AGENZIA REGIONALE PER LA PROTEZIONE DELL'AMBIENTE DELLA SARDEGNA

ARPAS

Direzione Tecnico Scientifica

Servizio Laboratori e misure in campo

Fornitura materiali diagnostici per la linea microbiologica da destinare ai laboratori dei Dipartimenti ARPAS

Capitolato Speciale d'appalto – Parte Tecnica

Allegato 2 - Schede tecniche

96 - **Acqua peptonata alcalina II**

peptone	g	20
cloruro di sodio	g	10
acqua distillata	ml	1000

pH 8,6

88 - **Acqua peptonata salina alcalina**

peptone	g	20
cloruro di sodio	g	30
acqua distillata	ml	1000

pH 8,6

19 - Acqua peptonata tamponata

peptone	g	10
cloruro di sodio	g	5
sodio fosfato monoacido	g	3,5
potassio fosfato biacido	g	1,5
acqua distillata	ml	1000

pH 7,2

Scheda 4: Agar OGYE

04 – Yeast – dextrose – oxitetracicline - gentamycina agar

Terreno base

estratto di levito	g	5
destrosio	g	20
agar	g	12-18
acqua distillata	ml	1000

pH 6,6

Soluzione di ossitetraciclina

ossitetraciclina	mg	50
acqua distillata	ml	50

Sciogliere l'ossitetraciclina in acqua e sterilizzare per filtrazione.

Soluzione gentamicina

gentamicina	mg	50
acqua distillata	ml	50

Sciogliere la gentamicina in acqua distillata e sterilizzare in autoclave a 121°C per 15 min.

Terreno completo

Terreno base	ml	100
Soluzione di ossitetraciclina	ml	10
Soluzione di gentamicina	ml	10

Scheda 5: Bacillus Cereus (PEMBA) agar

32 - Peptone mannitol bromothymol agar (PEMBA)

Terreno base

peptone	g	1
mannitolo	g	10
sodio cloruro	g	2
magnesio solfato	g	0,1
sodio fosfato monoacido	g	2,5
potassio fosfato biacido	g	0,25
sodio piruvato	g	10
blu di bromotimolo	g	0,12
agar	g	12-18
acqua distillata	ml	1000

pH 7,2

Supplementi da aggiungere asetticamente dopo sterilizzazione dei terreno base

Emulsione di rosso d'uovo
tuorlo d'uovo diluito 1:5 con acqua distillata sterile. Trattare 2 ore a 45°C in b.m. e conservare a 0-5°C per non più di 72 ore.

Soluzione di Polimixina B

polimixina B solfato (circa 100 mg, secondo il titolo della polvere)	U.I. 10^6
acqua distillata	ml 100

Sterilizzare per filtrazione.

Terreno completo

Terreno base	ml 900
Soluzione di Polimixina B	ml 10
Emulsione di rosso d'uovo	ml 100

Scheda 6 : Baird Parker agar

2.1.4. Terreni di coltura e reagenti.

2.1.4.1. Terreno di base Agar Baird Parker

Composizione

Triptone	10 g
Estratto di carne	5 g
Estratto di lievito	1 g
Glicina	12g
Piruvato di sodio	10 g
Cloruro di litio	5 g
Agar	20 g
Acqua distillata	1000 ml

pH 6,8±0,2

Il terreno si trova anche in commercio in forma disidratata. Reidratare il terreno in acqua distillata seguendo le istruzioni della ditta produttrice. Riscaldare fino ad ebollizione agitando frequentemente fino ad ottenere la completa dissoluzione dei componenti. Sterilizzare in autoclave a (121 ± 3) °C per 15 minuti. Lasciare raffreddare fino alla temperatura di (50 ± 5) °C prima di aggiungere il supplemento.

2.1.4.2. Emulsione di tuorlo d'uovo e tellurito di potassio al 3,5%

L'emulsione, già pronta, è disponibile in commercio. Il tellurito di potassio è classificato come Xi - Irritante. Nella manipolazione seguire le istruzioni della ditta produttrice e adottare precauzioni per la protezione respiratoria, delle mani e della pelle.

2.1.4.3. Terreno completo Agar Baird Parker

Composizione

Terreno di base (2.1.4.1.) 1000 mL
Emulsione di tuorlo d'uovo (2.1.4.2.) 50 mL

Aggiungere ad 1 L di terreno di base (2.1.4.1.) 50 ml di emulsione di tuorlo d'uovo al tellurito di potassio (2.1.4.2.). Agitare per ottenere una soluzione omogenea e distribuire, rispettando le comuni regole di asepsi, in capsule di Petri.
Il terreno, pronto per l'uso, può essere conservato a (5 ± 3) °C per non più di 7 giorni in condizioni ottimali.

Apat Irsa 29/2003 - 7040 Metodo A
Scheda 7 : Agar bile esculina azide

3. METODO A

3.1 Strumentazione e vetreria

Normale attrezzatura di laboratorio.

3.2 Reattivi e terreni di coltura

3.2.1 Terreni di isolamento

3.2.1.1 Terreno Bile Esculina Azide

Composizione:

Triptone	17 g
Peptone	3 g
Estratto di lievito	5 g
Bile	10 g
Esculina	1,0 g
Ferro (III) ammonio citrato	0,5 g
Sodio cloruro	5 g
Sodio azide	0,15 g
Agar	15 g
Acqua distillata	1000 mL

pH 7,1± 0,2

Il terreno si trova anche in commercio in forma disidratata e si prepara secondo le istruzioni della ditta produttrice. Nel caso sia necessario correggere il pH, utilizzare una soluzione di sodio carbonato (100 g/L). Adottare idonee precauzioni durante la preparazione del terreno che è tossico e mutageno per la presenza di azide sodica. Evitare il contatto e l'inalazione. Dopo avere sciolto la polvere, sterilizzare a 121± 3°C per 15 ±1 minuti. Distribuire in capsule Petri e lasciare solidificare. Conservare a circa +4°C per non più di 2 settimane in condizioni ottimali.

