TECHNICAL DATA SHEET

CFC AGAR

ENUMERATION OF *PSEUDOMONAS*

1 INTENDED USE

CFC Agar is a selective medium for the enumeration of *Pseudomonas* spp. that frequently contaminates meats and poultry-based products during their cold storage.
The typical composition responds to the Directive NF EN ISO 13720, for the enumeration of presumptive *Pseudomonas* in meats and meat-based products.

2 HISTORY

The formula of the base medium is a modification of King A medium, in which magnesium chloride and potassium sulfate favor pyocyanin production. CFC Supplement was developed by Mead and Adams in 1976 in order to enable the selective development of most psychrophilic *Pseudomonas* that contaminate poultry products.

3 PRINCIPLES

Pancreatic digest of gelatin and Tryptone are the nutrient substrates required for the rapid multiplication of *Pseudomonas*.

The production of pyocyanin (a blue, non-fluorescent pigment, soluble in water and in chloroform) is stimulated by magnesium chloride and potassium sulfate.

The concentration of cephalotin (an antibiotic of the cephalosporine family) allows the inhibition of a major contaminating flora, in particular the enterobacters, staphylococci and streptococci).

Fucidin inhibits the development of *Acinetobacter / Moraxella* without affecting the growth of *Pseudomonas*.

Contaminating yeasts are inhibited by cetrimide & quaternary ammonium salts.

4 TYPICAL COMPOSITION

The composition can be changed to obtain optimal performance.

Pour 1 liter of media :

- Pancreatic digest of gelatin ..... 16,0 g
- Tryptone ..... 10,0 g
- Potassium sulfate ..... 10,0 g
- Magnesium chloride ..... 1,4 g
- Bacteriological agar ..... 12,0 g
- Cetrimide ..... 10,0 mg
- Fucidin ..... 10,0 mg
- Cephalotin ..... 50,0 mg

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

For 49 g of base media BK118 or 1 liter of BM096

- Pancreatic digest of gelatin ..... 16,0 g
- Tryptone ..... 10,0 g
- Potassium sulfate ..... 10,0 g
- Magnesium chloride ..... 1,4 g
- Bacteriological agar ..... 12,0 g

For one vial of supplement BS022

- Cetrimide ..... 5,0 mg
- Fucidin ..... 5,0 mg
- Cephalotin ..... 25,0 mg

5 PREPARATION

Using dehydrated media and freeze-dried supplement :

- Suspend 49,4 g of dehydrated base medium (BK118) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense 100 mL in flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47 °C.

✓ **Reconstitution : 49,4 g/L**

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

- Reconstitute the freeze-dried CFC supplement (BS022) with 5 mL of sterile distilled water.
- Mix or vortex the vial in order to obtain a complete dissolution, without creating an abundance of foam.

- Aseptically add 1 mL of reconstituted to each 100 mL flask.
- Pour 15 mL of complete medium into sterile Petri dishes.
- Let solidify on a cold surface.

Using ready-to-melt media :

Melt the media (if it is prepared in advance) or melt the ready-to-melt media (BM096) for the least amount of time needed to achieve total reliquefaction.

- Cool and maintain at 44-47 °C.
- Aseptically add 2 mL of supplement to each 200 mL vial of media (BM096).
- Pour into sterile Petri dishes.
- Let solidify on a cold surface.

6 INSTRUCTIONS FOR USE

- Dry the recently poured plates, covers partially opened.
- On the surface of the media, inoculate 0,1 mL of the sample to test and its serial dilutions.
- Inoculate the sample with the aid of a sterile spreader on the surface.
- Incubate at 25 ± 1 °C for 44 ± 4 hours.

✓ **Inoculation : 0,1 mL on surface**

✓ **Incubation : 44 h at 25 °C**

7 RESULTS

Only count plates containing no more than 150 colonies.
Pseudomonas often present pigmented or fluorescent colonies, but all types of colonies must be confirmed by an Oxidase test.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.
Selective Supplement : white pellet, after reconstitution giving a whitish to yellow opaque solution.
Prepared media : Whitish agar.

Typical culture response on complete medium after 44 hours of incubation at 25 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)
Pseudomonas fluorescens	WDCM 00115	$P_R \geq 50$ %
Pseudomonas fragi	WDCM 00116	$P_R \geq 50$ %
Escherichia coli	WDCM 00013	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
CFC Selective supplement : 2-8 °C.
Ready-to-melt base media : 2-8 °C.
The expiration dates are indicated on the labels.

Base media prepared in vials (*) : 180 days at 2-8 °C.
Complete media prepared in plates (*) : 8 days at 2-8 °C.
Rehydrated CFC Selective supplement (*) : 30 days at 2-8 °C.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without cetrimide, nor Fucidin, nor cephalotin) :
500 g bottle BK118HA

CFC Selective Supplement (cetrimide, fucidin, cephalotin):
10 vials qsp 500 mL BS02208

Ready-to-melt base media (without cetrimide, nor Fucidin, nor cephalotin) :
10 vials x 200 mL BM09608

11 BIBLIOGRAPHY

Barnes, E.M. and Thorney, M.I.. 1966. The spoilage flora of eviscerated chickens stored at different temperatures. Journal of Food Technology, **1**:113-119.

Mead, G.C. and Adams, B.W.. 1977. A selective medium for the rapid isolation of pseudomonads associated with poultry meat spoilage. British Poultry Science, **18**:661-670.

NF EN ISO 13720. Novembre 2010. Viandes et produits à base de viandes. Dénombrement des *Pseudomonas* spp. présomptifs.

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

CFC Agar

Detection & enumeration of *Pseudomonas*.

Results :

Growth obtained after 48 hours of incubation at 25 °C.