Reference: 100158JF **Technical Data Sheet**

Product: **BRUCELLA AGAR + VIT.K + HEMIN.**

Specification

All purpouse primary isolation medium for the isolation of anaerobes and others fastidious microorganisms.

Presentation

20 Prepared Plates
90 mm
with: 21 ± 2 ml

Packaging Details
1 box with 2 packs of 10 plates/pack. Single cellophane.

Shelf Life	Storage
2,5 months	2-14 ºC

Composition

Composition (g/l):

Pancreatic digest of casein	10.0000
Peptic digest	10.0000
Hemin	0.0050
Menadione	0.0005
D(+)Glucose	1.0000
Yeast extract	2.0000
Sodium chloride	5.0000
Sodium bisulphite	0.1000
Agar	15.0000
Sheep blood	50 ml

Description /Technique

Brucella Agar with Hemin and Vitamin K is recommended for use in the primary isolation, quantitation, and partial identification of obligately anaerobic microorganisms from clinical specimens. This non-selective medium is also suitable for the growth of aerobic and microaerophilic bacteria when incubated under the appropriate conditions.
Brucella Agar with Hemin and Vitamin K is a modification of the formulation given by the American Society for Microbiology (ASM) According to Finegold, this medium is preferable to Heart Infusion Blood Agar Base for the cultivation of anaerobic bacteria.Onderdonk et al. and Weinstein et al. both reported the addition of hemin.Jousimies-Somer et al. further describe supplementing the medium with vitamin K. It has been shown that Brucella Agar supports the growth of anaerobic gram-negative bacilli better than CDC (trypticase soy) or Schaedler's agar.
Brucella Agar with Hemin and Vitamin K is an enriched medium designed to support and enhance the growth of fastidious microorganisms. The medium contains dextrose for energy, peptones to provide nitrogenous compounds, and yeast extract as a source of B vitamins for cell maintenance and metabolism. Hemin and vitamin K are nutritious supplements known to enhance the cultivation of some species of anaerobes, and to further promote the pigment production of *Prevotella melaninogenica*. Sheep blood provides additional growth factors required by some fastidious microorganisms and can also be used to assess hemolytic reactions as seen by the double zone beta-hemolysis of *Clostridium perfringens.* The medium is also suitable for use in susceptibility testing, differential disk, and spot biochemical testing.

Thecnique:
Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat, cold and oxygen exposure.
When possible, clinical specimens should be inoculated directly onto the medium to prevent loss of organism viability. Minimize specimen exposure to ambient oxygen levels in air.
Apply a liquid specimen directly to the agar surface; streak with a sterile inoculating loop to obtain isolated colonies. An enrichment broth, such as Thioglycollate Broth with Hemin and Vitamin K , should be inoculated concurrently with primary isolation plates. For best results, specimens should be plated onto non-selective and selective media.
Immediately after culture, incubate plates anaerobically at 35-37ºC for up to 72 hours. Some fastidious anaerobes may require additional periods of incubation for proper recovery. Regardless of atmospheric system used, it is important to confirm anaerobiosis.
Confirmation of obligate anaerobic microorganisms should be performed. A Chocolate Agar plate, incubated in 5-10% CO2 is required for aerotolerance testing to detect isolates that require CO2, especially slow-growing, fastidious, facultative or microaerophilic species that do not grow alone on media containing blood (such as *Haemophilus* and *Actinobacillus* spp.).
Use of traditional blood agar media alone for CO2 incubation may yield false-negative results. An additional Blood Agar plate incubated in air will further detail the atmospheric requirements and hemolytic properties of facultatively anaerobic microorganisms.

Quality control

Physical/Chemical control

Color : Red pH: 7.6 ± 0.2 at 25ºC

Microbiological control

Inoculate 30-300 CFU (productivity) 1.000-10.000 CFU (selectivity)

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

Anaerobiosis. Incubation at 35 ± 2 ºC, reading after 24-48 hours

Microorganism	Growth
Bacteroides fragilis ATCC® 25285, NCTC® 9343	Good -Grey colonies
Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237	Bueno - Grey-white colonies-beta hemolysis

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

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