Scheda 8 : Brain Hearth INFUSION

2.1.4.5. Infuso Cuore Cervello

Composizione

Infuso di cervello di vitello	200 g
Infuso di cuore di bue	250 g
Peptocomplex	10 g
Glucosio	2 g
Sodio cloruro	5 g
Sodio fosfato bibasico	2,5 g
Acqua distillata	1000 mL

pH 7,4±0,2

Apat Irsa 29/2003 - 7010 C
Scheda 9 : Chromogenic Coliform medium

Miscela cromogenica 20,3 g
Estratto di lievito 3 g
Peptone 5 g
Lattosio 2,5 g
Sodio cloruro 5 g
Sodio fosfato monoacido 3,5 g
Potassio fosfato biacido 1,5 g
Rosso neutro 0,03 g
Agar 15 g
Acqua distillata 1000 mL
pH 6,8±0,2

Il terreno si trova anche in commercio in forma disidratata e si prepara secondo le istruzioni della ditta produttrice. Dopo avere sciolto la polvere sterilizzare a 121±3°C per 15±1 minuti. Distribuire in capsule Petri e lasciare solidificare. Conservare a circa +4°C per non più di 2 settimane in condizioni ottimali.
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2.2.4.12. Brodo Indolo - Nitrato e per la motilità (alternativo)

Composizione

Triptone	20 g
Glucosio	1 g
Disodio idrogeno fosfato	2 g
Nitrato di potassio	1 g
Agar	1 g
Acqua distillata	1000 mL

pH 7,2 0,2

UNI EN ISO 11290-1: 1996
Scheda 11: Fraser broth

84 - Fraser broth

Terreno base		
peptone di carne	g	5
triptone	g	5
estratto di carne	g	5
estratto di lievito	g	5
cloruro di sodio	g	20
sodio fosfato monoacido	g	12
potassio fosfato biacido	g	1,35
esculina	g	1
cloruro di litio	g	3
acido nalidixico (sale sodico)	g	0,02
acqua distillata	ml	1000

Soluzione di acriflavina		
acriflavina cloridrato	g	0,25
acqua distillata	ml	100

Soluzione di citrato femco ammoniacale		
ammonio ferrico citrato	g	5
acqua distillata	ml	100

Terreno completo

Terreno base	ml	10
Soluzione di acriflavina	ml	0,1
Soluzione di citrato femco ammoniacale	ml	0,1

pH 7,2

Apat Irsa 29/2003 - 7020 B
Scheda 12: M-Faecal coliform agar

8. METODO B. Metodo della filtrazione su membrana (MF)

Con questo metodo viene calcolata la concentrazione dei coliformi fecali che, presenti in un campione di acqua, sulla superficie di una membrana, posta su terreno di coltura agarizzato, hanno formato colonie tipiche prodotte dai microrganismi ricercati. Di seguito sono proposti due substrati d'isolamento alternativi.

8.1 Volume da analizzare

Per l'analisi è necessario determinare il volume in base alla tipologia e alla qualità dell'acqua da esaminare. Per acque reflue o comunque di bassa qualità generalmente è necessario analizzare diluizioni scalari del campione; mentre per acque già sottoposte a trattamento possono essere analizzate diluizioni minori e comunque aliquote diverse.

9. Strumentazione e vetreria

Normale attrezzatura di laboratorio.

10. Reattivi e terreni di coltura

10.1 Terreni di isolamento

10.1.1 m-FC Agar

Composizione:

Triptosio	10	g
Proteose peptone n. 3 o polipeptone	5	g
Estratto di lievito	3	g
Sodio cloruro	5	g
Lattosio	12,5	g
Sali di bile	1,5	g
Blu di anilina	0,1	g
Agar	15	g
Acqua distillata	1000	mL

pH 7,4±0,2

Il terreno si trova anche in commercio in forma disidratata e si prepara secondo le istruzioni della ditta produttrice. Reidratare in acqua distillata contenente 10 mL di acido rosolico all'1% in NaOH 0,2 N. Non sterilizzare. Conservare il terreno distribuito in capsule Petri a circa +4°C per non più di 2 settimane in condizioni ottimali.

Scheda 13 : Nutriente agar salino

91 - Agar nutritivo salino

estratto di carne	g 3
peptone	g 5
cloruro di sodio	g 30
agar	g 12-18
acqua distillata	ml 1000

pH 8,5

Scheda 14 : Nutriente agar

63 - Agar nutritivo II

estratto di carne	g	3
peptone	g	5
agar	g	12-18
acqua distillata	ml	1000

pH 7,2

Scheda 15 : Tryptone Bile X-Glucuronide Agar

7.4.1 Tryptone Bile X-Glucuronide Agar

Composizione:

Triptone	20 g	
Sali di bile n. 3	1,5 g	
X-Gluc	0,075 g	(5-Br-4-Cl-3-indolilβ-D-glucuronide)
Agar	15 g	
Acqua distillata	1000 mL	

pH 7,2±0,2

Il terreno si trova anche in commercio in forma disidratata e si prepara secondo le istruzioni della ditta produttrice. Dopo avere sciolto la polvere sterilizzare a 121±3°C per 15±1 minuti. Distribuire in capsule Petri e lasciare solidificare. Conservare a circa +4°C per non più di 2 settimane in condizioni ottimali.

Rapporti ISTISAN 96/35
Scheda 16 : Tryptone soy agar

71 rapporti ISTISAN- Agar Triptone- Caseina - Soia

Composizione:

triptone	g	15
peptone di soia	g	5
cloruro di sodio	g	5
agar	g	12-18
acqua distillata	ml	1000

pH 7,3

Scheda 17 : TSC agar con cicloserina (Triptosio solfito cicloserina agar)

2.2.4.1 Terreno di base: Triptosio Solfito Cicloserina Agar

Composizione

Triptosio	15 g
Soia peptone	5 g
Estratto di lievito	5 g
Sodio metabisolfito	1 g
Ferro ammonio citrato	1 g
Agar	12 g
Acqua distillata	1000 mL

2.2.4.2. Soluzione di D-cicloserina

Composizione

D-cicloserina	4 g
Acqua distillata	100 mL

2.2.4.3. Terreno completo al Triptosio Solfito Cicloserina Agar

Composizione

Terreno di base (2.2.4.1.)	1000 mL
Soluzione di cicloserina (2.2.4.2.)	10 mL

pH 7,6±0,2

D.M. 13.01.1993 (G.U. n.14 del 19.01.93)
Scheda 18 : McConkey agar

5.3.7. McConkey agar

Composizione

Peptone	20 g
Lattosio	10 g
Sali biliari	5 g
Rosso neutro	75 mg
Agar	15 g
Sodio Cloruro	5 g
Acqua distillata	1000 mL

pH 7,4 ±0,1

D.M. 13.01.1993 (G.U. n.14 del 19.01.93)

Scheda 19 : SPS agar

Agar al Solfito Polimixina Solfadiazina

Composizione:

Solfito di sodio	0,5	g
Solfato di polimixina	0,01	g
Sulfodiazina	0,12	g
Triptone o peptone	15	g
Estratto di lievito	10	g
Citrato di ferro	0,5	g
Sodio tioglicollato	0,1	g
Sorbitan monooleato	0,05	g
Agar	15	g
Acqua distillata	1000	mL

pH 7,0±0,2

Scheda 20: Triple sugar iron agar salino inclinato

92 - Triple sugar saline iron agar

estratto di carne	g	3
estratto di lievito	g	3
peptone	g	20
cloruro di sodio	g	30
lattosio	g	10
saccarosio	g	10
glucosio	g	1
citrato ferrico	g	0,3
rosso fenolo	g	0,024
sodio tiosolfato	g	0,3
agar	g	12-18
acqua distillata	ml	1000

pH 7,4

Distribuire 10 ml di terreno per provetta. Sterilizzare per 10 min. a 121°C Lasciar solidificare il terreno a becco di clarino.

