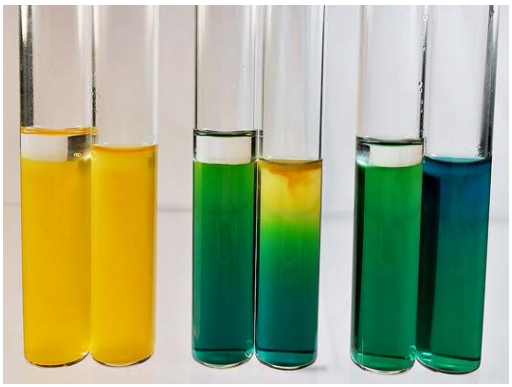
**INSTRUCTIONS FOR USE**

# OF HUGH LEIFSON BASE

**Dehydrated culture medium**



OF Hugh Leifson Glucose Agar – from left: *Ecoli, P..aeruginosa, A.faecalis*

## 1 - INTENDED USE

*In vitro* diagnostic. For the differentiation of Gram-negative bacilli, isolated from clinical samples or other materials, on the basis of the oxidative or fermentative metabolism of carbohydrates.

## 2 - COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

| | |
|---|---|
| Tryptone | 2.00 g |
| Sodium chloride | 5.00 g |
| Dipotassium phosphate | 0.30 g |
| Bromothymol blue | 0.03 g |
| Agar | 2.50 g |

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

O/F Hugh Leifson Base, prepared according to the formula proposed by Hugh and Leifson[1], is a medium to which various carbohydrates can be added for the study of oxidative or fermentative metabolism of microorganisms. The medium is intended for the differentiation of gram-negative bacilli isolated from clinical specimens and other materials.[2,3]

Bacteria utilise glucose and other carbohydrates through various metabolic pathways; some are oxidative routes but others involve fermentation reactions. During the anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids at high concentration, depending on the type of fermentation. Certain non-fermenting Gram-negative bacteria metabolize glucose using aerobic respiration and therefore only produce a small amount of weak acids during glycolysis and Krebs cycle. The complete medium will contain a high concentration of carbohydrate with a low content of peptone to avoid the utilisation of peptone by an aerobic organism and the resultant production of an alkaline reaction which would neutralise slight acidity produced by an oxidative organism.[3]

O/F Medium Base, supplemented with the suitable carbohydrate, allows to distinguish between the two metabolic pathways. The medium contains bromothymol blue as a pH indicator: the high concentration of acid produced during fermentation will turn the bromothymol blue indicator from green to yellow in the presence or absence of oxygen. The persistence, after incubation, of a green colour or the appearance of a blue colour, due to an alkaline transformation of the medium, indicates that the test is negative and that there was no degradation of the carbohydrate.

O/F Hugh Leifson Base is a semi-solid medium: the presence of agar at a concentration of 0.25% enables the determination of motility in addition to OF test and also aids in preventing the distribution of any acid produced towards the surface of the medium, with a consequent dilution; dipotassium phosphate promotes carbohydrate fermentation and acts as a pH control buffer[1]; tryptone provides carbon, nitrogen and trace elements for microbial growth; sodium chloride maintains the osmotic balance. Glucose is the carbohydrate most frequently used for the OF test; however, there are organisms being tested unable to metabolize glucose, which can attack other carbohydrates; a battery consisting of glucose, lactose and sucrose should then be employed and, sometimes, maltose, mannitol and xylose.[2,4,5]

## 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 9.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute in screw cap tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and add a sterile solution of the chosen carbohydrate at 1% (w/v) final concentration. Alternatively, and depending on heat stability, add 10 g/L of carbohydrate prior to sterilisation.

For each strain to be examined, prepare two test tubes and, after inoculation, cover one of them with liquid paraffin to create anaerobic conditions.

## 5 - PHYSICAL CHARACTERISTICS

| | |
|---|---|
| Dehydrated medium appearance | green, fine, homogeneous, free-flowing powder |
| Solution and prepared plates appearance | green, limpid |
| Final pH at 20-25 °C | 7.1 ± 0.2 |

## 6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| O/F Hugh Leifson Base | Dehydrated medium | 4018362 | 500 g (51 L) |

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave and water-bath, sterile needles, incubator and laboratory equipment as required, test tubes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies, carbohydrates and liquid paraffin.

## 8 - SPECIMENS

The sample consists of bacterial cultures isolated from clinical samples or other materials, purified on Tryptic Soy Agar or blood agar or other suitable medium.

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## 9 - TEST PROCEDURE

For each carbohydrate used, inoculate lightly a pair of OF tubes for each organism being tested, by inserting a straight needle vertically to approximately ¼ inch from the bottom.
Cover one tube of each pair with 3 cm layer of liquid paraffin to create anaerobic condition, leaving the other tube open to the air.
Also set up control sets: one inoculated set with no carbohydrate added and one uninoculated set with carbohydrate.
Incubate, with loose caps, at 35-37°C for 44-48 hours; slow-growing bacteria require longer incubations (3-4 days or even up to 14 days)[3]

## 10 - READING AND INTERPRETATION

Examine the tubes daily for colour change.
Oxidation: acid in aerobic tube only (yellow colour in aerobic tube, green in anaerobic tube)
Fermentation: acid in both tubes (yellow colour), with gas production (aerogenic strain) or without (anaerogenic strain).
No degradation of sugar (asaccharolytic strain): no yellow change of both test tubes that remain green (covered test tube) or turn green-blue (open test tube).
The following table, adapted from MacFaddin[3], summarizes the reactive patterns on OF Hugh Leifson Base supplemented with glucose.

