INSTRUCTIONS FOR USE

PHENYLALANINE AGAR

Dehydrated culture medium

Phenylalanine Agar: from left: uninoculated tube, *P.mirabilis* +, *Ecoli* -

1 - INTENDED USE

In vitro diagnostic. For the differentiation of *Proteus, Morganella* and *Providencia* from other members of the *Enterobacteriaceae* by the ability to deaminate phenylalanine.

2 - COMPOSITION - TYPICAL FORMULA*

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	3
DL phenylalanine	2
Disodium hydrogen phosphate	1
Sodium chloride	5
Agar	15

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Phenylalanine Agar is a differential medium developed by Buttieux[1] and subsequently modified by Ewing, Davis, and Reavis[2]. It is intended for the differentiation of the genera *Proteus, Providencia* and *Morganella* from the other members of *Enterobacteriaceae* by the ability to oxidatively deaminate phenylalanine to phenylpyruvic acid and ammonia (NH_3) by the enzymatic activity of phenylalanine deaminase. The phenylpyruvic acid is detected by adding a few drops of 10% ferric chloride which acts as a chelating agent; a green coloured complex is formed between these two compounds indicating a positive test. If the medium remains a pale yellow, the organism is negative for phenylalanine deaminase production. Yeast extract is a source of carbon, nitrogen, vitamins for bacterial growth, sodium phosphate acts as a buffer system, sodium chloride maintains the osmotic balance of the medium, phenylalanine serves as the substrate for the enzyme.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 26 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute in tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool in slanted position to obtain a long slant and a short butt.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Phenylalanine Agar	Dehydrated medium	4019162	500 g (19,2 L) CND: W0104010101; EDMA 14.01.01.01; RDM: 1874646/R

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave and water-bath, sterile loops, incubator and laboratory equipment as required, ancillary culture media and reagents for the complete identification of the culture (ferric chloride reagent: ferric chloride $FeCl_3$ 12 g, concentrated HCl 2.5 mL, purified water 100 mL).

8 - SPECIMENS

Phenylalanine Agar is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

9 - TEST PROCEDURE

Using a loopful of inoculum from an 18-24 hours pure culture, heavily streak the slant surface using a fishtail motion.
Incubate aerobically at 35-37°C for 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, add 4-5 drops of ferric chloride reagent to the growth on the slant and gently rotate the tube.
A positive test (deamination of phenylalanine) is indicated by the development of a light green to bright green colour on the slant and on the reagent, within 1-5 minutes after applying the ferric chloride reagent.

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A negative test is indicated by the absence of green colour: the reagent remains yellow.
Proteus spp., *Providencia* spp. and *Morganella* spp. are positive for the phenylalanine deamination test while the other *Enterobacteriaceae* are negative.

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, it is responsibility of the end-user to perform 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	EXPECTED RESULTS
P.mirabilis ATCC 25933	35-37°C / 18-24H / A	development of a light green to bright green colour after $FeCl_3$ reagent addition
E.coli ATCC 25922	35-37°C / 18-24H / A	the reagent remains yellow

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Phenylalanine Agar is tested for performances characteristics comparing the results with a previously approved Reference Batch.
Pure colonies cultured on Tryptic Soy Agar of 5 phenylanine deaminase positive strains ((*P.vulgaris* ATCC 9484, *P.mirabilis* ATCC 10005, *P.stuardii* ATCC 33682, *P.morgani* clinical isolate, *P.rettgeri* ATCC 39944) and 4 phenylalanine deaminase negative strains (*E.coli* ATCC 25922, *E.aerogenes* ATCC 13048, *K.pneumoniae* ATCC 27736, *S.*Thyphimurium ATCC 14028) are inoculated by streaking the medium in tubes. After incubation at 35-37°C for 18-24 hours aerobically, the ferric chloride reagent is added to each tube and the colour change is observed. All strains show reactivity according to the specifications for both batches tested.

13 - LIMITATIONS OF THE METHOD

- A positive phenylalanine reaction must be interpreted within 5 minutes upon addition of reagent as the green colour fades quickly.[3]
- Rolling the reagent over the slant aids in obtaining a faster reaction and a more intense colour.[3]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; 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.
- 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 inoculated tubes with microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredients for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet 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. This also applies in relation to any third-party rights. 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 were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

16 - REFERENCES

1. Buttiaux R, Osteux R, Fresnoy R, Moriamez J. Les propriétés biochimiques charachéristiques di genre Proteus: inclusion souhaitable des Providencia dans celui-ca. Ann Inst Pasteur Lille 1954; 87:375.
2. Ewing WH, Davis BR Reavis RW. Phenylalanine and malonate media and their use in enteric bacteriology. Publ Health Lab 1975; 15:153.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF OR REF 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	2020/06

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

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