PKU Test Agar Base

M282

Intended Use:

Recommended for estimation of phenylalanine in blood for detection of Phenylketonuria (PKU).

Composition**

Ingredients	g / L
L-Glutamic acid	0.500
DL-Alanine	0.500
Asparagine	0.500
Dextrose (Glucose)	10.000
Dipotassium hydrogen phosphate	15.000
Potassium dihydrogen phosphate	5.000
Ammonium chloride	2.500
Ammonium nitrate	0.500
Sodium sulphate	0.500
Magnesium sulphate	0.050
Manganese chloride	0.005
Ferric chloride	0.005
Calcium chloride anhydrous	0.0025
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.06 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE OR OVERHEAT.** Cool to 45-50°C and add *Bacillus subtilis* spores and 3.3 mg β-2-thienylalanine. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Phenylketonuria is a congenital defect caused due to absence of phenylalanine hydroxylase. As a result of this, phenylalanine accumulates in the blood, which is excreted via urine hence it is called as phenylketonuria. Subsequently this deficiency may cause brain damage resulting in mental retardation. Guthrie and Tiekelmann (1) devised a modified inhibition assay for early detection of PKU using blood/urine samples of newborn infants having low levels of phenylalanine by determining the serumphenylalanine levels or the level of phenylpyruvic acid in urine. PKU Test Agar Base, developed by Demain (2) is a chemically defined medium, which is supplemented with β-2-thienylalanine. It provides the agar bed for testing blood samples. The Guthrie test (1,3,4) was developed on the observation that *Bacillus subtilis* is normally inhibited in presence of β-2-thienylalanine but grows well when L-phenylalanine is added to the medium. Phenylalanine neutralizes the β-2-thienylalanine and allows bacteria to grow. The phenylalanine level can be read to determine the level of amino acid in blood. Other than phenylalanine, proline, phenylpyruvic acid or phenyllactic acid can be used.

Type of specimen

Clinical samples - Blood, Urine

Specimen Collection and Handling:

Small filter paper discs saturated with patients blood are placed on PKU Test Agar with β-2-thienylalanine inoculated with *Bacillus subtilis.* Control discs impregnated with different levels such as 2, 4, 6, 8,10,12 and 20 mg% of L-phenylalanine are also placed on the medium. After overnight incubation, zones of growth around the paper discs are observed and compared with zones around control discs. A response comparable to 4 mg% control disc is considered as presumptive positive. The results can be repeated using a duplicate test disc and a chemical or spectrofluorometric procedure (1,4).

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

N.A.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petriplates

Reaction

Reaction of 5% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed with added β-2-thienylalanine, after an incubation at 35-37°C for 12-16 hours

Organism	Growth w/2% Phenylalanine	Growth w/4% Phenylalanine	Growth w/6% Phenylalanine	Growth w/8% Phenylalanine	Growth w/10% Phenylalanine	Growth w/ 12% Phenylalanine
** *Bacillus spizizenii* ATCC 6633 (00003*)	none-poor	luxuriant	luxuriant	luxuriant	luxuriant	luxuriant

Key : *Corresponding WDCM numbers. **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Guthrie R. and Tiekelmann H., 1960, London Conference on the Scientific study of Mental Deficiency, London.
2. Demain A. L., 1958, J. Bacteriol., 75:517.
3. Guthrie R., 1961, J. Am. Med. Assoc., 178:863.
4. Guthrie R. and Susi A., 1963, Pediatrics, 32:338.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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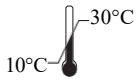
IVD *In vitro* **diagnostic medical device**



Storage temperature

EC REP

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CE **CE Marking**

Do not use if package is damaged

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