DEHYDRATED
CULTURE MEDIA FOR

HARMONISED PHARMACOPOEIA

ENHANCED PORTFOLIO

AVAILABLE NOW

SUPPORTING REGULATORY COMPLIANCE

LAB™
A Neogen® Company

THE GATEWAY TO MICROBIOLOGY™

INTRODUCTION

Harmonised Pharmacopoeia; USP/EP/JP

The European Pharmacopoeia 8.0 volume 1 (2014) is a standard ensuring the quality of medicines and their components. The European Pharmacopoeia (EP) is harmonised with the equivalent chapters of the United States (USP) and Japanese Pharmacopoeias (JP), allowing the free movement of medicinal products within Europe and beyond*.

Supporting Regulatory Compliance

The following range of dehydrated culture media from Lab M is formulated and performance tested according to the requirements specified within the European Pharmacopoeia 8.0 volume 1 (2014).

WHY LAB M?

Product Quality

Established in 1971, Lab M has a long history of providing culture media to laboratories globally. The quality of Lab M's products has a direct impact on the service their customers provide. As a result of this Lab M consider quality to be a shared responsibility.

Lab M is accredited to ISO 9001:2008 & ISO 13485:2003 for the design, manufacture and supply of microbiological culture media, antibiotic supplements and diagnostic products.
These accreditations, alongside Lab M's stringent quality management system, ensure the highest quality products are available with batch-to-batch consistency. Providing our customers with peace of mind and assurance that their results are reliable and reproducible is of critical importance to Lab M.

FOR MORE INFORMATION **Tel:** +44 (0) 161 820 3833 **Fax:** +44 (0) 161 820 5383

*European Pharmacopeia, 2014, 8th edition, European Directorate for the Quality of Medicine, Council of Europe, 226 Avenue de Colmar BP907, F-67029 Strasbourg Cedex 1, France.

PRODUCTS

Media for sterility testing:

According to the European Pharmacopoeia the following media are used to test sterility of substances, preparations or articles required to be sterile. A sample is considered sterile if no growth of micro-organisms occur in the incubated portion of media after 14 days. The following conditions refer to the requirements of the growth promotion test, other protocols are described for different procedures.

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP001 Fluid Thioglycollate Medium (USP/EP/JP)	Growth promoting	*Cl. sporogenes,* *P. aeruginosa,* *S. aureus*	30-35°C for ≤ 3 days
HP002 Casein Soya Bean Digest Broth (USP/EP/JP)	Growth promoting	*A. brasiliensis,* *B. subtilis,* *C. albicans*	20-25°C for ≤ 3 days (bacteria) & ≤ 5 days (fungi)

HP001

Typical apperance of uninoculated (1st left), *B. subtilis* (2nd left), *P. aeruginosa* (3rd left), and *S. aureus* (far right).

This sterility test medium not only supports luxuriant growth of a wide range of organisms but prevents the accumulations of reactive oxygen species that can be derogatory to recovery and growth of environmental contaminants. This is due to the low oxygen reduction potential and the nutritionally supportive base.

HP002

Turbidity (right) indicating growth in contrast to the clear uninoculated broth (left).

Enzymatic digests of casein and soya bean act as a source of nitrogen and glucose is a carbon source in the form of a fermentable carbohydrate. Sodium chloride maintains the osmotic balance and dipotassium hydrogen phosphate acts as a buffering agent. This nutritious base can support the growth of a wide range of micro-organisms.

Media for the examination of non-sterile products for specified micro-organisms:

The European Pharmacopoeia specifies the following culture media for the growth of specific organisms.

Bile-tolerant Gram-negative bacteria:

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP003 Enterobacteria Enrichment Broth – Mossel (USP/EP/JP)	Growth promoting	*E. coli,* *P. aeruginosa*	30-35°C for ≥ 24 hrs
	Inhibitory	*S. aureus*	30-35°C for 48 hrs
HP004 Violet Red Bile Glucose Agar (USP/EP/JP)	Growth promoting & indicative	*E.coli,* *P. aeruginosa*	30-35°C for ≥ 18 hrs

HP003

Typical *E. coli* growth (right) results in a colour change to yellow from the uninoculated green vial (left). *P. aeruginosa* (middle) results in turbid growth with no colour change.

The formulation stated by Mossel consists of a nutritious base made selective by bile acids and brilliant green, which is also strongly buffered to prevent auto sterilisation from the production of acid from fermentation.

HP004

Indicative *E. coli* colonies on VRBGA are pink/red with a pink/red precipitation zone. Indicative *P. aeruginosa* present colourless colonies without a precipitation zone.

Glucose fermenting bile-tolerant organisms produce acid from the fermentation which results in a pH drop indicated by neutral red resulting in pink colonies. Enough acid production will cause the precipitation of bile salts resulting in a bile precipitate or halo around colony. Non-glucose fermenting bile-tolerant bacteria grow but remain colourless with no bile precipitate.

