TECHNICAL DATA SHEET

TSC (TRYPTONE-SULFITE-CYCLOSERINE) AGAR

ENUMERATION OF ANAEROBIC SULFUR-REDUCING BACTERIA AND *CLOSTRIDIUM PERFRINGENS*

1 INTENDED USE

Tryptone Sulfite Cycloserine Agar was described by Harmon for the selective isolation and enumeration of *Clostridium perfringens* in water and food samples. The medium was recommended for the enumeration of sulfur-reducing anaerobes from foods of animal origin.

The typical composition corresponds to that defined in the standards ISO 14189, NF EN ISO 7937 and NF V08-061.

2 PRINCIPLES

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

For enumeration of *Clostridium perfringens*, it is recommended to incubate the inoculated medium at 37°C and to confirm characteristic colonies.

Contaminating flora are inhibited by D-Cycloserine which also reduces the size of the black halos around the colonies.

3 TYPICAL COMPOSITION

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

For 1 liter of media :

- Tryptone ..... 15,0 g
- Papaic digest of soybean meal ..... 5,0 g
- Yeast extract ..... 5,0 g
- Sodium metabisulfite ..... 1,0 g
- Ferric ammonium citrate ..... 1,0 g
- D cycloserine ..... 0,4 g
- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25°C : 7,6 ± 0,2.

For 42 g of BK031 (Sulfite-Iron agar, base TSC)

- Tryptone ..... 15,0 g
- Papaic digest of soybean meal ..... 5,0 g
- Yeast extract ..... 5,0 g
- Sodium metabisulfite ..... 1,0 g
- Ferric ammonium citrate ..... 1,0 g
- Bacteriological agar ..... 15,0 g

For one vial of supplement BS006 Cycloserine

- D cycloserine ..... 200 mg

For one vial of supplement BS092 Cycloserine

- D cycloserine ..... 3.6 g

For one vial of supplement BS094 Cycloserine

- D cycloserine ..... 2.0 g

4 PREPARATION

Preparation from dehydrated media :

- Dissolve 42,0 g of dehydrated media (BK031) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense 20 mL in 20 x 200 mm tubes or 100 mL per flask.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution : 42,0 g/L**

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

Preparation of supplement D cycloserine

- Rehydrate one vial of freeze-dried supplement (BS006) avec 5 mL of sterile distilled water.
- Add 1 mL of supplement for every 100 mL of agar maintained in molten state at 44-47 °C (or 0,2 mL per 20 mL tube).

Use of ready-to-melt media in vials or tubes :

- If the media has been prepared in advance as described above, or if using the ready-to-melt media (BM039 or BM077), melt the agar for the minimum amount of time necessary to achieve complete liquefaction.
- Cool and maintain at 44-47°C.

Preparation from complete media

- Just prior to inoculation, add 0,2 mL of supplement to each 20 mL tube of base media maintained at 44-47 °C or 1 mL of supplement to 100 mL of agar media.
- Mix well and use almost immediately.

5 INSTRUCTIONS FOR USE

Food microbiology, enumeration of anaerobic sulfur reducing bacteria (NF V08-061)

- The inoculation can be done in 20 mL tubes or in Petri plates, at the user's convenience.

- Heat, if needed, the product being tested in order to destroy the vegetative forms and activate the spores.

In tubes

- Transfer 1 mL of the inoculum and its serial dilution to each tube, while minimizing the incorporation of air into the media.
- Mix well.
- Cool in an ice water bath.
- Incubate at 46 ± 1 °C for 20 ± 2 hours.

✓ **Inoculation : 1 mL in tubes**

✓ **Incubation : 20 h at 46 °C**

In Petri plates

- Transfer 1 mL of the inoculum and its serial dilutions to empty, sterile Petri plates.
- Pour 15 to 20 mL of complete medium.
- Mix well.
- Let solidify on a cold, flat surface.
- Add a second layer of agar and let solidify.
- Incubate the plates in an anaerobic jar for 20 ± 2 hours at 46 °C in the presence of a mixture of hydrogen and carbon dioxide.

✓ **Inoculation : 1 mL in a double layer**

✓ **Incubation : Under anaerobic conditions 20 h at 46 °C**

Food microbiology, enumeration of *Clostridium perfringens* (NF EN ISO 7937)

- Transfer 1 mL of inoculum and its serial dilutions into sterile Petri plates.
- Pour 15 to 20 mL of complete media.
- Mix well.
- Let solidify on a flat, cool surface.
- Add a second layer of agar and let solidify.
- Incubate the plates in an anaerobic jar for (20 ± 2) hours in the presence of a mixture of hydrogen and carbon dioxide.

✓ **Inoculation : 1 mL in a double layer**

✓ **Incubation : Under anaerobic conditions 20 h at 37 °C**

Water quality, enumeration of *Clostridium perfringens* (ISO 14189)

- If needed, destroy the vegetative forms by heating 15 min at 60 ± 2 °C.
- Pour 15 to 20 mL of complete medium and let solidify on a cool, flat surface.
- Filter the appropriate amount of water onto each membrane.
- Deposit the membrane, filtered side down and making sure that the membrane and agar are in close contact.
- Incubate the plates in an anaerobic jar for 21 ± 3 hours at 44 ± 1 °C.

