

Culture Media as on Pharmacopoeia 7.3, Harmonized Method for Microbiological Examination of non sterile products

-FORMULATIONS

Buffered sodium chloride-peptone solution pH 7.0 Cat. N° 1401

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate	7.2 g, equivalent to 0.067 M phosphate
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Purified water	1000 ml

Sterilise in an autoclave using a validated cycle.

Casein soya bean digest broth Cat. N° 1224

Pancreatic digest of casein	17.0 g
Papaic digest of soya bean	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.3 ± 0.2 at 25 °C. Sterilise in an autoclave using a validated cycle.

Casein soya bean digest agar Cat. N° 1068

Pancreatic digest of casein	15.0 g
Papaic digest of soya bean	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.3 ± 0.2 at 25 °C. Sterilise in an autoclave using a validated cycle.

Sabouraud-dextrose agar Cat. N° 1024

Dextrose	40.0 g
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ± 0.2 at 25 °C. Sterilise in an autoclave using a validated cycle.

Potato dextrose agar Cat. N° 1022

Infusion from potatoes	200 g
Dextrose	20.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ± 0.2 at $25\text{ }^{\circ}\text{C}$. Sterilise in an autoclave using a validated cycle.

Sabouraud-dextrose broth Cat. N° 1205

Dextrose	20.0 g
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ± 0.2 at $25\text{ }^{\circ}\text{C}$. Sterilise in an autoclave using a validated cycle.

Enterobacteria enrichment broth-Mossel Cat. N°1202

Pancreatic digest of gelatin	10.0 g
Glucose monohydrate	5.0 g
Dehydrated ox bile	20.0 g
Potassium dihydrogen phosphate	2.0 g
Disodium hydrogen phosphate dihydrate	8.0 g
Brilliant green	15 mg
Purified water	1000 ml

Adjust the pH so that after heating it is 7.2 ± 0.2 at $25\text{ }^{\circ}\text{C}$. Heat at $100\text{ }^{\circ}\text{C}$ for 30 min and cool immediately.

Violet red bile glucose agar Cat. N° 1092

Yeast extract	3.0 g
Pancreatic digest of gelatin	7.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30 mg
Crystal violet	2 mg
Purified water	1000 ml

Adjust the pH so that after heating it is 7.4 ± 0.2 at $25\text{ }^{\circ}\text{C}$. Heat to boiling; do not heat in an autoclave.

MacConkey broth Cat. N° 1210

Pancreatic digest of gelatin	20.0 g
Lactose monohydrate	10.0 g
Dehydrated ox bile	5.0 g
Bromocresol purple	10 mg
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.3 ± 0.2 at $25\text{ }^{\circ}\text{C}$. Sterilise in an autoclave using a validated cycle.

MacConkey agar Cat. N° 1052

Pancreatic digest of gelatin	17.0 g
Peptones (meat and casein)	3.0 g
Lactose monohydrate	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	30.0 mg
Crystal violet	1 mg
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.1 ± 0.2 at $25\text{ }^{\circ}\text{C}$. Boil for 1 min with constant shaking then sterilise in an autoclave using a validated cycle.

Rappaport Vassiliadis *Salmonella* enrichment broth Cat. N° 1414

Soya peptone	4.5 g
Magnesium chloride hexahydrate	29.0 g
Sodium chloride	8.0 g
Dipotassium phosphate	0.4 g
Potassium dihydrogen phosphate	0.6 g
Malachite green	0.036 g
Purified water	1000 ml

Dissolve, warming gently. Sterilise in an autoclave using a validated cycle, at a temperature not exceeding $115\text{ }^{\circ}\text{C}$. The pH is to be 5.2 ± 0.2 at $25\text{ }^{\circ}\text{C}$ after heating and autoclaving.

Xylose, lysine, deoxycholate agar Cat. N° 1080

Xylose	3.5 g
L-Lysine	5.0 g
Lactose monohydrate	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	80 mg
Agar	13.5 g
Sodium deoxycholate	2.5 g
Sodium thiosulphate	6.8 g
Ferric ammonium citrate	0.8 g
Purified water	1000 ml

Adjust the pH so that after heating it is 7.4 ± 0.2 at $25\text{ }^{\circ}\text{C}$. Heat to boiling, cool to $50\text{ }^{\circ}\text{C}$ and pour into Petri dishes. Do not heat in an autoclave.

Cetrimide agar cat N° 1102

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Dipotassium sulphate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Purified water	1000 ml
Glycerol	10.0 ml

Heat to boiling for 1 min with shaking. Adjust the pH so that after sterilisation it is 7.2 ± 0.2 at 25 °C. Sterilise in an autoclave using a validated cycle.

Mannitol salt agar Cat. N° 1062

Pancreatic digest of casein	5.0 g
Peptic digest of animal tissue	5.0 g
Beef extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	0.025 g
Purified water	1000 ml

Heat to boiling for 1 min with shaking. Adjust the pH so that after sterilisation it is 7.4 ± 0.2 at 25 °C. Sterilise in an autoclave using a validated cycle.

Reinforced medium for clostridia Cat. N° 1007

Beef extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Glucose monohydrate	5.0 g
Cysteine hydrochloride	0.5 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
Agar	0.5 g
Purified water	1000 ml

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilisation it is 6.8 ± 0.2 at 25 °C. Sterilise in an autoclave using a validated cycle.