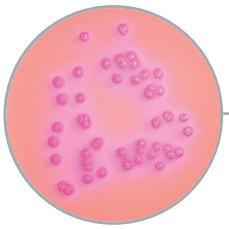
Escherichia coli:

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP005 MacConkey Broth (USP/EP/JP)	Growth promoting	*E. coli*	42-44°C for ≥ 24 hrs
	Inhibitory	*S. aureus*	42-44°C for 48 hrs
HP006 MacConkey Agar (USP/EP/JP)	Growth promoting & indicative	*E.coli*	30-35°C for ≥ 18 hrs

HP005

Typical *E. coli* growth (right) results in a colour change to yellow from the uninoculated purple (left).

Gelatin peptone provides a source of nitrogen, while lactose is a fermentable carbohydrate. Ox bile acts as a selective agent inhibiting most gram-positive organisms and bromocresol purple acts as a pH indicator. A colour change from purple to yellow indicates growth of a bile-tolerant, lactose-fermenting organism such as *Escherichia coli.*



HP006

Indicative *E. coli* colonies on MacConkey Agar presenting a pink/red colony colour with a bile precipitation zone.

Escherichia coli can ferment lactose to produce acid which results in a pH drop. This is indicated by neutral red resulting in pink colonies. Enough acid production will cause the precipitation of bile salts resulting in a bile precipitate or halo around lactose-fermenting bacteria. Non-lactose fermenting bacteria grow but remain colourless with no bile precipitate.

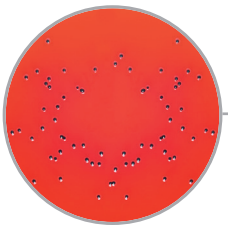
Salmonella:

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP007 Rappaport Vassiliadis Salmonella Enrichment Broth (USP/EP/JP)	Growth promoting	*Salmonella enterica subsp. enterica serovar Typhimurium*	30-35°C for ≥ 18 hrs
	Inhibitory	*S. aureus*	30-35°C for 24 hrs
HP008 Xylose Lysine Deoxycholate Agar (USP/EP/JP)	Growth promoting & indicative	*Salmonella enterica subsp. enterica serovar Typhimurium*	30-35°C for ≥ 18 hrs

HP007

Typical *Salmonella* growth (right) results in turbidity in contrast to the clear uninoculated (left) broth.

The finely poised selective system consisting of magnesium chloride, malachite green, high osmotic pressure and low pH effectively inhibit non target bacteria, whilst allowing rapid growth of *Salmonella*.



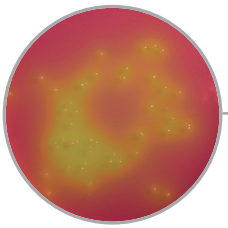
HP008

Indicative *Salmonella* on XLD Agar are red colonies with or without black centres.

Salmonella are able to ferment xylose to produce acid but not lactose or sucrose. When the xylose is exhausted *Salmonella* will decarboxylate lysine shifting the pH back to neutral. At near neutral pH *Salmonella* can reduce sodium thiosulfate producing hydrogen sulfide which creates a complex with ferric ammonium citrate to produce black or black centred colonies. Other organisms are able decarboxylate lysine but acid production from the fermentation of lactose and sucrose keeps the pH too acidic for H_2S production.

Staphylococcus aureus:

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP009 Mannitol Salt Agar (USP/EP/JP)	Growth promoting & indicative	*S. aureus*	30-35°C for ≥ 18 hrs
	Inhibitory	*E.coli*	30-35°C for 72 hrs



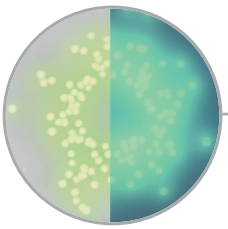
HP009

Indicative *S. aureus* presenting yellow/white colonies surrounded by a yellow zone.

Staphylococcus aureus ferment mannitol and drop the pH of the medium due to acid production. Phenol red indicates this resulting in yellow colonies. Selectivity is achieved through the high salt concentration which only halophilic organisms like *S. aureus* can tolerate.

Pseudomonas aeruginosa:

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP010 Cetrimide Agar (USP/EP/JP)	Growth promoting	*P. aeruginosa*	30-35°C for ≥ 18 hrs
	Inhibitory	*E.coli*	30-35°C for 72 hrs



HP010

Indicative *P. aeruginosa* on Cetrimide Agar exhibiting yellow/green pigmentation under normal light (left) and under UV light (right).

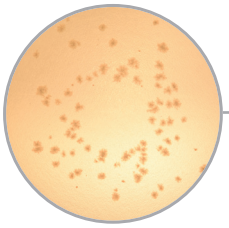
Cetrimide is a quarternary ammonium compound that inhibits the growth of a wide range of Gram-positive and some Gram-negative micro-organisms. Magnesium chloride and dipotassium sulphate improve the production of pyoverdin and pyocyanin pigments that combine to give *Pseudomonas aeruginosa* characteristic green colonies.

