TM 242 – OF BASAL MEDIUM

INTENDED USE
For differentiation of gram negative bacteria on the basis of fermentative and oxidative metabolism of carbohydrates.

PRODUCT SUMMARY AND EXPLANATION
Hugh and Leifson developed OF Medium to study oxidative and fermentative metabolism of carbohydrates by gram negative bacteria. This criterion is used during taxonomic studies of Enterobacteriaceae. The authors Hugh and Leifson showed that when a gram-negative organism is inoculated in this medium containing a carbohydrate 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 will produce an acid reaction in both the covered and uncovered medium. Oxidative organisms will 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.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	2.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	0.300
Bromo thymol blue	0.080
Agar	2.000

PRINCIPLE
The medium consists of Tryptone that provides the necessary carbon and nitrogen, vitamins etc. required for bacterial growth. A carbohydrate whose fermentation reaction is to be studied is added separately. Phosphate buffers the medium and the low agar concentration determines motility and dispersion of the acid produced on the surface. Bromothymol blue in the medium acts as the pH indicator. The low concentration of agar permits the determination of motility and aids in the even distribution of any acid produced at the surface of the medium. Motility is observed as diffused zone of flaring out from the line of inoculation. Non-motile organisms grow along the line of inoculation. Dextrose is the most important carbohydrate for use in OF Basal Medium. However, certain organisms may metabolize other carbohydrates even if they are unable to utilize dextrose. Degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its colour to yellow. Oxidative utilization takes place when the medium is exposed to air while fermentative utilization occurs under exclusion of air. OF Basal Medium can be supplemented with 2% serum or yeast extract (0.1%) to make the medium more nutritious for the growth of bacteria.

INSTRUCTION FOR USE
- Dissolve 9.38 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Dispense in 100 ml amounts and sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- To first 100 ml of sterile basal medium, aseptically add 10 ml of sterile 10% dextrose solution.
- To second 100 ml add 10 ml sterile 10% lactose solution.
- To third 100 ml add 10 ml sterile 10% saccharose solution.
- Mix and dispense aseptically in 5 ml amounts in sterile tubes in duplicate for aerobic and anaerobic fermentation.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to greenish yellow homogeneous free flowing powder.
Appearance of prepared medium : Green coloured clear to slightly opalescent gel forms in tubes.
pH (at 25°C) : 6.8 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Only Basal Medium (aerobic)	Only Basal Medium (overlayed with mineral oil)	W/ Dextrose (aerobic)	W/Dextrose (overlayed with mineral oil)	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	Acidic reaction, yellowing of the medium with gas formation	Acidic reaction, yellowing of the medium with gas formation	35-37°C	18-48 Hours
Enterobacter aerogenes	13048	50-100	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	Acidic reaction, yellowing of the medium with gas formation	Acidic reaction, yellowing of the medium with gas formation	35-37°C	18-48 Hours
Pseudomonas aeruginosa	9027	50-100	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	Acidic reaction, yellowing of the medium	Alkaline reaction, green colour of the medium	35-37°C	18-48 Hours
Shigella flexneri	12022	50-100	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	Acidic reaction, yellowing of the medium	Acidic reaction, yellowing of the medium	35-37°C	18-48 Hours
Alcaligenes faecalis	8750	50-100	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	35-37°C	18-48 Hours
Acinetobacter baumannii	19606	50-100	Alkaline reaction, green colour of the medium	Alkaline reaction, green colour of the medium	Acidic reaction, yellowing of the medium	Alkaline reaction, green colour of the medium	35-37°C	18-48 Hours

PACKAGING:

In pack size of 100 gm and 500 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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4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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6. MacFaddin J. F., 1985, Media for the Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
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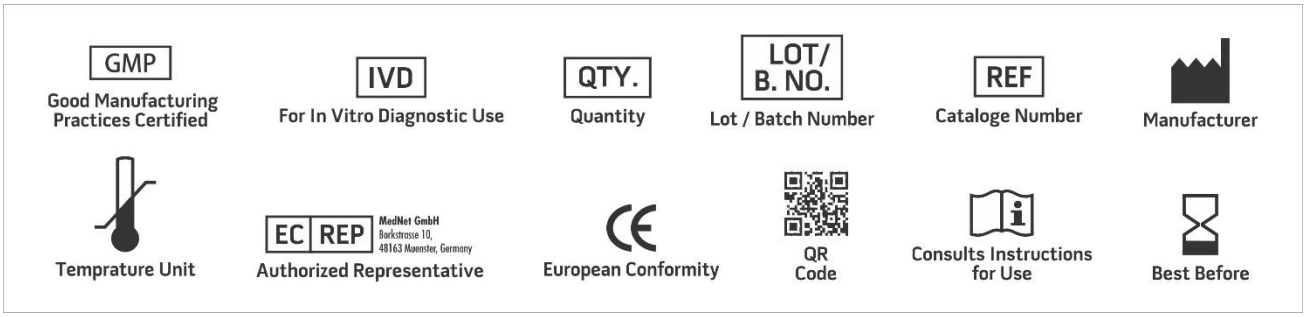

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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