

Technical Specification Sheet



Perfringens Agar Base (TSC) (NCM0077)

Intended Use

Perfringens Agar Base (TSC) is used with selective and differential supplements for the recovery of *Clostridium perfringens* and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Formulated according to ISO 14189:2013, Perfringens Agar Base (TSC) is a nutritious base medium used in the preparation of SFP Agar and TSC Agar. Depending upon the formula, supplements are added to increase the selectivity of the medium. Shahidi-Ferguson Perfringens (SFP) Agar is based on the formula by Shahidi and Ferguson and incorporates the supplements, Polymyxin B Sulfate and Kanamycin. Tryptose Sulphite Cycloserine (TSC) Agar is developed with the same base as SFP Agar, with the addition of Cycloserine. If desired, Egg Yolk Emulsion can be added to either formula.

Typical Formulation

Enzymatic Digest of Casein	15.0 g/L
Enzymatic Digest of Soya	5.0 g/L
Yeast Extract	5.0 g/L
Sodium Metabisulfite	1.0 g/L
Ferric Ammonium Citrate	1.0 g/L
Agar	14.0 g/L

Final pH: 7.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

NCM4022	TSC Perfringens Supplement
NCM4017	Egg Yolk Emulsion 50%

Precaution

Refer to SDS

Preparation

1. Suspend 41 grams of the medium in 900 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.
4. Add 100 mL of NCM4017* Egg Yolk Emulsion, 50%, and the desired antimicrobial supplement:

TSC Agar: Add 2 vials of NCM4022-0.5* TSC Perfringens Supplement, each reconstituted using 5 mL sterile deionized/RO water.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

Overlay Agar

1. Suspend 41 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.
4. Overlay agar does not get supplemented with Egg Yolk Emulsion. Overlay agar is held at 45 -50°C until used and should be poured right after inoculation.



620 Leshner Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodsafety@neogen.com • foodsafety.neogen.com

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Test Procedure

For a complete discussion on the isolation and identification of *C. perfringens* and other anaerobic bacteria refer to specific procedures in appropriate references.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light to medium beige.

Prepared Appearance: Prepared medium is light to medium beige and trace to slightly hazy.

Expected Cultural Response: Cultural response on Perfringens Agar Base, prepared as TSC Agar, supplemented as required. After inoculation of the base layer, medium was covered with overlay agar. *Clostridium* species were incubated under anaerobic conditions and all others under aerobic conditions at 35°C for 18 - 24 hours of incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Clostridium perfringens</i> ATCC® 10543	50-200	Suppressed to inhibited, black colonies where present
<i>Clostridium perfringens</i> ATCC® 13124	50-200	Black colonies
<i>Clostridium novyi</i> ATCC® 17861	>10 ⁴	Suppressed to inhibited
<i>Escherichia coli</i> ATCC® 25922	>10 ⁵	Complete Inhibition

Results

Clostridium perfringens produce black colonies on TSC Agar and SFP Agar. If Egg Yolk Emulsion is added, colonies may have an opaque halo around the black colony due to lecithinase activity. All black colonies should be confirmed. Cultures which are not overlaid with agar are unlikely to produce black colonies.

Expiration

Dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Both black lecithinase-positive and black lecithinase-negative colonies should be considered as presumptive *Clostridium perfringens* on TSC or SFP Agars. Perform confirmatory tests.
2. Egg yolk positive facultative anaerobes may grow on SFP Agar producing completely opaque plates and covering up the egg yolk reaction of *Clostridium perfringens*.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Shahidi, S. A., and A. R. Ferguson. 1971. Appl. Microbiol. 21: 500-506.
2. Harmon, S. M., and D. A. Kauttar, J. T. Peeler. 1971. Appl. Microbiol. 22:688-692.
3. Vanderzant, C., and D. F. Splitstoesser (eds.). 2015. Compendium of methods for the microbiological examination of food, 4th ed. American Public Health Association, Washington, D.C.
4. International Organization for Standardization (ISO): 1978. Meat and meat products. - Enumeration of *Clostridium perfringens* - Working Draft ISO/TC 34/SC 6.
5. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.



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