RIDA® Anreicherungsbouillon

mTSB for the enrichment of
shigatoxin-producing E. coli bacteria

Article no: Z1000
Article no: Z1003

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1. Intended use

For *in vitro* diagnostic use. The RIDA® Anreicherungsbouillon is used to enrich *E.coli* bacteria which produce shigatoxins (verotoxins) from stool specimens.

2. Summary and explanation of the test

Of the different human pathogenic variants of the intestine bacterium, Escherichia coli, one of the distinguishing features of EHEC bacteria is that they always produce so-called shigatoxins or verotoxins. Both names are used synonymously and refer to their high level of homology to shigellentoxin (the term Shiga-Like-Toxin is also commonly used) and their toxicity to verocells respectively. Such STEC / VTEC (shigatoxin-producing E. coli / verotoxin-producing E. coli) are now referred to as EHEC bacteria which trigger symptoms in humans and are therefore pathovars to humans. These again are classified into different serovars according to the antigenic structure. Some serovars are found more often among diseased patients than others. Furthermore, they do not all possess the same virulence markers, the main ones being intimin and enterohaemolysin beside the two shigatoxins Stx 1 and Stx 2. The information for producing the shigatoxin is located on a bacteriophage which is integrated in the bacterial chromosome. The amount of shigatoxin produced varies greatly between the different STEC strains. Screening for the presence of these toxins directly from the stool samples of patients with EHEC infection therefore cannot be recommended. The pathogens must first be enriched by culturing them from a patient stool sample in a suitable medium. The media which are suitable for this purpose are those which, in particular, activate the potential for producing shigatoxin as well as the selectivity factors for Escherichia coli. The ratio of EHEC organisms to the physiological, commensal E. coli intestinal flora is approximately 1 : 200. In this respect, an effective method of enrichment which induces the formation of shigatoxin is the basic requirement for successful screening in ELISA. This enrichment broth takes these requirements into consideration.

3. Test principle

The RIDA® Anreicherungsbouillon selectively promotes E. coli organisms because it contains bile salts and suppresses the growth of gram-positive organisms. The addition of Mitomycin C promotes the production of shigatoxin in particular by inducing the lambdoid prophage on the bacteria chromosome and its release by cell lysis so that, even with weak toxin producers, the toxins can be reliably determined from the supernatant of the enrichment culture in the screening ELISA which follows. This is especially important with HUS patients, where often only very few EHEC organisms are found in the stools.

4. Reagents provided

There are enough reagents in the kit for 100 enrichments.

Tube	100(25)	Test tube with 4 ml Anreicherungsbouillon; contains mTSB and Mitomycin C

5. Storage instructions

The test tubes with the enrichment broth must be stored at 2 – 8 °C and can be used until the expiry date printed on the label. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid. If the clear pale yellow enrichment broth has turned visibly turbid, this is an indication that it is contaminated with micro-organisms. Any test tubes which have turned visibly turbid must not be used and must be disposed of in accordance with Section 10.2.

6. Additional necessary reagents – and necessary equipment

6.1. Reagents

- PBS buffer or physiological saline solution (optional)
- Disposal solution (Section 10.1.)

6.2. Accessories

- Disposable pipettes (Article no: Z 0001)
- Micropipette for 100 µl volume
- Thin cotton swabs
- Horizontal shaker or rotary mixer with rack for 16.5 x 105 mm test tubes
- 37 °C incubator

7. Precautions for users

For *in vitro* diagnostic use only

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.
The RIDA® Anreicherungsbouillon contains Mitomycin C. Contact with the skin and mucous membranes must be avoided under all circumstances.
If the substance should come into contact with the skin or mucous membrane, rinse with plenty of water and change any contaminated clothing. Materials which come into contact with the enrichment broth, such as pipette tips and swabs, must be disposed of in the same way (Section 10.3.). Dispose of the enrichment broth in accordance with Section 10.2.

8. Specimen collection and storage

If the stool samples to be tested for shigatoxins (or verotoxins) are not used, they can be stored as follows before use in the enrichment broth:
– up to 24 hours at room temperature or at 2 – 8 °C
– up to 72 hours at 2 – 8 °C
Freezing the samples is not recommended since this will damage the EHEC bacteria and reduce their ability to multiply or even stop them from multiplying at all in the enrichment culture.

