TSN/Agar
(Tryptone-Sulfite-Neomycin)

356-4724

DEFINITION
Medium used in the testing of food products for the detection and enumeration of sulfite reducing anaerobes by depth inoculation.

STANDARDS
- **Compulsory microbiological criteria for foodstuffs of animal origin – General methods of bacteriological analysis** (Decree of 21 December 1979 in JO dated 19 January 1980, amended by Decrees of: 7 September 1984 in JO dated 29 September 1984; 5 March 1985 in JO dated 23 March 1985; 2 June 1988 in JO dated 8 July 1988; 13 March 1989 in JO dated 20 April 1989).

- **Mechanically-separated poultry meat – Specimen collection and analytical technique** (Circular DQ/N°171C dated 25 November 1977).

PRINCIPLE
The principle of the medium relies on the ability of sulfite-reducing anaerobic bacteria to reduce sulfites to sulphide which, in the presence of ferric citrate, forms a black precipitate of iron sulphide (black colonies).
Neomycin, polymyxin and sulfite render the medium inhibitor with regard to secondary flora.

PRESENTATION
Dehydrated
500 g **code 356-4724**

STORAGE
- +15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Tryptone	15 g
Yeast extract	10 g
Sodium sulfite	1 g
Neomycin sulfate	50 mg
Polymyxin sulfate	20 mg
Ferric citrate	500 mg
Agar	13.5 g
Distilled water	1,000 ml

Final pH (25°C) = 7.2 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Test tubes (16 x 160 mm) with autoclave proof stoppers
- 125 ml Pyrex bottles with autoclave-proof stoppers
- Sterile Petri dishes (ø = 90 mm)
- Sterile pipettes (1 ml, etc)
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM
Always shake before use.
Dissolve 40 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix thoroughly until a homogenous suspension is obtained.
Heat gently, swirling frequently, then bring to boiling point until completely dissolved.
Dispense 19 ml per tube or 100 ml per bottle. Sterilize in autoclave at 121°C (± 1°C) for 10 minutes.

Reconstitution ratio: 40 g/l.
500g of powder makes 12.5 liters of medium.

PROTOCOL
• **Preparation of samples**
According to the standards applicable to the product concerned.

• **Inoculation and incubation**
In tubes
Inoculate 5 ml of test specimen or of its decimal dilutions in a tube of medium, melted and cooled to 44°C - 47°C.
Homogenize, avoiding entrapping air bubbles. Incubate at 37°C ± 1°C and at 46°C ± 1°C for 24 hours.

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In Petri dishes
Inoculate 1 ml of test specimen or of its decimal dilutions in a dish. Pour medium melted and cooled to 44℃ - 47℃.
Homogenize, avoiding entrapping air bubbles.
Incubate at 37℃ ± 1℃ and at 46℃ ± 1℃ for 24 hours.
The temperature of 46℃ enables the more pecific detection of *Clostridium perfringens*.

READING AND INTERPRETATION

- **In tubes:** Only tubes presenting well-isolated, black colonies are considered for enumeration.

- **In Petri dishes:** Readings should be made as soon as the cloches are opened, as colonies may be discolored by oxidation on contact with the air.

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment hen the dilutions come into contact with the culture medium must not exceed 15 minutes.
- To avoid any re-oxygenation of the medium, it should not be stirred violently
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

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

STRAINS	Results of 24h culture at 37℃
Clostridium perfringens (3 strains amound which ATCC 13124)	Good growth
Clostridium sporogenes	Good growth
Escherichia coli ATCC 25922	Inhibition
Staphylococcus aureus ATCC 25923	Inhibition

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.

KEY WORDS

TSN / *Clostridium perfringens* / Sulfite-reducing anaerobes / Food products / Detection / Enumeration / Sulfite / Neomycin / Polymyxin / Medium.

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