

**Biolife**

## Technical Sheet

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### SHIGELLA BROTH BASE NOVOBIOCIN ANTIMICROBIC SUPPLEMENT

Powdered medium and selective supplement for the enrichment of food supply chain samples for the identification of *Shigella*

#### INTENDED USE

Shigella Broth Base with added novobiocin is used as a selective enrichment broth for the determination (presence or absence) of *Shigella* in samples of the food supply chain according to ISO 21567.

#### PRINCIPLE OF THE METHOD

*Shigella* is a Gram negative rod, anaerobic facultative, not motile, oxidase-negative, asporigenous. The genetic analysis of the genus *Shigella* has shown that its four species have biological and physiological characteristics similar to *Escherichia coli*. Bacteria of the genus *Shigella* are etiologic agents of the bacillary dysentery or shigellosis. Their pathogenic action is due to the marked invasiveness towards the intestinal epithelium of the ileum and the colon and to endotoxins and exotoxins production, in particular by *S.dysenteriae*.

The medium here described contains a casein peptone which provides nitrogen and carbon for bacterial growth, glucose as a carbohydrate for growth stimulation, a buffer system consisting of potassium salts and sodium chloride to maintain an adequate osmotic balance, novobiocin to inhibit especially the Gram positive bacteria and some Gram negative as *Proteus spp* and polysorbate 80 as supporting agent for the inhibitory properties of the medium.

#### TYPICAL FORMULA (G/L) \*

Enzymatic digest of casein	20,0
Potassium hydrogen phosphate (anhydrous)	2,0
Potassium dihydrogen phosphate (anhydrous)	2,0
Sodium chloride	5,0
Glucose	1,0
Tween 80	1,5

The culture medium can be adjusted to adapt its performance to specifications.

#### PREPARATION OF THE DEHYDRATED CULTURE MEDIUM

Dissolve 15,75 g. in 500 mL of cold, purified water. Heat under stirring to dissolve the powder, distribute in bottles (225 mL per bottle) and autoclave at 121 °C for 15 minutes. Store the bottles at 2-8 °C for up to a month. At the moment of use, add to each bottle Novobiocin in order to obtain an enrichment broth with final concentration of 0.5 mcg / mL and finally add 25 g or 25 ml of sample (total volume: 250 mL).

Using the selective supplement "Novobiocin Antimicrobial Supplement" (REF 4240045) proceed as follows: dissolve the contents of the vial with 4 ml of sterile purified water and add 50 µl of this solution to the 225 ml bottle (final concentration 0.125 mg / bottle or 0.5 mcg / mL in the broth complete with the sample). The remaining novobiocin solution can be stored at 2-8 °C for one month or at -20 °C, divided into aliquots to be thawed only once, for 6 months.

#### CHEMICAL AND PHYSICAL CHARACTERISTICS

Aspect of the Dehydrated Culture medium: grainy powder of greyish color.

Appearance of the medium in bottle: slightly opalescent yellow

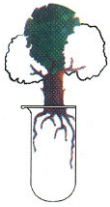
pH of the complete medium: 7,0 ± 0,2

#### METHOD

Prepare the sample aliquot according to the ISO 6887 standard.

Add 25 g or 25 ml of sample to 225 ml of Shigella Broth Base with added novobiocin as described above. Mix or homogenize the sample with the enrichment broth.

Incubate the bottles at 41.5 ± 1 °C for 16 -20 hours.



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Transfer a loopful of enrichment broth incubated as previously described on three different media plates, one moderately selective, one selective, one markedly selective: Mac Conkey Agar (Ref 401670), XLD Agar ISO Formulation (Ref 402208), Hektoen Enteric Agar (Ref 401541).

Incubate the inverted plates at  $37 \pm 1$  °C for  $22 \pm 2$  hours.

After incubation observe for the presence of typical *Shigella* colonies. A detailed description of the characteristics of *Shigella sonnei* and other *Shigella* species colonies, as well as the most common enterobacteria on the three plate media mentioned above, is reported in Annex C of ISO 21567.

Perform the biochemical and serological confirmation tests on the typical colonies as reported in the mentioned standard.

### QUALITY CONTROL

It is responsibility of the user to perform quality control tests in accordance with the regulations and according to his own Laboratory experience. The following table shows some useful strains for the quality control.

CONTROL STAINS	INCUBATION T°/t / ATM	EXPECTED RESULTS
<i>Shigella boydii</i> ATCC 11462	41,5°C ± 1°C / 18h ± 2h / A	Good growth
<i>Shigella flexneri</i> ATCC 12022	41,5°C ± 1°C / 18h ± 2h / A	Good growth
<i>Shigella sonnei</i> ATCC 25931	41,5°C ± 1°C / 18h ± 2h / A	Good growth
<i>Shigella dysenteriae</i> ATCC 9721	41,5°C ± 1°C / 18h ± 2h / A	Good growth
<i>Staphylococcus aureus</i> ATCC 25923	41,5°C ± 1°C / 18h ± 2h / A	Inhibited

#### Note

Incubation atmosphere A: aerobic incubation

ATCC is a registered trade mark of American Type Culture Collection; NCTC is a registered trade mark of National Collection of Type Cultures.

### STORAGE

**Dehydrated medium:** store at 10-30°C in the dark, in a dry place. When stored as directed, the medium remain stable until the expiry date shown on the label. Do not use beyond stated expiry date. Discard if there are any obvious signs of deterioration (color changes, hardening etc.).

**Selective supplement:** store at 2-8°C until the expiry date shown on the label.

### PRECAUTIONS

**Dehydrated medium:** The preparation described here is not classified as dangerous according to current legislation.

As for all dehydrated media, the handling must be carried out with adequate protection of the respiratory tract.

**Selective supplement :** the supplement contains novobiocin, Consult the Safety data Sheet before use.

### REFERENCES

• ISO 21567:2004 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Shigella* spp.

### PRODOTTI

Product	Type	Cat. N°	Package
SHIGELLA BROTH BASE	Dehydrated Culture medium	4020402	500 g (15.9 L)
NOVOBIOCIN ANTIMICROBIC SUPPLEMENT	Lyophilized supplement	4240045	10 vials (10 mg/vial)



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