UNI EN ISO 11290-2: 1998/Amd 2004
Scheda 21: Agar Listeria sec. Ottaviani e Agosti (ALOA)

B.3 Agar Listeria sec. Ottaviani e Agosti (ALOA)

B.3.1 Base medium

Enzymatic digest of animal tissues	g	18	
Enzymatic digest of casein	g	6	
Yeast extract	g	10	
Sodium pyruvate	g	2	
Glucose	g	2	
Magnesium glycerophosphate	g	1	
Magnesium sulfate (anhydrous)	g	0,5	
Sodium chloride	g	5	
Lithium chloride	g	10	
Disodium hydrogen phosphate (anhydrous)	g	2,5	
5-Bromo-4-chloro-3-indolyl-b-b-glucopyranoside	g	0,05	
Agar	g	12-18	(*)
Water	ml	930	(**)

(*) depending on the gel strength of the agar.
(**) 925 ml if Amphotencin B solution is used (see B 3.5.2).

Preparation

Dissolve the dehydrated components or dehydrated complete base in the water by boiling.
Sterilize for 15 min. in the autoclave set at 121 °C.
Adjust the pH, if necessary, so that after sterilization it is 7,2 ± 0,2.

B.3.2 Nalidixic acid solution

Nalidixic acid sodium salt	g	0,02
Sodium hydroxide	ml	5

Dissolve the Nalidixic acid sodium salt in 5 ml of sodium hydroxide and sterilize by filtration.

B.3.3 Ceftazidime solution

Ceftazidime	g	0,02
Water	ml	5

Dissolve the Ceftazidime in 5 ml of water and sterilize by filtration through a 0,45 mm membrane.

B.3.4 Polymiyxin B solution

Polymyxin B sulfate	IU	76700
Water	ml	5

Dissolve the Polymyxin B in 5 ml of water and sterilize by filtration through a 0,45 mm membrane.

B.3.5 Antibiotic supplement

B.3.5.1 Cycloheximide solution

Cycloheximide	g	0,05
Ethanol	ml	2,5
Water	ml	2,5

Dissolve the Cycloheximide in 2,5 ml of ethanol then add 2,5 ml of water. Sterilize by filtration through a 0,45 mm membrane.

B.3.5.2 Anphotericin B solution (as an alternative to Cycloheximide solution)

Anphotericin B	g	0,01
HCI (1mol/l)	ml	2,5
Dimethylformamide (DMF)	ml	7,5

Dissolve the Anphotericin in 2,5 ml of HCl/DMF solution. Sterilize by filtration through a 0,45 mm membrane.

WARNING: The HCl/DMF solution is toxic, handle with care.

B.3.6 Supplement

Dissolve 2 g of L-a-phosphatidylinositol (Sigma P6636) in 50 ml of cold water.

Stir for about 30 min. until a homogeneous suspension is obtained.

Autoclave at 121°C for 15 min. and cool to 48°C to 50°C.

B.3.7 Complete medium

Composition

B.3.1	Base medium	ml	930 (*)
B.3.2	Nalidixic acid solution	ml	5
B.3.3	Ceftazidime solution	ml	5
B.3.4	Polymiyxin B solution	ml	5
B.3.5.1	Cycloheximide solution	ml	5
or B.3.5.2	Anphotericin B solution	ml	10
B.3.6	Supplement	ml	50

(*) 925 ml if Anphotericin B solution is used.

Preparation

Add the solutions to the molten base at 50°C, mixed thoroughly between each addition..
The pH of the complete medium shall be 7,2 ± 0,2 at 25°C.
The medium shall be homogeneously opaque.

Preparation of agar plates

Place in each Petri dish 15 ml to 20 ml of the freshly prepared complete medium, then allow to solidify.

B.4 Tryptone Soya yeast extract agar (TSYEA)

Composition

Enzymatic digest of casein	g	17	
Enzymatic digest of soya meal	g	3	
Sodium chloride (NaCl)	g	5	
Depotassium hydrogen phosphate (K_2HPO_4)	g	2,5	
D-Glucose	g	2,5	
Yeast extract	g	6	
Agar	g	9-18	(*)
Water	ml	1000	

(*) depending on the gel strength of the agar.

Preparation

Dissolve the components or dehydrated complete medium in the water by boiling.
Adjust the pH, if necessary, so that after sterilization it is 7,3 ± 0,2 at 25°C.
Dispense the medium into tubes of suitable capacity to obtain portions appropriate for the test.
Sterilize for 15 min. in the autoclave set at 121 °C.
Allow to set in a sloping position.
For the preparation of the agar plates, dispense the medium into sterile Petri dishes in portions appropriate for the test. Allow to solidify.

UNI EN ISO 11290-2: 1998(E)
Scheda 23 : Blood agar +5% sangue di montone

B.6 Sheep blood agar

B.6.1 Base

Composition

Enzymatic digest of animal tissues	g 15
Enzymatic liver digest	g 2.5
Yeast extract	g 5
Sodium chloride (NaCl)	g 5
Agar	g 9-18 (*)
Water	ml 1000

(*) Depending on the gel strength of the agar.

Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.
Adjust the pH, if necessary, so that after sterilization it is 7,2 _+ 0,2 at 25 "C.
Dispense the medium into flasks of suitable capacity to obtain portions appropriate for the test.
Sterilize for 15 min in the autoclave set at 121 "C.

8.6.2 Complete base

Composition

Base (8.6.1)	ml 100
Defibrinated sheep blood	ml 5 to 7

Preparation

Add the blood to the base previously cooled to about 47 "C. Mix well.
Dispense the medium into sterile Petri dishes in portions appropriate for the test. Allow to solidify.

