TM 983 – DIFFERENTIAL BUFFERRED CHARCOAL YEAST EXTRACT AGAR BASE

INTENDED USE

For selective isolation and differentiation of *Legionella* species.

PRODUCT SUMMARY AND EXPLANATION

Legionella pneumophila is a gram-negative rod responsible for Legionnaires disease. It infects the respiratory passage when airborne droplets of water are inhaled. In nature, the bacterium lives within the cytoplasm of the waterborne protozoan *Hartmanella*. Common sources of *Legionella* include cooling towers used in industrial cooling water systems as well as in large central air conditioning systems, domestic hot water systems, fountains, and similar disseminators that draw upon a public water supply. Natural sources include freshwater ponds and creeks.

F-G Agar developed by Feelay et al was used for the isolation of *L. pneumophila*. F-G Agar was further modified by replacing beef extract and casein hydrolysate by yeast extract. Also starch was replaced by activated charcoal. The modified F-G Agar was improved by the addition of ACES Buffer (N-2-acetamido-2-aminoethane sulfonic acid). Sensitivity of the resulting Buffered Charcoal Yeast Extract Agar was increased by the addition of alpha-ketoglutarate. Differential Buffered Charcoal Yeast Extract Agar Base used for the selective isolation and differentiation of *Legionella* species is based on the formulation of Vickers containing the two dyes, bromocresol purple and bromothymol blue.

COMPOSITION

Ingredients	Gms / Ltr
Yeast extract	10.000
Charcoal activated	1.500
L-Cysteine hydrochloride	0.400
Ferric pyrophosphate, soluble	0.250
ACES buffer	10.000
Alpha - Ketoglutarate	0.200
Bromocresol purple	0.010
Bromothymol blue	0.010
Agar	15.000

PRINCIPLE

The medium consists of yeast extract, which provide necessary nutrients for bacterial growth. Ferric pyrophosphate, L-cysteine hydrochloride and alpha- Ketoglutarate stimulates the growth of *Legionella* species. Toxic metabolic products produced in the medium get neutralized by activated charcoal which modifies the surface tension of the medium. Bromocresol purple and bromothymol blue help in the identification of *Legionella* species based on colour and colony morphology. Polymyxin B inhibits most of the gram-negative bacilli while vancomycin suppresses the growth of most of the gram positive bacteria. ACES buffer helps to buffer the medium.

INSTRUCTION FOR USE

- Dissolve 37.37 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.

- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C. If desired aseptically add 50 units/ml of Polymyxin B and 1 mg/ ml Vancomycin or aseptically add the rehydrated contents of one vial of V.P. Supplement.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Light grey to black homogeneous free flowing powder.
Appearance of prepared medium	: Grey-black coloured, opaque gel forms in Petri plates.
pH (at 25°C)	: 6.9 ± 0.2

INTERPRETATION

Cultural characteristics observed with added 50 units/ml Polymyxin B and 1mg/ml Vancomycin after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
Legionella dumoffii	33343	50-100	Luxuriant	>=70%	Blue-grey	35-37°C	72- 96 Hours
Legionella pneumophila	33153	50-100	Luxuriant	>=70%	White-grey to blue-grey	35-37°C	72- 96 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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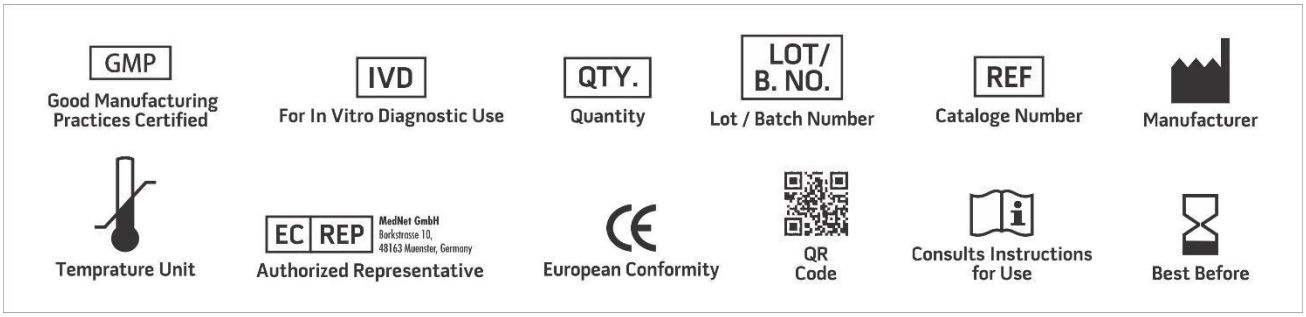

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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