Clostridia:

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP011 Reinforced Medium for Clostridia (USP/EP/JP)	Growth promoting	*Cl. sporogenes*	30-35°C for 48 hours (anaerobic conditions)
HP012 Columbia Agar (USP/EP/JP)	Growth promoting	*Cl. sporogenes*	30-35°C for ≥ 48 hrs (anaerobic conditions)

HP011
Typical *Clostridium sporogenes* growth (right) results in turbidity in contrast to the clear uninoculated broth (left).

The medium engineers an environment favourable to spore forming anaerobes, especially *Clostridium* spp. A combination of a nutritious base, osmotic balance, detoxifying and reducing components mean this medium is able to achieve fertile growth from a small inoculum.



HP012
Typical *Clostridium sporogenes* colonies on Columbia Agar.

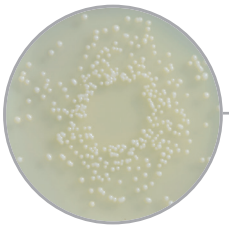
Originally described as a general purpose nutritious agar base by Ellner *et al.* at Columbia University, this agar can support the growth of *Clostridia* and other fastidious micro-organisms.

Candida albicans:

Recommended culture media	Property	Test Strains	Incubation time & temperature
HP013 Sabouraud Dextrose Broth (USP/EP/JP)	Growth promoting	*C. albicans*	30-35°C for ≥ 72 hrs
HP014 Sabouraud Dextrose Agar (USP/EP/JP)	Growth promoting & indicative	*C. albicans*	30-35°C for ≥ 72 hrs

HP013
Typical *Candida albicans* growth (right) results in turbidity in contrast to the clear uninoculated broth (left).

The peptone digests and dextrose provide a nutritious base for luxuriant fungal growth and the acidic pH affords selectivity against bacteria.



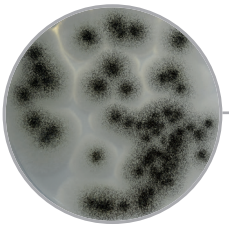
HP014
Indicative white colonies of *Candida albicans* on Sabouraud Dextrose Agar.

This nutritious base is able to reliably cultivate and differentiate fungi whilst the high dextrose and low pH effectively inhibit bacteria.

Support media for control culture cultivation inoculum preparation:

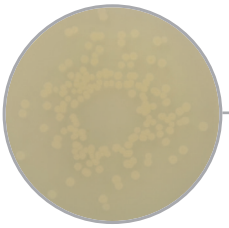
Further media are defined in the Harmonised European Pharmacopoeia that are used to support the described protocols. These media are used as part of specific protocols or as control or preparation media for performance and suitability testing. They are also used to prepare culture required to test culture media.

Recommended culture media	Use
HP015 Potato Dextrose Agar (USP/EP/JP)	As an alternative plating media for the preparation of *Aspergillus brasiliensis* test strain.
HP016 Casein Soya Bean Digest Agar (USP/EP/JP)	As a plating media for the growth promotion & total aerobic microbial count test. Also used as a medium for preparation of bacterial test strains.
HP002 Casein Soya Bean Digest Broth (USP/EP/JP)	As a liquid media for the growth promotion & total aerobic microbial count test. Also used as a medium for preparation of bacterial test strains and preparation of samples.
HP017 Buffered Sodium Chloride-Peptone Broth pH 7.0 (USP/EP/JP)	As a medium for the preparation of samples and as a diluent for preparation of microbial test suspensions.



HP015
Typical *Aspergillus brasiliensis* growth on Potato Dextrose Agar.

The extract from potato and dextrose provide a nutritionally rich base that encourages mould sporulation and pigment production.



HP016
Bacillus subtilis growing on Casein Soya Bean Digest Agar.

The medium is commonly referred to as tryptone (or tryptic) soy agar and abbreviated to TSA. Enzymatic digests of casein and soya bean act as a source of nitrogen and amino acid and sodium chloride maintain the osmotic balance.

HP002
Turbidity (right) indicating growth in contrast to the clear uninoculated broth (left).

Enzymatic digests of casein and soya bean act as a source of nitrogen and glucose is a carbon source in the form of a fermentable carbohydrate. Sodium chloride maintanis the osmotic balance and dipotassium hydrogen phosphate acts as a buffering agent. This nutritious base can support the growth of a wide range of micro-organisms.

HP017
Buffered Sodium Chloride-Peptone Solution used as a medium for the preperation of samples and as a diluent for the preperation of microbial test suspensions.

The low level of peptone lessens the physiological shock experienced by micro-organism when suspended in a diluent. The dual phosphate components create a buffered environment and sodium chloride maintains the osmotic balance.

LAB™
A Neogen® Company

1 Quest Park, Moss Hall Road, Heywood, Lancashire, BL9 7JJ, UK
Tel: +44 (0) 161 820 3833 **Fax:** +44 (0) 161 820 5383
E-mail: labm@neogeneurope.com **Web:** www.labm.com

NE8501