

TECHNICAL DATA SHEET **OF Basal Medium**

Principle

OF (Oxidation Fermentation) Basal medium is developed by Hugh and Leifson (1953) to study oxidative and carbohydrate fermentative metabolism of gram-negative bacteria. The media is composed of tryptone, sodium chloride, Dipotassium hydrogen phosphate bromothymol blue and agar. Tryptone provides nitrogen, vitamins and necessary essential elements. Sodium chloride maintains osmotic equilibrium. Phosphates buffers the medium and bromothymol blue act as pH indicator. The low concentration of agar facilitates determination of motility and enables to visualize acid production at the surface of the medium. The motility is determined by the diffused zone of blazing out from the line of inoculation. Non-motile organisms grow along the line of inoculation. Degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its color to yellow. Oxidative utilization takes place when the medium is exposed to air while fermentative utilization occurs under exclusion of air. Generally, the test is performed in duplicate, of which one tube is covered with mineral oil to exclude oxygen and the second tube is uncovered; reactions of differential value can be observed. Fermentative organisms produce an acid reaction in both the covered and uncovered medium. Oxidative organisms produce an acid reaction in the uncovered medium and give slight growth without change in the covered medium. Organisms which are not classified either as oxidative or fermentative show no change in the covered medium and an alkaline reaction in the uncovered medium. The acidic reaction of oxidative organisms is more apparent at the surface of the medium that gradually spreads throughout the medium. If the oxidation reaction is weak or slow, an initial alkaline reaction at the surface of the uncovered tube may persist for several days and eventually convert to an acid reaction. The media can be incorporated with 2% serum or yeast extract (0.1%) to make the medium more nutritious.

Use: For differentiation of gram negative bacteria on the basis of fermentative and oxidative metabolism of carbohydrates.

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Contents*

Ingredients	Gram/Liter
Tryptone	2.000
Sodium chloride	5.000
Dipotassium phosphate	0.300
Bromothymol blue	0.080
Agar	2.000
pH at 25°C	6.8 ±0.2

* Formula adjusted for optimum performance and parameters

Directions: Dissolve 9.38 grams in 1000 ml distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add 10 ml of 10% sterile carbohydrate solution (10% Dextrose, 10% lactose or 10% Saccharose solution). Mix well and aseptically transfer 5 ml in sterile test tube to form stab. Inoculate test sample aseptically.

Specimens' types analyzed

Clinical samples: Faeces or fecal and rectal screening swab samples, pure isolates etc.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

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Quality Control

Appearance	Light beige to greenish yellow colored, free-flowing, homogeneous
Reaction of 0.94 % solution	6.8 ±0.2 at 25 °C
pH	6.60- 7.00
Gelling	Semisolid comparable with 0.2% agar gel
Color and clarity of ready medium	Green color, clear opalescent semi solid gel
Growth Promotion properties	Best at ≤ 100 CFU at 32-37 °C for 18-72 h
Indicative properties	Optimum at ≤ 100 CFU at 32-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

Different Microbial Response: Cultural characteristics observed after incubation at 33-37°C for 18-48 hours. Inoculum 50-100 CFU.

Organism	ATCC	Basal medium (Aerobic)	Basal medium (Anaerobic)	with dextrose (Aerobic)	with dextrose (Anaerobic)
<i>Escherichia coli</i>	8739	Green color (alkaline reaction)	Green color (alkaline reaction)	Yellow color (acidic reaction with gas formation)	Yellow color (acidic reaction with gas formation)
<i>Klebsiella aerogenes</i>	13048	Green color (alkaline reaction)	Green color (alkaline reaction)	Yellow color (acidic reaction with gas formation)	Yellow color (acidic reaction with gas formation)
<i>Pseudomonas aeruginosa</i>	27853	Green color (alkaline reaction)	Green color (alkaline reaction)	Yellow color (acidic reaction)	Yellow color (acidic reaction)
<i>Salmonella enteritidis</i>	13076	Green color (alkaline reaction)	Green color (alkaline reaction)	Yellow color (acidic reaction with gas formation)	Yellow color (acidic reaction with gas formation)
<i>Shigella flexneri</i>	9199	Green color (alkaline reaction)	Green color (alkaline reaction)	Yellow color (acidic reaction)	Yellow color (acidic reaction)

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Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. American Public Health Association, (1978) Standard Methods for the Examination of Dairy Products, 14th Ed., Washington D.C.
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4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J. H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015), Manual of Clinical Microbiology, 11th Ed. Vol. 1 Wastewater, 23rd Ed., APHA, Washington, D.C.
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7. MacFaddin J. F., (2000), Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams & Wilkins, Baltimore, Md.
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