CM 20326 - BUFFERED CHARCOAL YEAST EXTRACT AGAR MEDIUM (ISO 11731-2:2017)

INTENDED USE

For selective isolation and cultivation of Legionella species from cooling towers, water samples, clinical and other materials.

PRODUCT SUMMARY AND EXPLANATION

Bufferedcharcoalyeast extractagar mediumwas a modification of the existing F-G Agar. F-G Agar had starch and casein enzymic hydrolysate as ingredients inthe composition. Feely et al replaced these two with charcoal and yeast extract respectively, and reported betterrecovery of Legionella pneumophilla. Later Paseulle reported that supplementation of the Charcoal Yeast Agarwith ACES buffer improved the performance of the medium. Edelstein further modified the medium by adding alpha-ketoglutarate. This addition helped in improving the sensitivity of the medium. The formulation of Buffered CharcoalYeast Extract Agar Base is as per specification laid in ISO 11731- 2017.

COMPOSITION

Ingredients	Gms / Ltr
Agar	12.000
Yeast extract	10.000
ACES Buffer	10.000
Activated Charcoal	2.000
Alpha ketoglutarate, monopotassium salt	1.000

PRINCIPLE

The media contains Yeast extract which acts as a rich source of vitamins, nitrogen as well as carbon. ACES Buffer and potassium hydroxide maintain optimal pH for growth while L-cystine hydrochloride; ferric pyrophosphate and -ketoglutarate stimulate growth of Legionella species. Activated charcoal is a protective agent neutralizing and absorbing toxic metabolites produced by bacterial growth. It decomposes hydrogen peroxide, a toxic metabolic product, and can also collect CO_2 and modify surface tension. Agar is the solidifying agent. For selective isolation, antibiotic supplements can be used to suppress contaminating microorganisms. PCP Supplement (TS 254) containing Polymyxin B, Sodium cefazolin and Pimaricin or Legionella (GVPC) Selective Supplement (TS 115) containing glycine, Polymyxin B sulphate, vancomycin and cycloheximide or Legionella Selective Supplement IV (MWY) (TS 018) containing glycine, polymyxin B, anisomycin, vancomycin, bromothymol blue and bromocresol purple are often used.

INSTRUCTIONFOR USE

Dissolve 35.00 grams in 1000ml distilled water.
Add 2.8 grams KOH pellets and mix to dissolve.
Heat to boiling to dissolve the medium completely.
Sterilize by autoclaving at (121 ± 3) °C for (15 ± 1) minutes.
Cool to 45-51°C.
Aseptically add sterile rehydrated contents of 2 vials each of Legionella Supplement (TS 019).
Mix well and pour with constant stirring to ensure that charcoal particles get evenly distributed.

For additional selectivity:

For BCYE+AB Agar: Aseptically add the rehydrated contents of one vial of PCP Supplement (TS 254)
For GVPC Agar: Aseptically add rehydrated contents of 2 vials of Legionella (GVPC) Selective Supplement (TS 115).

- For Modified Wadowsky Yee (MWY) Agar: Aseptically add the rehydrated contents of one vial of Legionella Selective Supplement IV (MWY) (TS 018).

QUALITY CONTROL SPECIFICATIONS

Appearance of Dehydratedpowder	:	Grey to black, Homogeneous free flowing powder
Appearance of Prepared medium	:	Grey-black coloured, opalescent gel
pH (at 25°C)	:	6.8± 0.2

INTERPRETATION

Cultural characteristics observed after incubation with added supplements Legionella Supplement (TS 019) and Legionella Selective Supplement IV (MWY) (TS 018) or Legionella (GVPC) Selective Supplement (TS 115) or PCP Supplement (TS 254) (TS 254) in 90% humid temperature. Recovery rate is considered 100% for bacteria growth on Soya Agar.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Colour of colony	Recovery	Incubation Temp	Incubation Period
Legionella pneumophila	33152	50-100	Luxuriant	white-blue fluorescence under long wavelength UV light with an entire edge exhibiting a characteristic ground glass appearance white-blue fluorescence under long wavelength UV light with an entire edge exhibiting a characteristic ground glass appearance white-blue fluorescence under long wavelength UV light with an entire edge exhibiting a characteristic ground glass appearance -	>=50%	34-38°C	2-5 Days
Legionella anisa	35292	50-100	Luxuriant		>=50%	34-38°C	5-10 Days
Legionella dumofi	33343	50-100	Luxuriant		>=50%	34-38°C	2-5 Days
Escherichia coli	25922	50-100	None-Poor		<=10%	34-38°C	2-5 Days
Enterococcus faecalis	29212	≥1000	Inhibited		0%	34-38°C	2-5 Days

PACKAGING

In100&500gm packaging size.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 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 powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

DISPOSAL

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

REFERENCES

1. Feeley J. C., Gorman G. W., Weaver R. E. et al, 1978, J. Clin. Microbiol., 8 : 320-325.
2. Feeley J. C., Gibson R. J., Gorman G. W. et al, 1979, J. Clin. Microbiol., 10:437.

3. Paseulle, Feely et al, 1980, J. Infect. Dis., 191:727.
4. Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.
5. Vicker R., Brown and Garrity, 1981, J. Clin. Microbiol., 13:380.
6. Water quality-Detection and enumeration of Legionella-Part 2 Direct membrane filtration method for waters with low bacterial counts International Organization for Standardization (ISO), 2017, Draft ISO/DIS, 11731-2.

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

*ForLab Use Only