UNI EN ISO 11290-1: 1996
Scheda 24 : Half fraser broth + supplementi

B.1 Selective primary enrichment medium- Half Fraser broth

B.1.1 Base

Composition

Meat peptone (peptic digest of animal tissue)	g	5
Tryptone (peptic digest of casein)	g	5
Beef extract	g	5
Yeast extract	g	5
Sodium chloride	g	20
Disodium hydrogen phosphate dehydrate	g	12
Potassium dihydrogen phosphate	g	1,35
Aesculin	g	1
Water	ml	1000

Preparation

Dissolve the base components or the dehydrated complete base in the water by heating if necessary.
Adjust the pH, if necessary, so that after sterilization it is 7,2 ± 0,2 at 25 °C.
Dispense the base in flasks (6.7) of suitable capacity to obtain portions appropriate for the test (see 9.1).
Sterilize for 15 min in the autoclave (6.1) set at 121°C.
NOTE 11 The lithium chloride solution (8.1.2) and nalidixic acid solution (8.1.3) may be added to the base (6.1 .l) be- . fore autoclaving.

B.1.2 Lithium chloride solution

Composition

Lithium chloride	g	3
Water	ml	10

B.1.3 Solution of sodium salt of nalidixic acid

Composition

Sodium salt of nalidixic acid	g	0,1
Sodium hydroxide, 0,05 rnol/l solution	ml	10

B.1.4 Acriflavine hydrochloride solution

Composition

Acriflavine hydrochloride	g	0,25
Water	ml	100

B.1.5 Ammonium iron(III) citrate solution

Composition

Ammonium iron (III) citrate	g	5
Water	ml	100

B.1.6 Complete medium

Composition

Base (B.1.1)	ml	100
Lithium chloride solution (8.1.2)	ml	1,0
Sodium salt of nalidixic acid (B.1.3)	ml	0,1
Acriflavine hydrochloride (B.1.4)	ml	0,5
Ammonium iron(III) citrate (B.1.5)	ml	1,0

UNI EN ISO 11290-1: 1996
Scheda 25 : Listeria Oxford agar base + selective supplement

B.3 First selective plating-out medium: Oxford agar

B.3.1 Agar base

Composition

Columbia agar (*)	g	39
Aesculin	g	1
Ammonium iron(III) citrate	g	0,5
Lithium chloride	g	15
Water	ml	1000

(*)	Proteose peptone	g	23
	Starch	g	1
	Sodium chloride	g	5
	Agar (depending on the gel strength of the agar)	g	9-18

Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.
Adjust the pH, if necessary, so that after sterilization it is 7,0 + 0,2 at 25 °C.
Sterilize for 15 min in the autoclave set at 121 °C.

B.3.2 Supplement for 1000 ml medium

Composition

Cycloheximide	mg	400
Colistin sulfate	mg	20
Acriflavine hydrochloride	mg	5
Cefotetan	mg	2
Fosfomycin	mg	10
Ethanol	ml	5
Water	ml	5

Preparation

Dissolve the components or the dehydrated complete medium in the ethanol/water mixture.
Sterilize by filtration.

B.3.3 Preparation of complete medium

Cool the base (8.3.1) to about 47 °C and aseptically add the supplement (8.3.2).
Dispense the medium into sterile Petri dishes in quantities of about 15 ml and allow to solidify.
Store the medium away from light.

UNI 7937: 2004
Scheda 26 : TSC agar con cicloserina (Sulfite cicloserina agar)

5.2 Sulfite cicloserina agar (SC)

5.2.1 Base

Composition

Enzymatic digest of protein	g	15
Enzymatic digest of soya	g	5
Yeast extract	g	5
Disodium disulfite ($Na_2S_20_3$), anhydrous	g	1
Ammonium iron(III) citrate (*)	g	1
Agar	g	9-18 (**)
Water	ml	1000

(*) This reagent should contain at least 15 % (mass fraction) of iron. I
(**) Depending on the gei strength of the agar.

Preparation

Dissolve the components in the water by boiling. Adjust the pH so that after sterilization it will be 7,6±0,2 at 25°C. Dispense the base into flasks or bottles of appropriate capacity. Sterilize for 15 min at 121°C. Store in a refrigerator at 5°C± 3°C. Discard unused medium 2 weeks after preparation.
In some cases (see 9.4.3.1), it may be necessary to prepare dishes of SC agar base medium for confìmation with the nitrate motility medium (5.5) and the lactose--gelatin medium (5.8). For this purpose, transfer portions of about 15 ml of the base [melted and cooled to approximately 44°C to 47°C using a water bath (6.10)] into Petri dishes and allow to solidify. Immediately before use, dry the plates (see ISO 7218).

5.2.2 D-Cycloserine solution

Composition

D-Cycloserine (*)	g	4
Water	ml	100

(*) Use white crystalline powder only.

Preparation

Dissolve the O-cycloserine in the water and sterilize the solution by filtration. Store in a refrigerator at 3°C±2°C. Discard unused solution 4 weeks after preparation.

5.2.3 Complete medium

Immediately before use in the pour-plate method (see 9.2), to each 100 ml of sterile molten base (5.2.1) cooled to 44°C to 47°C, add 1 ml of D-cycloserine solution (5.2.2).

UNI 7937: 2004
Scheda 27 : Thioglycollate medium (USP)

5.3 Fluid thioglycollate medium

Composition

Enzymatic digest of casein	15,0 g
L-Cystine	0,5 g
D-Glucose	5,5 g
Yeast exiract	5,0 g
Sodium chloride	2,5 g
Sodium thioglycollate (mercaptoacetate)	0,5 g
Agar	0,5 g to 2,0 g (*)
Resazurin	0,001 g
Water	1000 ml

(*) Depending on the gei strength of the agar.

Preparation

Dissolve the components in the water by boiling. Adjust the pH so that after sterilization it will be 7,1±0,2 at 25°C.
Dispense 10 ml portions into tubes and sterilize at 121°C for 15 min.
Before use, this medium shall be de-aerated.

Performance testing for the quality assurance of thioglycollate broth

For the definition of selectivity and productivity, refer to ISO/TS 11133-1. To check the performance, refer to ISO/TS 11133-2:2003, Table B.4

UNI 7937: 2004
Scheda 28 : Nitrate motility medium

5.5 Nitrate motility medium (optional)

Composition

Enzymatic digest of casein	5,0 g
Meat extract	3,0 g
Galactose	5,0 g
Glycerol	5,0 g
Potassiurn nitrate ($KN0_3$	1,0 g
Disodium hydrogen orthophosphate (Na,HPO,)	2,5 g
Agar	1 to 5 g (*)
Water	1000 ml

(*) Depending on the gel strength of the agar.

Preparation

Dissolve the components in the water by boiling. Adjust the pH so that it will be 7,3 $\pm$ 0,2 at 25 °C after sterilization.

Transfer the rnedium to culture tubes in 10 ml quantities and sterilize at 121 °C for 15 min. If not used the -same day, store in a refrigerator at 5 °C $\pm$ 3 °C. Just prior to use, heat in boiling water or steam for 15 min and then d rapidly to the incubation temperature.

