PHENOL RED BROTH BASE

Dehydrated culture medium

Phenol Red Broth w/Lactose: from left: uninoculated tube, *E.coli* (lac+), *S.*Enteritidis (lac-)

1 - INTENDED USE

Basal medium to aid in differentiation between genera and species of bacteria by their ability to ferment (degrade) specific carbohydrates.

2 - COMPOSITION - TYPICAL FORMULA*

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.000 g
Beef extract	3.000 g
Sodium chloride	5.000 g
Phenol red	0.018 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

Phenol Red Broth Base, prepared according to a modification of the formula proposed by Vera[1] in 1950, is used for the differentiation between genera and species of bacteria, by their ability to ferment (degrade) specific carbohydrates incorporated in the basal medium [2]. Phenol Red Broth Base supplemented, in separate tubes, with 5 g/L of dulcitol, 10 g/L of sucrose and 10 g/L of lactose, is reported by FDA-BAM[3] among the tests for the identification of *Salmonella*.

The basal medium contains a peptone with a low carbohydrates content and beef extract which are sources of nitrogen, carbon and minerals for bacterial growth; sodium chloride maintains the osmotic balance; phenol red is a pH indicator: when Phenol Red Broth is prepared with a final concentration of 0.5-1 % carbohydrate, most of the end products of its fermentation are organic acids, which produce a colour change of the pH indicator from red to yellow; if gas is produced during the fermentation reaction, it is collected in the inverted Durham tube. If the test is negative, a catabolic attack of peptones will occur with the formation of ammonia, the alkalinisation of the medium and a colour change of phenol red from red-orange to reddish-pink.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 18 g in 1000 mL of cold purified water and add the chosen carbohydrate to the final concentration of 0.5-1% (5-10 g/L). Heat if necessary to dissolve the medium completely, dispense 3-5 mL in suitable tubes and insert Durham tubes when gas production must be recorded. Sterilize by autoclaving at 118°C for 15 minutes. Alternatively, to the autoclaved and cooled base, add a filter sterilized solution of carbohydrate so to obtain a final concentration of 0.5-1%.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	red-orange, limpid
Final pH at 20-25 °C	7.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Phenol Red Broth Base	Dehydrated medium	4019102	500 g (27,8 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, test tubes, Durham tubes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Phenol Red Broth Base is not intended for primary isolation from clinical specimens; it is inoculated with 18-24 h pure culture from solid media such as Tryptic Soy Agar or blood agar, isolated from clinical specimens or other materials.

9 - TEST PROCEDURE

With a heavy inoculum, inoculate tubes of Phenol Red Carbohydrate Broth with pure culture using an inoculating loop or swab. Inoculate a carbohydrate-free test tube too.

Incubate tubes with loosened caps, aerobically or anaerobically depending on suspected microorganism(s) at 35-37°C for 18-48 hours. Prolonged incubation may be required, up to 30 days to be considered a negative result.[2]

10 - READING AND INTERPRETATION

After incubation observe the presence of growth (turbidity) and the colour change of the medium.

Positive reaction (carbohydrate degradation): the medium turns yellow and the formation of gas bubbles can be observed.

Negative reaction: the medium is turbid reddish-pink in colour.

No yellow colour should occur in the control tube.

After a positive reaction has been observed, discard the tube; by prolonging the incubation, an inversion of the reaction may be observed.

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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 testing 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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	GLUCOSE
E. coli ATCC 25922	35-37° / 18-24H /AE	AG
S. Typhimurium ATCC 14028	35-37° / 18-24H /AE	AG
P.aeruginosa ATCC 14207	35-37° / 18-24H /AE	K

AE: aerobic incubation; ATCC is a trademark of American Type Culture Collection; A: acid production, yellow colour; G: gas production; K: alkalinity, reddish-pink

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Phenol Red Broth Base supplemented with glucose, lactose, mannose and sucrose is tested for performances characteristics comparing the results with a previously approved Reference Batch.
Pure colonies cultivated on Tryptic Soy Agar of *E.coli* ATCC 25922, *S.*Typhimurium ATCC 14028, *C.freundii* ATCC 8090, *P.aeruginosa* ATCC 14207, *E.faecalis* ATCC 19433 are heavily inoculated into test tubes . After incubation at 35-37°C for 18-24 hours aerobically, the colour change of the medium is observed and recorded. All strains show performances characteristics according to the specifications for both batches.

13 - LIMITATIONS OF THE METHOD

- "Carbohydrate" are collectively termed "sugars". However, the test can be performed with "real sugars" whose names end with "ose" (lactose, glucose, sucrose etc.) or with alcohols, whose names end with "ol" (dulcitol, mannitol). A few exceptions exist such as the sugar salicin (glycoside).
- The concentration of carbohydrates incorporated into the medium base is usually 1% (exception salicin and dulcitol: 0.5%). 1% concentration reduces the possibility of alkaline re-inversion of positive reactions.[2]
- Certain carbohydrates can withstand autoclaving at 116-118°C for 15 minutes with little or no breakdown. Autoclaving is not advisable for the following: arabinoses, lactose, maltose, salicin, sucrose, trehalose, xylose.[2]
- The medium after autoclaving, when hot, appears a light orange colour; this will change to an orangish-red colour after cooling.
- The addition of certain carbohydrates to the medium can cause a decrease in pH. If this occur add 0.1N NaOH drop by drop until the desired pH.
- The inverted Durham tube, in the examination of *Enterobacteriaceae*, is necessary only for the medium incorporating glucose: if the strain produces gas from glucose, it will also produce gas from all other degraded carbohydrates.[2]
- Even the observation of a single bubble makes the test positive for the production of gas (CO_2 and H_2) by the aerogenic strains.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and 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.
- 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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 plates 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.
- 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. Vera HD. Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am.J.PublicHealth 1950; 40:1267-1272..
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/05
Revision 2	Update of “intended use”, “precautions and warnings” and “storage conditions and shelf life”	2022/05

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

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