9. Test procedure

For successful enrichment, the following procedure and pipetting volumes must be followed as precisely as possible. These correspond to the current state of knowledge and have been adjusted in close collaboration with the appropriate reference laboratories of the RKI (Robert Koch Institute) and the BfR (German Institute for Risk Evaluation). Only those stool samples which are fresh or have been stored at 2 – 8 °C for 3 days maximum should be used.

9.1. Liquid or aqueous stool samples

Transfer 100 µl (when using disposable pipettes Article no. Z 0001, corresponds to a height slightly above the second thickening) or a thin swab soaked in the sample to 4 ml RIDA® Anreicherungsbouillon and shake the test tube until the sample is sufficiently in suspension.

9.2. Solid stool samples

Collect 50 - 100 mg solid stool with a spatula or a disposable inoculation loop and suspend it in 4 ml RIDA® Anreicherungsbouillon. Alternatively, an equivalent quantity can be collected by wiping it from the stool with a thin swab (preferably at different places on the stool sample) and suspending it by thoroughly shaking it in 4 ml RIDA® Anreicherungsbouillon. Likewise, a stool sample previously suspended in PBS (50 - 100 mg in 1 ml buffer) is also suitable from which 500 µl can be used to inoculate 4 ml RIDA® Anreicherungsbouillon.

9.3. Enrichment

Incubate the enrichment broth inoculated according to Section 9.1 or 9.2 for 18 - 24 h at 37 °C with shaking (120 - 160 rpm) with the test tube inclined and with an adequate supply of oxygen (half turn of the turn-lock fastener). Make sure that none of the liquid escapes. A horizontal shaker or rotary mixer are equally suitable for this purpose. After 24 h maximum, centrifuge down the entire enrichment broth at 2500 g for 5 min. Use 100 µl of the undiluted supernatant in the RIDASCREEN® Verotoxin ELISA.

Note:

If scum has formed on the enrichment broth, this should be carefully removed so that it will not be transferred to the microwell plate. This scum can lead to false positive results due to its high propensity to stick in the wells of the microwell plate.

The following procedure is recommended for dispatching material for confirmation of a positive ELISA result by an authorised reference laboratory (PCR and organism identification):

1. Decant the remaining supernatant from the test tube after centrifugation
2. Remove the entire pellet with a swab
3. Introduce the swab material into a suitable transport medium (Cary Blair, Stuart or Amies)
4. Dispatch (preferably with cooling) to the appropriate specialist laboratory for further analysis

Note :
A swab loaded with the stool sample can also be dispatched directly in the above mentioned transport media for further analysis.

10. Disposal of media and materials containing Mitomycin C

Since Mitomycin C is considered to be carcinogenic, the corresponding growth media containing Mitomycin and materials which have come into contact with it must be disposed of in accordance with the dangerous material regulations (special refuse) within Germany or relevant national guidelines outside Germany.

10.1. Disposal solution

0.5 % Wofasteril® (or 0.2 % peracetic acid) in 5 % acetic acid

i.e. 50 ml acetic acid
+ 5 ml Wofasteril® (or 2 ml peracetic acid)
to 1 l distilled water
Storage at 2 – 8 °C

Note :
Wofasteril® is available from Kesla Hygiene AG, D-06803 Greppin, Germany
Tel: +49 (0) 3494 69950 ; www.kesla.de/hygiene

10.2. Disposal of enrichment broth

Add an equivalent volume of disposal solution to the enrichment culture (i.e. 4 ml culture + 4 ml disposal solution), allow to act overnight at room temperature and then autoclave at 121 °C for at least one hour.

10.3. Disposal of materials

Pipette tips and swabs can be disposed of together with the enrichment broth under Section 10.2.

11. Clinical results

In an enrichment media validation test carried out by the BGVV, the RIDA® Anreicherungsbouillon which contained Mitomycin was found to be significantly superior to media which did not contain Mitomycin, whether the media were supplemented with antibiotics (Novobiocin and Cefsoludin) alone or bile salts alone or supplemented with both together.
With clinical stool samples from HUS patients, the Mitomycin additive yielded an average increase of 37.5 %.
With strain isolates (well-known producers of shigatoxin) from artificially inoculated stool samples, the addition of Mitomycin yielded an increase in the shigatoxin found by between 40 and 50 % in comparison to enrichment media without Mitomycin.

The concentration of Mitomycin used in the enrichment broth was found to be a very low-grade mutagen in a recently unpublished study on mutagenicity, which means that its use as a means of providing a sensitive method of shigatoxin diagnosis is justified. At present, no better but less harmful inductors for the release of shigatoxin are known.