TM 759 – LEGIONELLA AGAR BASE

INTENDED USE
For cultivation of *Legionella* species.

PRODUCT SUMMARY AND EXPLANATION
Legionella is a gram-negative bacterium and is the causative agent of Legionnaires disease. Natural sources of *Legionella* are fresh water ponds and creeks. Transmission to humans takes place via inhalation of aerosols from cooling towers, hot water systems or fountains containing the bacteria.
Legionella Agar initially called as F-G Agar was modified by Feely et al by replacing starch by charcoal and casein hydrolysate by yeast extract which resulted in better recovery of *Legionella pneumophila*. Pasculle et al reported that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of the medium. Edelstein suggested addition of alpha-ketoglutarate to increase the sensitivity of this medium. For the isolation of *Legionella* species from clinical samples, homogenize the specimens in sterile distilled water, examine microscopically for Legionella by fluorescent antibody (FA) method. Inoculate FA positive cultures on Legionella Agar Base. Incubate the plates at 35°C in 90% relative humidity atmosphere. Growth usually appears in 2-3 days but continue to examine the plates daily for 14 days before discarding them.

COMPOSITION

Ingredients	Gms / Ltr
Yeast extract	41.000
Charcoal activated	20.000
ACES buffer	2.000
Alpha-Ketoglutarate	1.100
Potassium hydroxide	5.000
Agar	15.000

PRINCIPLE
This medium consists of yeast extract to provide the necessary nitrogenous nutrients for *Legionella* growth. Alpha-Ketoglutarate satisfies the specific nutritional requirements of Legionella species. Activated charcoal nullifies toxic compounds that either accumulate in the medium during growth or develop during sterilization of medium. Addition of ACES buffer helps in maintaining proper pH of the medium for optimal growth of *Legionella*. Antibiotics in the supplement inhibit the growth of various contaminating bacteria and fungi.

INSTRUCTION FOR USE
- Dissolve 18.5 grams in 500 ml purified/distilled water.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Do not heat prior to sterilization.
- Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Legionella Growth Supplement or Legionella Supplement and Legionella Selective Supplement.
- Mix well and pour into sterile Petri plates. Stir the medium during dispensing to prevent settling of charcoal particles.

QUALITY CONTROL SPECIFICATIONS

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

INTERPRETATION

Cultural characteristics observed with added Legionella Growth Supplement, or Legionella Selective Supplement and Legionella supplement after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
Legionella dumoffii	33343	50-100	Good-luxuriant	>=50%	Light blue to grey white	25-30°C	48-72 Hours
Legionella pneumophila	33153	50-100	Good-luxuriant	>=50%	Light blue to grey white	25-30°C	48-72 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 25-30°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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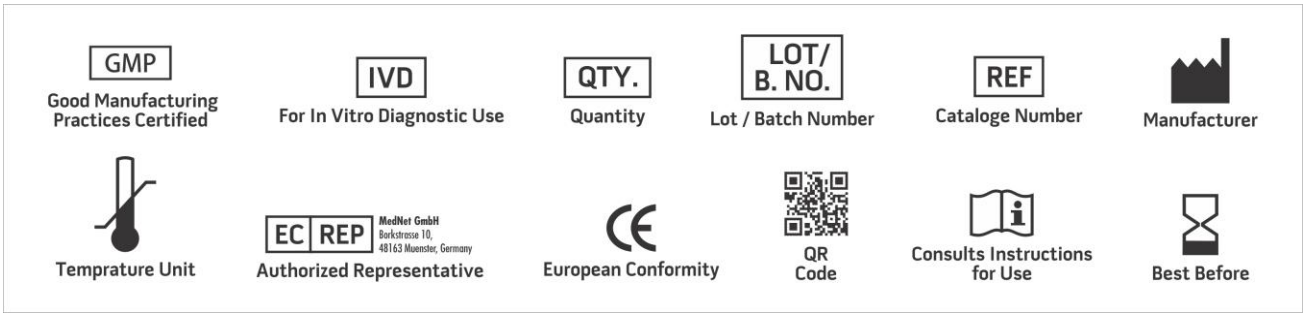

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

Revision: 08 Nov., 2019