

Phadebact® ETEC-LT Test

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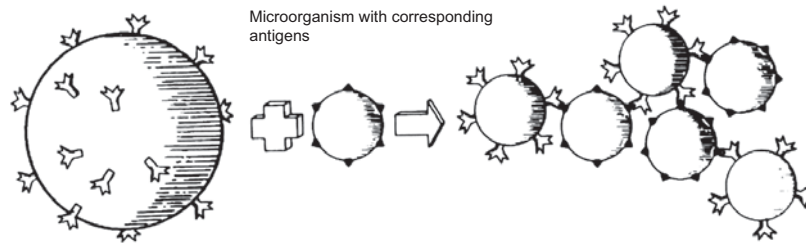
Directions for Use

Bactus AB
Lunastigen 3
SE-141 44 Huddinge
Sweden



Antibody-coated staphylococci

Co-agglutination



INTENDED USE

Phadebact® ETEC-LT Test is intended for the identification of heat-labile (LT) enterotoxin produced by *E. coli* from stool cultures.

SUMMARY AND EXPLANATION OF THE TEST

Enterotoxigenic *Escherichia coli* (ETEC) is a frequent cause of diarrhea in developing countries and of traveller's diarrhea. ETEC produces one or two distinct enterotoxins, heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) both of which cause diarrhea.

Laboratory diagnosis of diarrhea due to *E. coli* presents a particular problem because of the ability of the organisms to cause diarrhea by several different mechanisms. The inability of traditional culture and serological methods to detect several pathogenic strains further complicates this problem. To detect most of the pathogenic *E. coli* strains methods for toxin testing are required. Since many of these test methods require animals, cell cultures or equipment for nucleic-acid based assays they are not applicable to routine use in clinical laboratories (1). A simple gel immunodiffusion test, the Biken test, has been developed and reported to be more suited for laboratories in need of simple tests for *E. coli* LT for routine diagnostic purposes. The main disadvantage of the Biken test (2) is that results are obtained after 3-4 days.

Phadebact® ETEC-LT Test is based on the co-agglutination technique, a rapid and simple immunoassay, requiring a minimum of laboratory equipment and no special education of laboratory personnel.

PRINCIPLE OF THE PROCEDURE

Phadebact® ETEC-LT Test is a co-agglutination test containing ETEC-LT Reagent, ETEC-LT Control and Extraction Solution. The ETEC-LT Reagent is composed of antibodies against highly purified LT enterotoxin, raised in rabbits and coupled to the Protein A of non-viable staphylococci (3). The ETEC-LT Control is composed of gammaglobulin from non-immunized rabbits, coupled to the staphylococci. When a drop of a cell extraction of suspended bacterial colonies from LT-producing *E. coli* strains is mixed with the ETEC-LT Reagent, specific antigens bind to corresponding antibodies on the surface of the staphylococci. In this way a co-agglutination lattice is formed, which is visible to the naked eye.

REAGENTS

Each Phadebact® ETEC-LT Test package contains reagents sufficient for 50 determinations. The reagents are coloured blue (Methylene blue) to facilitate interpretation of results.

Reactive ingredients

- | | |
|---|---------|
| • ETEC-LT Reagent
Specific antibodies raised in rabbit,
bound to non-viable staphylococci. | 1 vial |
| • ETEC-LT Control, negative
Gammaglobulin from non-immunized rabbit,
bound to non-viable staphylococci. | 1 vial |
| • Extraction Solution | 2 vials |

Other components

- Droppers
- Disposable slides
- Directions for Use

Precaution

For *in vitro* diagnostic use.

Warning! The reagents contain sodium azide (NaN₃) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to the decontamination procedures as outlined by CDC.

Preparation of reagents

The reagents are READY TO USE.

Shelf life and storage

The expiration date is stated on the outer label and the vial labels. It is recommended that the kit be stored at 2-8°C. Reagents must be protected from freezing.

SPECIMEN COLLECTION AND HANDLING

Please refer to a standard microbiology textbook regarding information on specimen collection, handling and transportation. A battery of media for routine use in the culture from stool specimens should include a non-selective medium such as blood agar, moderately selective differential medium such as Mac Conkey agar, a more selective differential medium, such as xylose-lysine-deoxycholate agar, and a broth enrichment (1). The use of such a battery will provide for the detection of the *Enterobacteriaceae* most commonly associated with enteric infection, including *Salmonella* and *Shigella* species and *E. coli*. However, toxin production may be optimized by the addition of lincomycin or clindamycin (9,10), e.g. 5% horse agar, pH 8.0, with clindamycin (45 µg/mL) is recommended (10).

PROCEDURE

Materials provided

See under REAGENTS.

Materials required but not provided

- Plastic tubes
- Pipettes
- Waterbath or heating device
- Centrifuge
- Disposable loops
- Timer with easily-read minute indicator

Parameters of the method

Reaction temperature	room temperature
Volume of reagents	one drop
Reaction time	1 minute

Preparation of samples

Please refer to a standard microbiology textbook regarding detailed information on preparation of primary cultures. After inoculation, the media should be incubated at 35-37°C for 18-24 hours (4). Colonies suspected of being *E. coli* may then be tested with Phadebact® ETEC-LT Test for identification:

- Remove a loopful of bacteria suspected to be *E. coli* from the plate.
- The bacteria are suspended in a small tube in 100 µl of Extraction Solution.
- Incubate at 37°C for 30 minutes in a water bath or by using a heating device.
- Centrifuge the suspension at 2000xg for 5 minutes.
- A drop of the supernatant is used for testing.