Columbia agar Cat. N° 1104

Pancreatic digest of casein	10.0 g
Meat peptic digest	5.0 g
Heart pancreatic digest	3.0 g
Yeast extract	5.0 g
Maize starch	1.0 g
Sodium chloride	5.0 g



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Agar, according to gelling power

10.0-15.0 g

Purified water

1000 ml

Hydrate the agar; dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilisation it is 7.3 ± 0.2 at 25 °C. Sterilise in an autoclave using a validated cycle. Allow to cool to 45-50 °C; add, where necessary, gentamicin sulphate corresponding to 20 mg of gentamicin base and pour into Petri dishes.

Tests for specified microorganisms

	Media	Property	Test strains	Conda
Test for bile-tolerant gram-negative bacteria	Enterobacteria enrichment broth-Mossel	Growth promoting	<i>E. coli</i> <i>P. aeruginosa</i>	1202
		Inhibitory	<i>S. aureus</i>	
	Violet red bile glucose agar	Growth promoting + indicative	<i>E. coli</i> <i>P. aeruginosa</i>	1092
Test for <i>Escherichia coli</i>	MacConkey broth	Growth promoting	<i>E. coli</i>	
		Inhibitory	<i>S. aureus</i>	
	MacConkey agar	Growth promoting + indicative	<i>E. coli</i>	1052
Test for <i>Salmonella</i>	Rappaport Vassiliadis <i>Salmonella</i> enrichment broth	Growth promoting	<i>Salmonella enterica</i> ssp. <i>enterica</i> serotype typhimurium or <i>Salmonella enterica</i> ssp. <i>enterica</i> serotype abony	1414
		Inhibitory	<i>S. aureus</i>	
	Xylose, lysine, deoxycholate agar	Growth promoting + indicative	<i>Salmonella enterica</i> ssp. <i>enterica</i> serotype typhimurium or <i>Salmonella enterica</i> ssp. <i>enterica</i> serotype abony	1080
Indicative	<i>E. coli</i>			
Test for <i>Pseudomonas aeruginosa</i>	Cetrimide agar	Growth promoting	<i>P. aeruginosa</i>	1102
		Inhibitory	<i>E. coli</i>	
Test for <i>Staphylococcus aureus</i>	Mannitol salt agar	Growth promoting + indicative	<i>S. aureus</i>	1062
		Inhibitory	<i>E. coli</i>	
Test for clostridia	Reinforced medium for clostridia	Growth promoting	<i>Cl. sporogenes</i>	1007
	Columbia agar	Growth promoting	<i>Cl. sporogenes</i>	
Test for <i>Candida albicans</i>	Sabouraud dextrose broth	Growth promoting	<i>C. albicans</i>	1205
	Sabouraud dextrose agar	Growth promoting + indicative	<i>C. albicans</i>	1024

Temperatures of Incubation (as reported on “Testing of Products”):

- Enterobacteria enrichment broth-Mossel: incubation at 30-35°C for 24-48 hr
- VRBGAgar: incubation at 30-35°C for 18-24 hr
- MacConkey Broth: 42-44°C for 24-48 hr
- MacConkey Agar: 30-35°C for 18-72 hr
- RVS: 30-35°C for 18-24 hr
- XLD: 30-35 °C for 18-48 h.
- Cetrimide Agar: 30-35°C 18-72 hr
- Mannitol Salt Agar: 30-35°C 18-72 hr
- Reinforced Medium for Clostridium: 30-35°C for 48 hr
- Columbia Agar: 30-35°C for 48-72 hr
- SDB: 30-35°C for 3-5 days
- SDA: 30-35°C for 24-48 hr

Details about Performance Test of Culture Media

Test for growth promoting properties, liquid media: inoculate a portion of the appropriate medium with a **small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test.** Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for growth promoting properties, solid media: perform the surface-spread method, inoculating each plate with a **small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test.** Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with **at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test.** No growth of the test micro-organism occurs.

Test for indicative properties: perform the surface-spread method, inoculating each plate with a small number (**not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test.** Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium

Test for Total Count

	Test strains
TSA Cat. N° 1068	<i>P. aeruginosa</i> <i>S. aureus</i> <i>B. subtilis</i> <i>C. albicans</i>
TSB Cat. N° 1224	<i>P. aeruginosa</i> <i>S. aureus</i> <i>B. subtilis</i> <i>A. brasiliensis</i>
SDA Cat. N° 1024	<i>C. albicans</i> <i>A. brasiliensis</i>

Strains of the test micro-organisms suitable for use in the growth promotion test and the method suitability test:

Staphylococcus aureus ATCC 6538
Pseudomonas aeruginosa ATCC 9027
Escherichia coli ATCC 8739
Candida albicans ATCC 10231
Clostridium sporogenes ATCC 11437
Clostridium sporogenes ATCC 19404
Clostridium perfringens ATCC 13124
Candida albicans 10231
Salmonella typhimurium ATCC 14028
Bacillus subtilis 6633
Aspergillus brasiliensis ATCC 16404

STERILITY

Fluid thioglycollate medium Cat. 1508

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate/anhydrous	5.5 g/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or Thioglycollic acid	0.5 g 0.3 mL
Resazurin sodium solution (1 g/L of resazurin sodium), freshly prepared	1.0 mL
Water R	1000 mL

pH after sterilisation 7.1 ± 0.2

Fluid thioglycollate medium is to be incubated at 30-35 °C.

For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20-25 °C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

Soya-bean casein digest medium Cat. 1068

Pancreatic digest of casein	17.0 g
Papaic digest of soya-bean meal	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate/anhydrous	2.5 g/2.3 g
Water R	1000 mL

pH after sterilisation 7.3 ± 0.2

Soya-bean casein digest medium is to be incubated at 20-25 °C.