

## Specification

Solid medium base used for the detection, isolation and enumeration of *Legionella* spp. from water according to the ISO Standards.

## Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90 mm with: 23 ± 1 ml	1 box with 2 packs of 10 plates/pack. Single cellophane. Side labeling.	3 months	2-14 °C

## Composition

Composition (g/l):	
Activated Charcoal.....	2.0
Yeast extract.....	10.0
Aces buffer.....	10.0
Potassium hydroxide.....	2.8
Alfa-ketoglutarate.....	1.0
Cysteine.....	0.4
Ferric pyrophosphate.....	0.25
Agar.....	15.0

## Description /Technique

### Description:

The actual formulation of this medium is according to the ISO Standards 11731, but BCYE Agar is based in a modification of a previously described media. In 1979 Feeley and collaborators described Charcoal Yeast Extract (CYE) Agar as a modification of the F-G Agar. They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in a better recovery of *Legionella pneumophila*. Pasculle, in 1980, reported that CYE Agar could be improved by buffering the medium with ACES buffer and a year later Edelstein increased the sensitivity of the medium by adding a-ketoglutarate which is the present formulation (BCYE Agar).

The medium consist of a Medium base supplemented with growth factors (BCYE Agar) and the Selective Medium supplemented with inhibitors of undesirable accompanying flora. The yeast Extract supplies the basic nutrients as the medium contains no fermentable carbohydrates. L-Cysteine, Ferric pyrophosphate and a-ketoglutarate are incorporate to satisfy the specific nutritional requirements of *Legionella* species.

The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO<sub>2</sub> and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth. The selectivity is increased by the addition of Vancomycin and polymyxin B which inhibit Gram positive bacteria and cycloheximide or natamycin which are antifungal agents and inhibits the yeast growth.

### Technique:

Refer to the ISO Standards 11731 or other standard procedures to obtain isolated colonies from specimens and samples. Allow the inoculated plates to stand until the inocula has been absorbed. Invert the plates and incubate at 36 ± 2°C for up to 5 days. To ensure the atmosphere in the incubator is humid, place a tray of water in the bottom of the incubator. Top up this tray with fresh water (if necessary) each time the plates are examined. Incubation in an atmosphere of air with 2,5% (volume fraction) CO<sub>2</sub> may be beneficial for the growth of some *Legionella*, but it is not essential.

Examine the plates with a plate microscope on at least three occasions at intervals of 2 to 4 days during the 10-day incubation period, as *Legionella* grow slowly an can be masked by the growth of other organisms. Record the number of each type of colony present.

Colonies of *Legionella* are often white-grey-blue-purple in colour, but may be brown, pink, lime-green or deep-red. They are smooth with a smooth edges and exhibit a characteristic ground-glass appearance. Under ultraviolet light colonies of several species autofluoresce brilliant white, but others are red and *L. pneumophila* appear dull green often tinged with yellow. All presumptive colonies must be confirmed by cultural, biochemical, serological or genetic methods.

## Quality control

### Physical/Chemical control

Color : Black

pH:  $6.8 \pm 0.2$  at 25°C

### Microbiological control

Inoculate: Practical range  $100 \pm 20$  CFU. Min. 50 CFU (Productivity).

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at  $36 \pm 2$  °C. Reading 2 - 5 days.

### Microorganism

*Legionella pneumophila* ATCC® 33152, WDCM 00107

*Legionella anisa* ATCC® 35292, WDCM 00106

### Growth

Good ( $\geq 70\%$ )

Good ( $\geq 70\%$ )

### Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

## Bibliography

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## Storage

Storage conditions: 2-14°C

Avoid direct contact with surfaces that can freeze product.