Discard unused medium 4 weeks after preparation.

UNI 7937: 2004
Scheda 29 : Lactose Gelatina medium

5.8 Lactose-gelatin mediurn

Composition

Enzymatic digest of casein	15,0	g
Yeast extract	10,0	g
Lactose	10,0	g
Gelatin	120,0	g
Phenol red	0,05	g
Water	1000	ml

Preparation

Dissolve the components, except the lactose and phenol red in the water. Adjust the pH so that after sterilization it will be 7,5 $\pm$ 0.2 at 25 °C.
Add the lactose and phenol red, dispense 10 ml portions into test tubes and sterilize at 121 °C for 15 min. if not used the same day, store in a refrigerator at 5 °C $\pm$ 3 °C.
Just prior to use, heat in boiling water or flowing steam for 15 min, then cool rapidly to the incubation temperature
Discard unused medium 3 weeks after preparation,

UNI 11731: 1998
Scheda 30 : Legionella BCYE agar + BCYE agar senza L-cysteine

6.2.1 Buffered Charcoal Yeast Extract agar medium (BCYE)

Composition

Yeast extract (bacteriological grade)	10,0	g
Agar	12,0	g
Activated charcoal	2,0	g
Alpha-ketoglutarate, monopotassium salt	1,0	g
ACES buffer (N-2-acetamido-2-aminoethanesulfanie acid)	10,0	g
Potassium hydroxide (KOH) (pellets)	2,8	g
L-cysteine hydrochloride monohydrate	0,4	g
Iron (III) pyrophosphate [$Fe_4(P_2O_7)_3$]	0,25	g
Distilled water	to 1000	ml

Preparation

a) Cysteine and iron solutions.
Prepare fresh solutions of L-cysteine hydrachloride and iron(III) pyrophosphate by adding 0.4 g and 0.25 g
respectively to 10 ml volumes of distilled water. Decontaminate each solution by filtration through a membrane filter with an average pore size of 0,22 pm. Store in clean sterile containers at -(20 $\pm$ 3)°C for not more than 3 months.
b) ACES buffer
Add the ACES granules to 500 ml of distilled water and dissolve by standing in a water bath at (45 to 60) °C. To a separate 480 ml of distilled water, add all the potassium hydroxide pellets and dissolve with gentle shaking. To
prepare the ACES buffer, mix the two solutions.
NOTE - ACES buffer can cause denaturation of the yeast extract if the following sequence is not followed.
c) Final medium.
Add sequentially to the 980 ml of ACES buffer, the charcoal, yeast extract and a-ketoglutarate.
Prepare a 0,1 mol/l solution of potassium hydroxide (KOH) by dissolving 5,6 g in 1 litre of distilled water.
Prepare a 0,1 mol/l solution of sulfuric acid (H_2S0_4) by carefully adding 5,3 ml of H_2S0_4 to 1 litre of distilled water. Use the solutions of 0,1 mol/l potassium hydroxide or 0,1 mol/l sulfuric acid as appropriate to adjust the pH to 6,9 $\pm$ 0,2. Add the agar, mix and autoclave at (121 $\pm$ 1) °C far (15 $\pm$ 1) min (see 6.2.4. first paragraph). After autoclaving, allow to cool to (50 $\pm$ 2) °C in a water bath.
Add the L-cysteine and the iron (III) pyrophosphate solutions aseptically, mixing well between additions.
Dispense in 20 ml volumes into Petri dishes of 90 mm to 100 mm diameter. The pH of the final medium is 6.9 $\pm$ 4
at 25 °C. Allow excess moisture on the plates to dry and store at (4 $\pm$ 2)°C in airtight containers in the dark for up to 4 week.

Buffered Charcoal Yeast Extract agar medium (BCYE) senza L-cysteine

Prepared this medium in the identical manner to BCYE but omit the L–cysteina.

6.2.3 Selective medium:
Buffered Charcoal Yeast Extract medium with selective supplements (GVPC medium)
NOTE - This medium is identical to BCYE except that three antibiotic supplements and glycine are added to the BCYE medium

6.2.3.1 Selective supplements
The final concentrations in the GVPC medium shall be:

Ammonium-free glycine	3 g/l
Polymyxin B sulfate	80 000 iu/l
Vancomycin hydrochloride	0,001 g/l
Cycloheximide	0,08 g/l

6.2.3.2 Preparation of antibiotic supplements
Add the appropriate amount (usually 200 mg) of polymyxin B sulfate to 100 ml of distilled water to achieve a
concentration of 14545 iu/ml. Mix and decontaminate by membrane filtration as described in 6,2.1.2. Dispense
....ml volumes into sterile containers and store a1 -(20 ± 3) °C. For use, thaw at room temperature.
Add 20 mg of vancomycin hydrochloride to 20 ml of distilled water, rnix and decontaminate by membrane filtration. Dispense in 1 ml volumes in sterile containers and store at -(20 ± 3) °C. For use, thaw at room temperature.
Add 2 g of cycloheximide to 100 ml of distilled water and decontarninate by membrane filtration as described in
6.2.1.2. Dispense in 4 ml volumes in sterile containers and store at -(20 ± 3) 'C. For use, thaw at room temperature.
NOTE - Antibiotic supplements may be stored for up to 6 months when frozen.
WARMING - Cycloheximide is hepatatoxic. Wear gloves and dust rnask when handling this powder form.

6.2.3.3 Preparation of GVPC medium
Follow the instructions for preparation of BCYE medium given in 6.2.1.2, but add 3 g of ammonium-free glycine after the addition of the a-ketoglutarate and then adjust the pH to 6,9 ± 0,4.
After the addition of the L-cysteine and iron, add one volume of each of the above three antibiotic supplements
(6.2.3.2) to the final medium. Mix well.

6.2.4 Quality control of media
Prolonged heating during sterilization or heating at too high a temperature shell be avoided, as it can affect the
nutritional qualities of BCYE medium. Batch-to-batch variation of the ingredients of the medium (particular)
a-ketoglutarate) can also affect its performance. Therefore it is essential to check the quality of each new
prepared batch of media for its ability to support the growth *of L. pnsumophila* serogroup 1within three days
incubation..

