

Formula * in g/L

Casein peptone.....	20,00
Dextrose.....	1,00
Potassium hydrogen phosphate.....	2,00
Potassium dihydrogen phosphate.....	2,00
Sodium chloride.....	5,00
Polysorbate 80.....	1,50

Final pH 7,0 ±0,2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 31,5 g of powder in 1 L of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes. Cool to 45°C and aseptically add novobiocin to obtain a final concentration of 0,5 mcg/mL. The complete medium must be used on the day of preparation. The broth base without antibiotic can be stored in the refrigerator for 4 weeks.

Description

Shigella Broth is a liquid culture medium that complies with ISO standard 21567:2004 for the detection of *Shigella* spp. in food and animal feeding stuffs. It is used in the preparation and dilution of the sample and as the selective enrichment step. It is a highly nutritive medium (peptone and glucose), with a strong phosphate buffer. The toxic-neutralizing and surfactant capacity of the polysorbate allows good growth of *Shigella*. The concentration of novobiocin used does not inhibit *Shigella* but inhibits the companion flora.

Technique

Prepare a 1:10 dilution of the sample in *Shigella* Broth with novobiocin and homogenize. A portion of 25 g or mL of the sample diluted in 225 mL of broth is recommended. Readjustment of the pH may be necessary after the addition of food to the broth.

The inoculated medium is incubated in anaerobic conditions with loose caps at 41,5 ± 1°C for 16-20 hours before streaking solid media with different selectivity levels: MacConkey Agar (Low Selectivity); XLD Agar (Medium Selectivity) and Hektoen Enteric Agar (High Selectivity).

The FDA proposes a slightly different technique: For *S. sonnei* the incubation temperature is 44°C and the novobiocin concentration is 0,5 µg/mL and for all other species the antibiotic concentration is increased to 3 µg/mL whilst the incubation temperature is lowered to 42°C.

Quality control

Incubation temperature: 41,5°C ±1 / ANAE **Incubation time:** 16-20 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (productivity) / 10⁴-10⁶ CFU (selectivity) according to ISO 11133:2014/Amd 1:2018 .

Microorganism

Shigella flexneri ATCC® 12022

Shigella sonnei ATCC® 9290

Salmonella typhimurium ATCC® 14028

Escherichia coli ATCC® 8739

Enterococcus faecalis ATCC® 29212

Growth

Good

Good

Good

Good (w/o Antibiotic)

Inhibited

Remarks

Recovery in XLD

Recovery in XLD

Recovery in XLD

Recovery in XLD

Recovery in XLD

References

- ATLAS, R.M. (1995) Handbook of Microbiological Media for the Examination of Food. C.R.C Press. Boca Raton. Fla. USA.
- FDA (Food and Drug Administrations) (1998) Bacteriological Analytical Manual 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 21567:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella* spp.
- VAN DER ZEE, H. (2003) Media for the isolation of *Shigella* spp. In "Handbook of Culture Media for Food Microbiology" (Corry et al. Eds.) Elsevier-Sci B.V. Amsterdam.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).