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 (Enterobacteriaceae).

Formula * in g/L
Casein peptone..... 2.00
Sodium chloride..... 5.00
Dipotassium phosphate.....0.30
Bromothymol Blue..... 0.08
Agar.....3.00

Final pH 6,8 ± 0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 10.38 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. Add to half of them vaseline seals or vaspar®. Sterilize for 15 minutes at 121°C.
Note: the usual sugar concentration is 10 g/l.

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 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.

Note: The current formulation has been adapted to the ISO standard in the Bibliography.

Quality control
Incubation temperature: 35°C ±2,0 **Incubation time:** 18-24 h

Inoculum: Pure cultures using and inoculating needle (C.H.: Dextrose 10 g/l) + vaselin, according to ISO 11133:2014/Amd 1:2018 & Adm 2:2020

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC® 27853	Good	O / F: + / -
Escherichia coli ATCC® 25922	Good	O / F: + / + Yellow medium
Salmonella typhimurium ATCC® 14028	Good	O / F: + / + Yellow medium
Salmonella enteritidis ATCC® 13076	Good	O / F: + / + Yellow medium
Pseudomonas fluorescens ATCC® 13525	Good	O / F: + / -

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).