For most bacteria, it is usual to assess the suitability of culture media to support their growth by using cultures
previously isolated organisms, rnaintained in the laboratory. For Legionellas this method rnay be misleading, as they can easily adapt to grow on culture media that would not support the primary isolation of 'wild' strains. The following procedure is therefore recommended for assessing the suitability of GVPC selective agar medium for Legionellas organisms.
Either
a) use plates of a previous batch of GVPC rnedium known to support the growth af Legionella together with plates
from the new batch of medium and inoculate them with a water sample known to contain Legionella organism
b) from a nationally recognized source of reference cultures, obtain a lyophilized strain of Leg serogroup 1. Reconstitute and recover as recommended, and subculture onto BYCE (6.2.
culture is not available, use a freshly isolated and confirmed strain of L. pneumophila serogrou
L. pneumophila shall be replaced aiter not more than 10 subcultures. After incubation, mak
the resulting growth just visible to the naked eye and dispense in 1 ml volumes in sterile glycerol b
storage at -(20 2 3)T, or alternatively in Page's Saline (6.3.2.1) or distilled water for storage
Plate out one suspension of each isolate onto BCYE rnedium for subsequent identification and
Legionella species and serogroup (see 9.3). For use, allow a stock suspencion of one (or more) i
room temperature. Shake thoroughly, wait 5 min to 10 min to allow aerosols to settle, and inoc
volume (e.g. 0,1 ml) onto each of two plates of GVPC medium from the batch to be tested.
Mer incubation, record and compare the results to ensure that the colonial rnorphology (9.2.6) and n
ire similar.

Scheda 32: Reinforced Clostridial medium

Reinforced Clostridial medium (RCM)

estratto di lievito	g/l	3
Lab-Lemco (estratto di carne)	g/l	10
Peptone	g/l	10
Destrosio	g/l	5
Amido (solubile)	g/l	1
Sodio cloruro	g/l	5
Sodio acetato	g/l	3
Cisteina cloridrato	g/l	0,5
agar	g	0,5

pH finale 6,8 $\pm$ 0,2

ISO 6579:2008
Scheda 33: Agar verde brillante modificato

Brilliant Green Agar (modified)

Lab-Lemco (estratto di carne)	g/l	5
Peptone batteriologico	g/l	10
estratto di lievito	g/l	3
Sodio fosfato monoacido	g/l	1
Sodio fosfato biacido	g/l	0,6
Lattosio	g/l	10
Saccarosio	g/l	10
Rosso fenolo	g/l	0,09
Verde brillante	g/l	0,0047
Lemco ammonio citrato	g/l	0,02
Agar n.1	g	12

pH finale 6,9 $\pm$ 0,2

Scheda 34: Maximum recovery diluent

Maximum recovery diluent

Peptone	g/l	1
Sodio cloruro	g/l	8,5

pH finale 7 ± 0,2

preparazione
sospendere 9,5 g di polvere in 1 litro di acqua distillata.
Distribuire nei contenitori finali e sterilizzare in autoclave a 121°C per 15 min.

ISO 16654:2003
Scheda 35: Cromogeno per isolamento E.coli 0157

Cromogenic E.coli 0157 agar

Peptone	g/l	17
Sali biliari n.3	g/l	1,5
Agar	g/l	12
Miscela di cromogeni	g/l	0,5

pH finale 7,2 ± 0,2

preparazione
Sospendere 31 g in 1000 ml di acqua distillata fredda. Portare ad ebollizione sotto agitazione, autcllavare a 121°C per 15 minuti. Raffreddare a 50°C e trasferire in piastre di Petri sterili. Per campioni alimentari fortemente contaminati impiegare il terreno con l'aggiunta del supplemento selettivo con cetixime e potassio tellurito (cat. n" 421SEC).

Unichim 1039:2002
Scheda 36 : Aeromonas agar

Terreno selettivo per Aeromonas

xilosio	g	3,75
lattosio	g	1,5
inositolo	g	2,5
sorbitolo	g	3
l-lisina monocloroidato	g	3,5
estratto di lievito	g	3
peptone proteosio	g	5
tiosolfato di sodio	g	10,67
sali biliari	g	3
citrato di ferro ammonico (III)	g	0,8
cloruro di sodio	g	5
blu bromotimolo	mg	40
blu timolo	mg	40
l-arginina monocloroidrato	g	2
agar	g	12,5
acqua	l	1

pH finale 8,0 $\pm$ 0,2

preparazione
Il pH della soluzione deve essere 8,0 $\pm$ 0,2 a 25°C, correggere eventualmente mediante aggiunta di idrato di sodio 0,1 M o acido cloridrico 0,1 M.
Nota: La presenza dl antibiotico migliora la selettività del mezzo, favorendo lo sviluppo delle specie microbiche ricercate in assenza di eventuali crescite concomitanti.

Scheda 37 : Modified lauryl sulfate tryptose broth vancomycin medium (mLST)

Modified lauryl sulfate tryptose broth (mLST)

composizione

Sodium chloride (NaCl)	g	34
Enzymatic digest of animal and plant tissue	g	20
Lactose ($C_{12}H_{22}0_{11}$)	g	5
Potassium dihydrogen phosphate (KH_2PO_4)	g	2,75
Dipotassium hydrogen phosphate (K_2HPO_4)	g	2,75
Sodiurn lauryl sulfate ($C_{12}H_{25}NaO_5S$)	g	0,1
Water	ml	1000

pH 6,8 $\pm$ 0,2

preparazione

Dissolve each of the components in the water. by heating if necessary.
Adjust the pH, if necessary, to 6,8 $\pm$ 0,2 at 25°C. Dispense 10 ml of mLST into tubes of dimension 18 mm x 160 mm.
Sterilize the tubes at 121°C for 15 min.

Vancomycin solution

composizione

Vancomycin	mg	10
Water	ml	10

preparazione

Dissolve the vancomycin in the distilled water. Mix and sterilize by filtration
The vancomycin solution rnay be kept at 0 °C to 5 °C for 15 days.

mLST/vancornycin medium

Add 0,1 ml of vancornycin solution to 10 ml of mLST solution so as to obtain a fi....
vancomycin concentration of 10 mg per millilitre of mLST.
The complete mLST/vancomycin medium may be kept at 0 °C to 5 °C for 1 day.

ISO/TS 22964:2006

Scheda 38 : Enterobacter Sakazakii isolation agar (ESIA)

Enterobacter Sakazakii isolation agar (ESIA)

composizione

Pancreatic peptone of casein	g	7
Yeast extract	g	3
Sodium choride (NaCl)	g	5
Sodium desoxycholate	g	0,6
5-Bromo-4-chloro-3-indolyl cx-D-glucopyranoside ($C_{14}H_{15}BrClNO_6$)	g	0,15
Crystal violet	mg	2
Agar	g	12-18 (*)
Water	ml	1000

pH 7 $\pm$ 0,2

(*) Depending on the gel strength of the agar.

Preparazione

Dissolve each of the components in the water by boiling. Adjust the pH, if necessary, to 7,0 $\pm$ 0,2 at 25 °C.