Test protocol

Note! Suspend reagents thoroughly by shaking or vortexing.

Put one drop of the ETEC-LT Reagent in one of the circles on the slide and one drop of the ETEC-LT Control in another circle. Fig. 1.

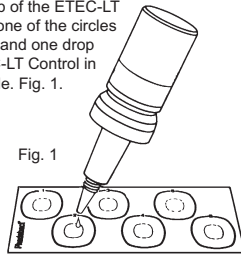


Fig. 1

Place one drop of the centrifuged supernatant alongside the drop of the ETEC-LT Reagent and one drop alongside the ETEC-LT Control. Fig. 2.

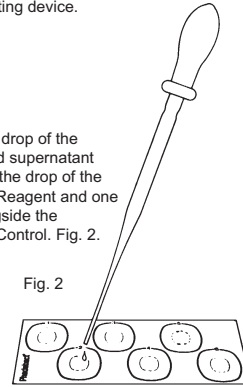


Fig. 2

Mix the drops. By starting with the Negative Control, the same disposable loop can be used for mixing of both Control and Reagent. Fig. 3.

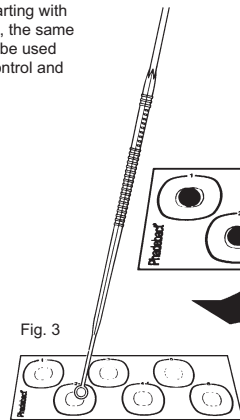


Fig. 3

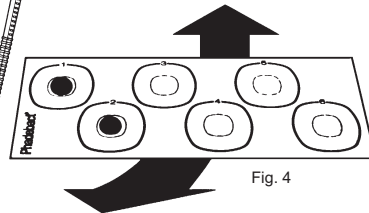


Fig. 4

Rock the slide and read the result within 1 minute. Fig. 4.

Stability of the final reaction mixture

The co-agglutination reaction is stable, but good laboratory practice dictates that the result be read within 1 minute (observe the risk of drying out of the reagents which may be misinterpreted as a positive reaction).

Calibration

No calibration is needed.

Quality control

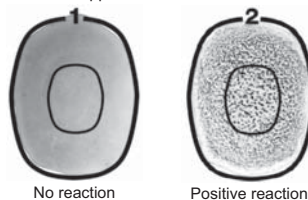
Positive control

As a control, an established LT⁺ *E. coli* strain should be used (e.g. NIH 2011 (8)). The control strain is treated in an identical manner as the unknown bacteria in the test procedure.

Negative control

As a control, an established LT⁻ *E. coli* strain should be used (e.g. ATCC 8739). The control strain is treated in an identical manner as the unknown bacteria in the test procedure.

Appearance of reaction



No reaction

Positive reaction

RESULTS

Positive result

A blue precipitate, co-agglutination, seen in the ETEC-LT reagent circle with no or a very weak reaction in the Control, confirms the identity of the specimen as a LT enterotoxin producing *E. coli*.

Negative result

A very weak reaction in the ETEC-LT Reagent together with a very weak reaction in ETEC-LT Control or no reaction in both, strongly suggests that the bacteria tested is not a LT enterotoxin producing *E. coli*.

LIMITATIONS OF PROCEDURE

Due to both biological and immunological similarities of the toxins, *V. cholerae* and *E. coli* will cross-react. However, by following the general guidelines as outlined in handbooks of microbiology when samples are cultivated, this problem will be avoided. Due to molecular heterogeneity between human and animal isolates (7), tests of veterinary isolates may result in false negatives.

PERFORMANCE CHARACTERISTICS

Specificity and sensitivity

In total 436 strains suspected of LT enterotoxin producing *E. coli* were investigated (5, 6, 7, 8). The reference methods were Biken test, ELISA and cell test. The overall performance of Phadebact® ETEC-LT Test was as follows.

1. No. of strains	COA	Biken Test
45	+	+
50	-	-
1	+	-
4	-	+

2. No. of strains	COA	ELISA
58	+	+
71	-	-
6	+	-
5	-	+

3. No. of strains	COA	Cell Test
100	+	+
210	-	-
16	+	-
10	-	+

In total, 665 isolates of human or veterinary origin (incl. laboratory strains) were investigated (5-10) against reference methods with a sensitivity of 90.0% (271/301) and a specificity of 95.1% (346/364).

		Reference	
		+	-
Phadebact®	+	271	18
	-	30	346

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Bactus AB may affect the results, in which event Bactus AB disclaims all warranties, expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. Bactus AB and its authorized distributors, in such event, shall not be liable for any damages, whether direct, indirect or consequential.

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PRODUCTS

Phadebact® COA System

Phadebact® Streptococcus Tests
Phadebact® Streptococcus Respiratory Test
Phadebact® Strep A Test
Phadebact® Strep B Test
Phadebact® Strep D Tests
Phadebact® Strep F Test
Phadebact® Strep Positive Controls
Phadebact® Pneumococcus Test
Phadebact® Haemophilus Test
Phadebact® GC Positive Controls
Phadebact® CSF Test
Phadebact® CSF Positive Controls
Phadebact® Extraction Solutions
Phadebact® Monoclonal GC Test
Phadebact® ETEC-LT Test
Phadebact® Salmonella Test
Phadebact® Staph Aureus Test

Near Patient Testing

Phadirect® Strep A
Phadirect® Rapid CRP Test

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