures.

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#### References

- Guerrant RL. Microbial toxins and diarrhoeal disease: introduction and overview. In: Evered D, Whelan J, eds. *Microbial toxins and diarrhoeal disease*. Ciba Foundation Symposium No 112. London: Pitman Medical, 1985:1-27.
- Chapman PA, Mitchelmore DL. A two-year survey of heat-labile enterotoxin-producing *Escherichia coli* and other enteric pathogens in travellers returning to the Sheffield area. *Epidemiol Infect* 1988;**101**:239-47.
- Echeverria P, Blacklow NR, Smith DH. Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agent in diarrhoea in Boston children. *Lancet* 1975;ii:1113-16.
- Back E, Blomberg T, Wadstrom T. Enterotoxigenic *Escherichia coli* in Sweden. *Infection* 1977;**5**:2-5.
- Brunton J, Hinde D, Langston C, Gross RJ, Rowe B, Gurwith M. Enterotoxigenic *Escherichia coli* in central Canada. *J Clin Microbiol* 1980;**11**:343-8.
- Chapman PA, Swift DL. A simplified method for detecting the heat-labile enterotoxin of *Escherichia coli*. *J Med Microbiol* 1984;**18**:399-403.
- Gross RJ. *Escherichia coli* diarrhoea. *J Infection* 1983;**7**:177-92.
- Chapman PA. Detection of heat-labile enterotoxin-producing *Escherichia coli* and their prevalence in acute diarrhoea. [Thesis]. University of Sheffield, 1987:98-104.
- Guerrant RL, Brunton LL, Schnaitman TC, Rebhun LI, Gilman AG. Cyclic adenosine monophosphate and alteration of chinese hamster ovary cell morphology: a rapid, sensitive in vitro assay for the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. *Infect Immun* 1974;**10**:320-7.
- Johnson WM, Lior H. Response of chinese hamster ovary cells to cytolethal distending toxin (CDT) of *Escherichia coli* and possible misinterpretation as heat-labile enterotoxin. *FEMS Microbiol Letts* 1987;**43**:19-23.
- Sack DA, Huda S, Neogi PKB, Daniel RR, Spira WM. Microtiter ganglioside enzyme-linked immunosorbent assay for *Vibrio* and *Escherichia coli* heat-labile enterotoxins and antitoxin. *J Clin Microbiol* 1980;**11**:35-40.
- Ronnberg B, Wadstrom T. Rapid detection by a coagglutination test of heat-labile enterotoxin in cell lysates from blood agar-grown *Escherichia coli*. *J Clin Microbiol* 1983;**17**:1021-5.
- Finkelstein RA, Yang Z. Rapid test for identification of heat-labile

- enterotoxin-producing *Escherichia coli* colonies. *J Clin Microbiol* 1983;18:23-8.
- 14 Bramucci MG, Holmes RK. Radial passive immune haemolysis assay for detection of heat-labile enterotoxin produced by individual colonies of *Escherichia coli* or *Vibrio cholerae*. *J Clin Microbiol* 1978;8:252-5.
  - 15 Honda T, Akhtar Q, Glass RI, Golam Kibriya AKM. A simple assay to detect *Escherichia coli* producing heat-labile enterotoxin: results of a field study of the Biken test in Bangladesh. *Lancet* 1981;i:609-10.
  - 16 Levner M, Wiener FP, Rubin BA. Induction of *Escherichia coli* and *Vibrio cholerae* enterotoxins by an inhibitor of protein synthesis. *Infect Immun* 1977;15:132-7.
  - 17 Gilligan PH, Robertson DC. Nutritional requirements for synthesis of heat-labile enterotoxin by enterotoxigenic strains of *Escherichia coli*. *Infect Immun* 1979;23:99-107.
  - 18 Business Statistics Office. *Business monitor annual statistics. Overseas travel and tourism*. London: HMSO, 1985.
  - 19 World Tourism Organisation. *Tourism Compendium*. Madrid: Graficas Fcc, SL, 1985.

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