Sterilize at 121 °C for 15 min.

Cool to between 44 °C and 47 °C. Pour about 15 ml of E S I A ™ medium into sterile empty Petri dishes and

allow to solidify on a cool even surface.

The medium may be kept at 0 °C to 5 °C for up to 14 days.

Scheda 39 : Lactose broth

5.1 – Brodo lattosato (Lactose broth)

Estratto di carne	g	3
Peptone	g	5
Lattosio	g	5
acqua distillata	ml	1000

pH 6,8 – 7,0

Il terreno si conserva a temperatura ambiente (evitare l'uso del frigorifero) per circa 1 settimana.

Scheda 40 : Brodo bile verde brillante 2%

5.2 – Brodo lattosio bile verde brillante (Brillant green lactose bile broth)

Peptone	g	10
Lattosio	g	10
Bile disidratata in polvere	g	20
Verde Brillante	g	0,0133
acqua distillata	ml	1000

pH 7,0 – 7,4

Conservare a 4°C – 10°C per non più di 2 settimane.

5.7 – Brodo azide etil violetto (Ethil violet azide broth)

Peptone (Tryptone, Trypticase o altro equivalente)	g	20
Glucosio	g	5
Sodio cloruro	g	5
Fosfato bipotassico	g	2,7
Fostato monopotassico	g	2,7
Sodio azide (azoturo di sodio)	g	0,4
Violetto di etile	g	0,00083
Acqua distillata	ml	1000
pH (dopo sterilizzazione)	7,0	

Conservare a 4°C - 10°C per non più di 2 settimane.

D.M. 13.01.1993 (G.U. n.14 del 19.01.93)
Scheda 42 : KF Streptococcus agar

5.8 – KF Streptococcus agar

Peptone (Proteose peptone Polypeptone o altro equivalente)	g	10
Estratto di lievito	g	10
Sodio cloruro	g	5
Sodio glicerofosfato	g	10
Maltosio	g	20
Lattosio	g	1
Sodio azide (azoturo di sodio)	g	0,4
Bromocresolporpora	g	0,015
Agar	g	20
Acqua distillata	ml	1000
pH finale (non sterilizzare in autoclave)		7,1 - 7,3

5.6 – Brodo azide destrosio (Azide destrose broth)

Estratto di carne	g	4,5
Peptone (Tryptone, Trypticase o altro equivalente)	g	15
Glucosio	g	7,5
Sodio cloruro	g	7,5
Sodio azide (azoturo di sodio)	g	0,2
Acqua distillata	ml	1000
pH (dopo sterilizzazione)		± 7,2

Conservare a 4°C - 10°C per non più di 2 settimane.

5.5 – Hektoen enteric agar

Peptone	g	12
Estratto di lievito	g	3
Lattosio	g	12
Saccarosio	g	12
Salicina	g	2
Sali biliari n. 3	g	9
Sodio cloruro	g	5
Sodio tiosolfato	g	5
Ferro ammonio citrato (ico)	g	1,5
Fucsina acida	g	0,1
Blu di Bromotimolo	g	0,065
Agar	g	14
Acqua distillata	ml	1000

Preparazione:
Sospendere 75 g di polvere in 1000 ml di acqua distillata. Non autoclavare.
Preparare al momento dell'uso.

Scheda 45 : Selenite cistine broth

5.2 – Brodo Selenite cistina (Selenite cistine broth)

Peptone	g 5
Lattosio	g 4
Sodio fosfato bibasico dodecaidrato	g 10
Sodio selenio acido	g 4
L-cistina	g 0,01
Acqua distillata	ml 1000

Preparazione:
Sciogliere 23 g di terreno in polvere in 1000 ml di acqua distillata, riscaldare a meno di 95°C. Non autoclavare. Preparare al momento dell'uso.

UNI ISO 9308-1:2002
Scheda 46 : Lactose TTC Tergitolo 7 Agar

B.1 Lactose TTC agar with sodium heptadecylsulfate

B.1.1 Basal medium

Composition

Lactose	20 g
Peptone	10 g
Yeast extract	6 g
Meat extract	5 g
Bromothymol blue	0,05 g
Agar (in powder or flake form)	15 g to 25 g(*)
Distilled water	1000 ml

(*) Depending on the gelling power of the agar.

Dissolve the ingredients in water by heating. If necessary, adjust the pH so that after sterilization it has a value
corresponding to 7,2 ± 0,1 at 25 °C. Dispense the medium into bottles, in volumes of maximum 250 ml, and
sterilize in the autoclave at (121± 3) °C for 15 min

B.1.2 TTC solution

2,3,5-Triphenyltetrazolium chloride (TTC)	0,05 g
Distilled water	100 ml

Dissolve the TTC in some of the water and make up to 100 mi. Sterilize by filtration through a membrane of
0,2 mm nominal pore size.

B.1.3 Sodiurn heptadecylsulfate solution

Sodium heptadecylsulfate (Tergitol (**) 7)	0,2 g
Distilled water	100 ml

Dissolve the sodium heptadecylsulfate in some of the water and make up to 100 ml. Sterilize in the autoclave at
(121± 3) °C for 15 min.

(**) Tergitol is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 9308 and does not constitute an endorsement by ISO of this product.

B.1.4 Complete medium

Basal medium (B.1.1)	100 ml
TTC solution (B.1.2)	5 ml
Sodiurn heptadecylsulfate solution (B.1.3)	5 ml

Melt the basal medium and cool to (50 ± 5) °C. Add the TTC and sodium heptadecylsulfate solutions aseptically,
mix thoroughly but avoid the formation of bubbles after each addition. Dispense in Petri dishes to a depth of at least 5 mm. If not for immediate use, store at (5 ± 3) °C in the dark for not longer than 10 d.

