OF Basal HiCynth™ Medium

Intended use

OF (Oxidation Fermentation) Basal medium is used for the determination of oxidative and fermentative metabolism of carbohydrates by gram-negative bacteria.

Composition**

**Formula adjusted, standardized to suit performance parameters
* Chemically defined peptone

Directions

Suspend 9.38 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in 100 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50ºC. 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.

Principle And Interpretation

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 (3). OF Basal HiCynth™ Medium is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. HiCynth™ Peptone No.4 in the medium provides the necessary carbon and nitrogen, vitamins etc. required for bacterial growth. Phosphate buffers the medium and the low agar concentration determines motility and dispersion of the acid produced on the surface (6). Bromothymol blue acts as the pH indicator. A carbohydrate whose fermentation reaction is to be studied is added separately.

Motility is observed as diffused zone of flaring out from the line of inoculation. Non-motile organisms grow along the line of inoculation. Dextrose (Glucose) is the most important carbohydrate for use in OF Basal HiCynth™ 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. 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 (6). Prepare the medium with 1% dextrose and without 1% dextrose. Two tubes of each carbohydrate are used per organism and inoculated by stabbing. One of the inoculated tubes of each carbohydrate medium is covered with 2 ml of sterile mineral oil and the other is left uncovered. The tubes are incubated at 35-37°C for 18-48 hours or longer. The results are read after 48 hours. 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.

OF Basal HiCynth™ Medium can be supplemented with 2% serum or yeast extract (0.1%) to make the medium more nutritious for the growth of bacteria (2).
**Type of specimen**

Isolated Microorganism from clinical and non-clinical samples.

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations**

1. Since the medium is pH dependent, pH must be monitored.
2. Certain fastidious organisms may not grow and require supplementation of 2% serum or yeast extract (0.1%)

**Performance and Evaluation**

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

**Quality Control**

**Appearance**

Cream to greenish yellow homogeneous free flowing powder

**Gelling**

Semisolid, comparable with 0.2% Agar gel.

**Colour and Clarity of Prepared medium**

Green coloured clear to slightly opalescent gel forms in tubes.

**Reaction**

Reaction of 0.94% w/v aqueous solution at 25°C. pH : 6.8±0.2

**pH**

6.60-7.00

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Only Basal Medium (aerobic)</th>
<th>Only Basal Medium (overlayed with mineral oil)</th>
<th>w/Dextrose (aerobic)</th>
<th>w/Dextrose (overlayed with mineral oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii ATCC 19606</td>
<td>50-100</td>
<td>alkaline reaction, green colour of the medium</td>
<td>alkaline reaction, green colour of the medium</td>
<td>acidic reaction, yellowing of the medium</td>
<td>alkaline reaction, green colour of the medium</td>
</tr>
<tr>
<td>Alcaligenes faecalis ATCC 8750</td>
<td>50-100</td>
<td>alkaline reaction, green colour of the medium</td>
<td>alkaline reaction, green colour of the medium</td>
<td>alkaline reaction, green colour of the medium</td>
<td>alkaline reaction, green colour of the medium</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>alkaline reaction, green colour of the medium</td>
<td>alkaline reaction, green colour of the medium</td>
<td>acidic reaction, yellowing of the medium with gas formation</td>
<td>acidic reaction, yellowing of the medium with gas formation</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
### Klebsiella aerogenes
ATCC 13048 (00175*)
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

### Pseudomonas aeruginosa
ATCC 9027 (00026*)
50-100 alkaline reaction, green colour of the medium
alkaline reaction, green colour of the medium
acidic reaction, yellowing of the medium
acidic reaction, green colour of the medium

### Salmonella Enteritidis
ATCC 13076 (00030*)
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

### Shigella flexneri ATCC 12022 (00126*)
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

### Vibrio cholerae ATCC 15748
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

**Key:** * Corresponding WDCM numbers

# Formerly known as Enterobacter aerogenes

### Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

### Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

### Reference

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Disclaimer:

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