| Reaction | Tube with reaction | Reaction in open tube | Reaction in covered tube |
|---|---|---|---|
| Oxidation<br>E.g. *P.aeruginosa* | Open | Yellow (A) | Green (-) |
| Fermentation | | | |
| Anaerogenic<br>E.g. *S.dysenteriae* | Covered | Yellow (A) | Yellow (A) |
| Aerogenic<br>E.g. *E.coli* | Covered | Yellow and gas (AG) | Yellow and gas (AG) |
| Neither fermentation nor oxidation<br>E.g. *A. faecalis* | Neither* | Blu or Green (-) | Green (-) |
| Both Fermentation and oxidation<br>E.g. *Citrobacter* | Both | Yellow (A o AG) | Yellow (A o AG) |

A: acid production; G: gas production *: Uninoculated carbohydrate control reading: no change in colour

OF Hugh Leifson is also useful for detecting bacterial mobility (diffuse growth starting from the inoculum line).

## 11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

| TEST STRAINS | INCUBATION | EXPECTED RESULTS | |
|---|---|---|---|
| | T°/ t | OPEN TUBE | COVERED TUBE |
| *P.aeruginosa* ATCC 14207 | 35-37°C / 44-48 h | yellow | green |
| *A.faecalis* ATCC 35655 | 35-37°C / 44-48 h | green/blu | green |
| *E.coli* ATCC 25922 | 35-37°C / 44-48 h | yellow | yellow |

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Type Culture Collection of the UK Health Protection Agency

## 12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated OF Hugh Leifson Base supplemented with glucose is tested for specific performance characteristics by comparing the results with a previously approved Reference Batch. Samples are inoculated directly by stabbing two tubes of the medium with cultures of *P.aeruginosa* ATCC 27853, *P.aeruginosa* ATCC 14207, *Acinetobacter calcoaceticus* ATCC 19606, *Alkaligenes faecalis* ATCC 35655, *E.coli* ATCC 25922, grown for 18-24 h on Tryptic Soy Agar. One tube for each organism is covered with liquid paraffin. Tubes are incubated with loose caps at 35-37 °C for 44-48 hours in aerobic atmosphere. The colour changes of tubed media are observed and recorded: the bacterial oxidative/fermentative reactions are conformed to the specifications.

## 13 - LIMITATIONS OF THE METHOD

- Mineral oil is not recommended because it is a heavy liquid petroleum which allows oxygen diffusion in the medium.[2,3]
- Some organisms are unable to grow on OF Hugh Leifson Medium; if this occurs, repeat the OF test with the medium enriched either with 2% serum or 0,1% yeast extract.[4]
- An organism that is neither oxidative or fermentative will produce a Slight alkalinity (blue-green) in the open tube but the covered tube will not exhibit a colour change (green).[3]
- Some bacteria produce an atypical reaction: acid in the closed tube but not in the open tube; *Chromobacterium violaceum* develops these atypical reactions with starch and maltose.[6]
- Some organisms require prolonged incubation before acid production is visible. Lederberg[7] states that the delayed reaction is due to the inability of a carbohydrate to penetrate the bacterial cell. He recommends to perform ONPG test to determine potential fermentative ability.
- A fermentative organism will exhibit an acid reaction throughout the medium in both tubes. However, acid production of an oxidative organism is evident only at the surface of the open tube and gradually spreads throughout the tube. If the oxidative reaction is delayed

or weak, an alkalinity may be observed on the surface of the open tube, often resulting in a misinterpretation of the OF reaction as being negative. However, on prolonged incubation of several days, the alkaline reaction reverts to acid.[3]

- Hugh and Leifson[1] report that some so-called "paracolon bacilli" can have both an oxidative and fermentative metabolism. In these cases, oxidation is not evident unless the fermentation is slow or delayed. This group of gram negative bacilli (*Citrobacter*, *S.arizonae*) is capable of oxidation or fermentation of lactose, or both.
- Even if the microbial colonies are differentiated on the basis of OF test, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

## 14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the production process of prepared media.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

## 15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging).

## 16 - REFERENCES

1. Hugh R, Leifson E. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J Bacteriol 1953; 66:24-26
2. Public Health England. Oxidation/Fermentation of Glucose Test. UK Standards for Microbiology Investigations. TP 27 Issue 3, 2015
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5. Finegold SM, Martin WJ, Scott EG. Bailey and Scots Diagnostic Microbiology, 5th ed., St.Louis: The C.V. Mosby Company, 1978, pp 124, 184, 453.
6. Sivendra R. One-tube oxidation-fermentation methods: limitations posed by atypical fermentative reactions. Appl Environ Microbiol 1976: 31(5):778-80
7. Lederberg J. The beta-D-galactosidase of Escherichia coli, strain K-12. J.Bacteriol 1950; 60: 381

**TABLE OF APPLICABLE SYMBOLS**

| | | | | |
|---|---|---|---|---|
| REF o REF<br>Catalogue number | LOT Batch code | IVD *In vitro* Diagnostic Medical Device | Manufacturer | Use by |
| Temperature limitation | Contents sufficient for <n> tests | Consult Instructions for Use | Keep away from direct light | Store in a dry place |

**REVISION HISTORY**

| Version | Description of changes | Date |
|---|---|---|
| Revision 1 | Updated layout and content | 2020/05 |
| Revision 2 | Update of "precautions and warnings" and "storage conditions and shelf life" | 2022/03 |
| Revision 3 | Removal of obsolete classification | 2023/04 |
| Revision 4 | Change of *A.faecalis* strain NCTC 655 with ATCC 35655 | 2024/12 |

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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