

Yersinia ITC/Broth

356-3513

DEFINITION

Medium used for the enrichment, isolation and presumptive identification of *Yersinia enterocolitica* in the analysis of food products.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 10273 (December 2003):** Food Microbiology - Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*.

PRINCIPLE

• ITC Both

The presence of green malachite and Irgasan inhibits the growth of Gram-positive bacteria, and the presence of Ticarcillin (or Carbecillin) and potassium chloride inhibits the growth of some Gram-negative bacteria.

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PRESENTATION

• Yersinia ITC Both

9 ml x 25 tubes

code 356-3513

STORAGE

- Ready-to-use: + 2°C to 8°C.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

ITC Broth (according to Wauters, Goossens, Jenessens and Vandepitte)

Tryptone	10 g
Yeast extract	1 g
Magnesium chloride	60 g
Sodium chloride	5 g
Green malachite	10 mg
Potassium chloride	1 g
Ticarcillin	1 mg
Irgasan	1 mg
Distilled water	1,000 ml

Final pH (25°C) = 6.9 ± 0.1

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

• Monovalent agglutinating sera for typing *Y. enterocolitica*:

- serogroup O3 (40 tests): 2 ml x 1 bottle (code 356-3501)
- serogroup O9 (40 tests): 2 ml x 1 bottle (code 356-3502)

• Positive control serum: 1 ml x 3 bottle (code 356-3505)

• Serodiagnosis: *Yersinia* kit (code 356-3500)

Detection and titration of anti-*yersinia* antibodies by direct agglutination in tube or microplate of antigens colored with:

- dark blue antigen suspension of *Yersinia enterocolitica* serotype O: 5 ml x 3 vials
- blue-green antigen suspension of *Yersinia enterocolitica* O: 9: 5 ml x 1 vial
- pink antigen suspension of *Yersinia pseudotuberculosis* serotype I: 5 ml x 1 vial

PROTOCOL

• Preparation of samples

Enrichment is essential for detecting slight contamination of food products by *Yersinia enterocolitica*. The sample is diluted at the rate of 1/10th or 1/100th in the ITC broth, then incubated for 2 days at 25°C.

• Inoculation and incubation

Depending on the origin of the sample, the associated flora is more or less abundant, and it is sometimes necessary to carry out a treatment of 20 seconds with 0.25% potassium prior to inoculation onto the CIN agar.

Using the culture obtained on ITC medium, inoculate the surface of a dish of CIN agar by means of an inoculating loop.

READING AND INTERPRETATION

On CIN agar, after 24 hours cells are small (1 to 1.5 mm), smooth, with a red center and translucent border (rosette appearance); after 48 hours a halo of precipitation of bile salts may turn this zone opaque.

The size and appearance of colonies are similar to those of *Enterobacteria*. Other bacterial

Yersinia/Broth-Agar

genera (*Aeromonas*, *Pseudomonas*) occasionally yield larger pink colonies.

• Identification

Identification of *Yersinia* genus and species requires further biochemical tests. Among these, *Y. enterocolitica* possesses very active urease, so detection of this enzyme in suspected colonies very rapidly guides diagnosis if it is shown to be present; the same applies to absence of tryptophan desaminase and constant mobility when incubation temperature is below 28°C.

• Typing of *Y. enterocolitica*

Identification of the serogroup is carried out once the species has been diagnosed. This biochemical identification (Kligler medium - mobility - indole - urea) is essential due to the antigenic communities between *Y. enterocolitica* serogroup O: 9 and *Brucella*.

Typing must be performed on colonies less than 48 hours old (incubated at less than 30°C) after the absence of spontaneous auto-agglutination of the test strain in isotonic saline is confirmed.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product.

Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24h culture at 25-29°C
<i>Staphylococcus aureus</i> ATCC 25923	Inhibition
<i>Escherichia coli</i> ATCC 25922	Inhibition
<i>Salmonella</i> <i>Typhimurium</i> ATCC 14028	Inhibition

<i>Yersinia enterocolitica</i> ATCC 23715	Red bull's eye Transparent border
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KEY WORDS

Yersinia / *Yersinia enterocolitica* / Enrichment / Isolation / Identification / Food products / Medium.

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