Oxidation-Fermentation Fluid Medium Base (O/F Medium)
Art. No. 03-037

Also known as
O/F Enteric Medium; O/F Basal Medium according to Hugh & Leifson

Specification
Fluid medium used for determining the oxidative and/or fermentative metabolism of Gram negative bacilli.

Formula* in g/L
Casein peptone .......................................................... 2,00
Sodium chloride .......................................................... 5,00
Dipotassium phosphate ................................................. 0,20
Bromothymol Blue ....................................................... 0,08
Agar ........................................................................... 2,50
Final pH 7.1 ± 0.2 at 25°C
* Adjusted and/or supplemented as required to meet performance criteria

Directions
Suspend 9.8 g of powder in 1 L of distilled water and bring to the boil. Add sugar in the desired concentration and distribute in fermentation tubes. Sterilize in the autoclave at 121°C for 15 min.

Description
Using this medium Hugh and Leifson were able to differentiate Gram negative bacteria into three categories: fermentative, oxidative and inactive. The organism to be studied is inoculated in two long narrow tubes (12x120 mm) by deep stab inoculation. One tube is covered with oil or a Vaseline® layer to induce an anaerobic environment that forces the strain to carry out fermentation. Fermentative organisms produce a large amount of acid in both the tubes, and this is indicated by the yellow colouration of the Bromothymol Blue indicator. Bacteria that utilise an oxidative metabolic pathway carry out this reaction only in the tube without the oil/Vaseline. Inactive strains do not use sugars and therefore do not induce any change in either tube. In some instances a slight blue colouration, probably due to alkalinization by peptone degradation, can occur. Some authors have proposed the usage of just one tube for this assay, but the medium must be modified by solidifying (with 1.5% agar) and the addition of yeast extract and/or cystine. In these tubes the stab must be, at least, 8 cm deep. Hugh and Leifson recommend simultaneous assay with glucose, lactose and sucrose of 1% concentration, adding the sugars, sterilised by filtration, to the medium.

References

Storage
For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Quality control

**Incubation temperature:** 35°C ± 2.0
**Incubation time:** 24 h
**Inoculum:** Pure cultures using an inoculating needle

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Growth</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>Enterococcus faecalis ATCC 29212</td>
<td>Poor</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853</td>
<td>Good</td>
<td>O / F: + / -</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>Good</td>
<td>O / F: + / + Yellow medium</td>
</tr>
<tr>
<td>Salmonella typhimurium ATCC 14028</td>
<td>Good</td>
<td>O / F: + / + Yellow medium</td>
</tr>
</tbody>
</table>

Left: *Escherichia coli* ATCC 25922
Right: *Salmonella typhimurium* ATCC 14028

*Pseudomonas aeruginosa* ATCC 27853