✓ **Inoculation :**
Membrane filtration

✓ **Incubation :**
Anaerobic conditions
21 h at 44 °C

NOTE :
To improve the blackening of the colonies, it is possible to pour a second layer of complete media onto the filter.

6 RESULTS

Count the colonies surrounded by a black halo when the inoculation is done in deeps or pour plates.
Enumerate all the colonies when the inoculation is done via membrane filtration (ISO 14189) as the anaerobic sulfur reducers tend to present a yellow, maroon or grey-black coloration.
Perform readings as soon as the jar is opened, as the colonies can turn pale and fade due to oxidation of iron sulfide.
In light of the confluence of halos, it can be necessary to perform intermediary counts.
Proceed with confirmation tests for the enumeration of *Clostridium perfringens*.

See ANNEX 1 : PHOTO SUPPORT.

7 QUALITY CONTROL

Dehydrated base media : beige powder, free-flowing and homogeneous.
Freeze-dried supplement : white pellet, giving rise after reconstitution to a colorless, slight amber solution which may have a slight precipitate.
Liquid supplement : white to yellowish, opalescent solution.
Prepared media : amber agar.

Typical culture response (in complete TSC with D-Cycloserine) after 20 hours of incubation at 37°C (NF EN ISO 11133 :

Microorganisms		Growth (Productivity Ratio P_R)	Characteristic colonies
Clostridium perfringens	WDCM 00007	$P_R \geq 50$ %	Black
Clostridium perfringens	WDCM 00080	$P_R \geq 50$ %	Black
Escherichia coli	WDCM 00013	Inhibied $, score 0	-

Typical culture response (Complete TSC agar with D-Cycloserine) after 24 hours incubation at 44 °C (ISO 14189) :

Microorganisms		Growth (Productivity Ratio P_R)	Characteristic colonies
Clostridium perfringens	WDCM 00007	$P_R \geq 50$ %	Black
Clostridium perfringens	WDCM 00080	$P_R \geq 50$ %	Black
Clostridium perfringens	WDCM 00174	$P_R \geq 50$ %	Black
Bacillus subtilis	WDCM 00003	Inhibited, score 0	-

8 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Ready-to-melt media : 2-25 °C.
D-Cycloserine 200 mg Selective Supplement : 2-8 °C.
D-Cycloserine Liquid supplement : 2-8°C, shielded from the light.
The expiration dates are on the labels.

Base media prepared in vials (*) : 180 days at 2-25 °C.
Rehydrated Cycloserine selective Supplement (*) : 20 days at 2-8°C
Prepared, complete media with supplement (*) : Use immediately after preparation.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated base media (without D-Cycloserine) :
500 g bottle BK031HA

D-Cycloserine lyophilisate Selective Supplement :
10 vials qsp 500 mL BS00608

D-Cycloserine Liquid Selective Supplement :
10 vials of 90 mL (qsp 9 Liters) BS09208
1 vial of 50 mL (qsp 5 Liters) BS09408

Ready-to-melt media (base without D-Cycloserine) :
10 x 200 mL vials BM07708
50 x 20 mL tubes BM03908

10 BIBLIOGRAPHY

Harmon, S.M., Kanter, D.A., and Peeler, J.T. 1971. Comparison of media for enumeration of *Clostridium perfringens*. Appl. Microb., 21: 922-927.

Hauschild, A.H.W., Hilsheimer, R., and Griffith, D.W. 1974. Enumeration of faecal *Clostridium perfringens* spores in eggyolk-free Tryptose-Sulfite-Cycloserine Agar. Appl. Microb., 27: 527-530.

Orth, D.S. 1977. Comparison of sulfite-polymyxin-sulfadiazine medium and tryptose-sulfite-cycloserine medium without egg yolk for recovering *Clostridium perfringens*. Appl. Envir. Microbiol., 33: 986-988.

ISO 14189. Novembre 2013. Qualité de l'eau – Dénombrement de *Clostridium perfringens* – Méthode de filtration sur membrane.

NF T90-415. Octobre 1985. Essais des eaux. Recherche et dénombrement des spores de bactéries anaérobies sulfito-réductrices et de *Clostridium* sulfito-réducteurs. Méthode générale par incorporation en gélose en tubes profonds.

NF EN ISO 7937. Février 2005. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Clostridium perfringens*. Technique par comptage des colonies.

NF V08-061. Décembre 2009. Microbiologie des aliments. Dénombrement en anaérobiose des bactéries sulfito-réductrices par comptage des colonies à 46 °C.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau. Préparation, production, stockage et essais de performance des milieux de culture.

11 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.

Document code : TSC AGAR WITH CYCLOSERINE_ENv14
Creation date : 10-2015
Updated : 12-2017
Origin of revision : Addition of liquid supplement BS094

ANNEX 1 : PHOTO SUPPORT

TSC Agar

Detection and enumeration of anaerobic sulfur reducing bacteria

Results :

Growth obtained after 24 hours of incubation at 37 °C, under anaerobic conditions.