TECHNICAL DATA SHEET

TSN AGAR

ENUMERATION OF SULFUR-REDUCING MICROORGANISMS

1 INTENDED USE

TSN agar is primarily used for the detection and enumeration of sulfur-reducing microorganisms at 46°C in certain food products, notably in pre-cooked packaged meals.

2 HISTORY

Tryptone Sulfite Neomycin Agar was recommended by Mossel and developed by Marschall *et al.* in 1965 for the selective isolation and enumeration of *Clostridium perfringens* in food products and other samples of animal origin, primarily when contaminated by considerable accompanying microflora.

The detection of *Clostridium perfringens* is based on the following characteristics :

- optimal growth at 46°C,
- tolerance to neomycin and polymyxin,
- high capacity to reduce sulfite

3 PRINCIPLES

The simultaneous presence of neomycin and polymixin inhibit the growth of enterobacteria.

Neomycin inhibits the growth of most strains of *Clostridium bifermentans*.

Sulfur-reducing microorganisms reduce sulfite to sulfide, which with ferric citrate causes a black iron sulfide precipitate around the colonies.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone ..... 15,0 g
- Yeast extract ..... 10,0 g
- Sodium sulfite ..... 1,0 g
- Ferric ammonium citrate ..... 0,5 g
- Neomycin sulfate ..... 50 mg
- Polymyxin B sulfate ..... 20 mg
- Bacteriological agar ..... 13,5 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

5 PREPARATION

- Dissolve 40,0 g of dehydrated media (BK001) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, with constant agitation until complete dissolution.
- Dispense in tubes or in vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution : 40,0 g/L**

✓ **Sterilization : 15 min at 121 °C**

6 INSTRUCTIONS FOR USE

Use in tubes :

- Heat the product to analyze in order to destroy vegetative cells and activate spores.
- Transfer 1 mL of the inoculum and its serial dilutions to the tubes, avoiding the incorporation of air into the medium.
- Homogenize thoroughly by inversion.
- Cool in an ice water bath.
- Incubate at 46 ± 1 °C for 24 ± 2 hours.

✓ **Inoculation : In pour plates**

✓ **Incubation : 24 h at 46°C**

Note : Do not overheat the medium. Avoid heating the tubes after inoculating.

Use in plates :

- Transfer 1 mL of the inoculum and its serial dilutions to empty, sterile Petri plates.
- Pour roughly 15 mL of media per plate.
- Homogenize well and let solidify on a flat, cold surface.
- Incubate the plates in an anaerobic jar in the presence of a mixture of hydrogen and carbon dioxide.

7 RESULTS

The plates are read immediately after opening the jar, since the colonies may become pale by the oxidation of iron sulfide by the outside air.
Count the colonies surrounded by a black halo.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : beige powder, free-flowing and homogeneous.
Prepared media : amber agar.

Typical culture response after 24 hours of incubation under anaerobic conditions at 46 °C :

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
Clostridium perfringens	WDCM 00007	$P_R \geq 70$ %	Black colonies
Clostridium perfringens	WDCM 00080	$P_R \geq 70$ %	Black colonies
Bacillus cereus	WDCM 00001	Inhibited	-
Escherichia coli	WDCM 00013	Inhibited	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media : Not recommended ; use immediately after preparation.

10 PACKAGING

Dehydrated media :
500 g bottle BK001HA

11 BIBLIOGRAPHY

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Arbuckle, R. E.. 1960. The influence of temperature on the growth of *Clostridium perfringens*. M.A. Thesis, Indiana Univ., Bloomington.

Collee, J.G., Knowlden, J.A., and Hobbs, B.C.. 1961. Studies on the growth, sporulation and carriage of *Clostridium welchii* with special reference to food poisonning strains. J.ournal of Applied Bacteriology, **24** : 326-339.

Marschall, R.S., Steenbergen, J.F., and McClung, L.S.. 1965. Rapide technique for the enumeration of *Clostridium perfringens*. Applied Microbiogy, **13(4)** : 559-563.

RODIER, J.. 1984. L'analyse de l'eau. Recherche et dénombrement des *Clostridium perfringens*. Dunod 7ème Ed., 855-857.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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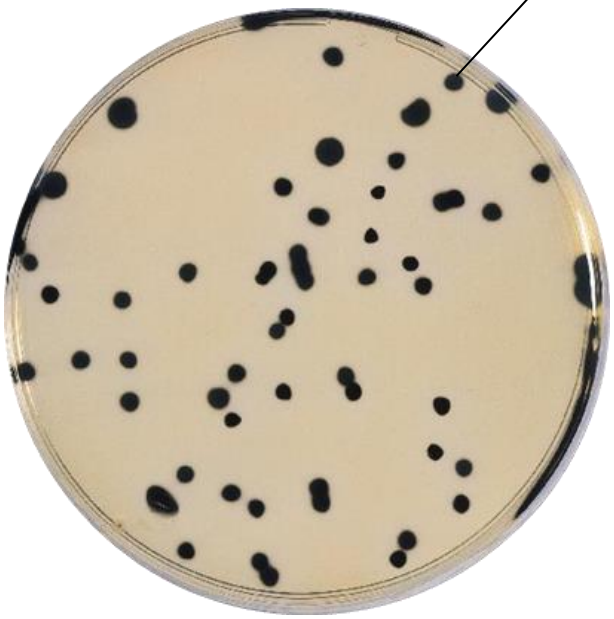
ANNEX 1 : PHOTO SUPPORT

TSN Agar

Enumeration of sulfur-reducing microorganisms

Results :

Growth obtained after 24 hours of incubation at 46 °C.



Clostridium perfringens

Characteristic colony :
black color