Scheda 47 : TCBS agar

Agar Tiosolfato Citrato Bile Saccarosio (TCBS)

Estratto di lievito	5	g/l
Peptone	10	g/l
Sodio tiosolfato	10	g/l
Sodio citrato	10	g/l
Bile di bue	8	g/l
Saccarosio	20	g/l
Cloruro di sodio	10	g/l
Citrato ferrico	1	g/l
Blu di bromotimolo	0,04	g/l
Blu timolo	0,04	g/l
Agar	16	g/l

preparazione
Sospendere i componenti in 1000 ml di acqua distillata fredda. Portare ad ebollizione sotto agitazione, raffreddare a circa 50°C e trasferire in piastre sterili da 90/100mm.
pH finale 8.4 a 25°C

ISO 16654:2003
Scheda 48: Triptosio soia novobiocina brodo + supplementi

5.1 Terreno di arricchimento: brodo triptosio soia modificato arricchito con novobiocina (mTSB t N)

5.1.1 Brodo triptosio soia modificato (mTSB)

Digerito enzimatico di caseina	17,0 g
Digerito enzimatico di soia	3,0 g
D(+)-glucosio	2,5 g
Sali biliari n. 3	1,5 g
Cloruro di sodio	5,0 g
Fosfato idrogeno di potassio (K_2HPO_4)	4,0 g
Acqua	1000 ml

preparazione
Sciogliere i componenti o il terreno completo disidratato nell'acqua, riscaldando se necessario.
Regolare il pH utilizzando il pH-metro (6.6), se necessario, in modo che dopo la sterilizzazione sia 7,4 $\pm$ 0,2 a 25 °C.
Distribuire il terreno in quantità appropriate in palloni o bottiglie (6.7).
Sterilizzare per 15 min. in autoclave (6.1) impostata a 121 °C

5.1.2 Soluzione di novobiocina

Novobiocina	0,45 g
Acqua	100 ml

Preparazione
Sciogliere la novobiocina nell'acqua e sterilizzare mediante filtrazione a membrana.
Preparare il giorno dell'utilizzo.

Preparazione del terreno completo
Immediatamente prima dell'utilizzo, aggiungere 1 ml o 4 ml di soluzione di novobiocina (5.1.2) a 225 ml o 900 ml di mTSB raffreddata (5.1.1).
La concentrazione finale di novobiocina è 20 mg per litro di mTSB.

ISO 16654:2003
Scheda 49: Mac-Conkey sorbitolo cefixime tellurito agar

5.2 Primo terreno di isolamento selettivo: agar al sorbitolo di MacConkey con cefixime e tellurito (CT-SMAC)

5.2.1 Terreno di base

Digerito enzimatico di caseina	17,0	g
Digerito enzimatico di tessuti animali	3,0	g
Sorbitolo	10,0	g
Sali biliari n. 3	1,5	g
Cloruro di sodio	5,0	g
Rosso neutro	0,03	g
Violetto di metile	0,001	g
Agar	9-18	g (*)
Acqua	1000	ml

(*) A seconda del potere gelificante dell'agar.

preparazione
Sciogliere i componenti base o la base disidratata completa nell'acqua per ebollizione.
Regolare il pH (6.6), se necessario, in modo che dopo la sterilizzazione sia 7,1 $\pm$ 0,2 a 25 °C.
Sterilizzare per 15 min in autoclave (6.1) impostata a 121 °C.

5.2.2 Soluzione di tellurito di potassio

Tellurito di potassio per uso batteriologico	0,25 g
Acqua	100 ml

Preparazione
Sciogliere il potassio nell'acqua e sterilizzare mediante filtrazione a membrana. Tale soluzione può essere conservata a temperatura ambiente fino a un 1 mese, ma scartarla se si forma un precipitato bianco.

5.2.3 Soluzione di cefixime

Cefixime	5,0 mg
Acqua	100 ml

Preparazione del terreno completo
Sciogliere la cefixime nell'acqua e sterilizzare mediante filtrazione a membrana.
Può essere necessario sciogliere la cefixime in etanolo.
Tale soluzione può essere conservata a (3 $\pm$ 2) °C per 1 settimana.

5.2.4 Terreno completo

Terreno di base (5.2.1)	1000 ml
Soluzione di tellurito di potassio (5.2.2)	1,0 ml
Soluzione di cefixime (5.2.3)	1,0 ml

ISO 6888-2:2004
Scheda 50 : Baird Parker RPF agar + suppl. RPF

Baird Parker agar (RPF) base

Composizione

Digerito pancreatico di caseina	10 g
Estratto di carne	5 g
Estratto di lievito	1 g
Glicina	12g
Piruvato di sodio	10 g
Cloruro di litio	5 g
Agar	20 g
Acqua distillata	1000 ml

pH 7.2 ± 0.2 @ 25ºC

Supplemento RPF per litro	
Bovine fibrinogen	3,75 g
Rabbit plasma	25,0 ml
Trypsin inhibitor	25,0 mg
Potassium tellurite	25,0 mg

Terreno completo per litro

Terreno base + Supplemento RPF

ISO 4833 :2004
Scheda 51: Plate count agar

Conta in piastra con agar (PCA)

Composizione

Digerito enzimatico di caseina	5,0 g
Estratto di lievito	2,5 g
Glucosio anidro ($C_6H_{12}O_6$)	1,0 g
Agar	9-18 g (*)
Acqua	1000 ml

pH 7,0 $\pm$ 0,2 a 25 °C.

(*) A seconda del potere gelificante dell'agar.

ISO 4832 :2006
Scheda 52 : Violet red bile lactose agar

Crystal violet neutral red bile lactose (VRBL) agar

Enzymatic digest of animal tissues	7 g
Yeast extract	3 g
Lactose ($C_{12}H_{22}O_{11}H_2O$)	10 g
Sodium chloride	5 g
Bile salts	1.5 g
Neutral red	0.03 g
Crystal violet	0,002 g
Agar	12-18 g (*)
Acqua	1000 ml

(*) A seconda del potere gelificante dell'agar.

Preparation

Proceed as follows in order to conserve the selectivity power and specificity of the medium.
Thoroughly mix the components or the dehydrated complete medium in the water and leave to stand for severa1
minutes. Adjust the pH so that. alter boiling. it is 7,4 $\pm$ 0,2 at 25 °C. Heat until boiling. stirring from time to time.
Allow to boil for 2 min. Immediately cool the medium in the water bath (6.5) at 44 °C to 47 °C
To avoid overheating, do not heat the medium far too long nor reheat it. Consequently, do not sterilize it in the
autoclave. and check the sterility of the medium at the time of use (see 9.2.2).
Use the medium within 4 h of its preparation.

Performance testing for the quality assurance of the culture medium
For the definitions of selectivity and productivity, refer to ISO/TS 11 133-1. Performance testing relating to crystal
violet neutra1 red bile lactose (VRBL) agar is given in ISO/TS 11 133-2:2003. Table B.1

ISO 15214 :1998

Scheda 53 : MRS medium (de Man, Rogosa and Sharpe) at pH 5,7

MRS medium (de Man, Rogosa and Sharpe) at pH 5,7

Enzymatic digest of casein	10.0 g
Meat extract	10.0 g
Yeast extract	4,0 g
Triammonium citrate ($(NH_4)_3C_6H_5O_7$)	2,0 g
Sodium acetate (CH_3COONa)	5,0 g
Magnesium sulfate heptahydrate ($MgS0_4.7H_20$)	0,2 g
Manganese sulfate tetrahydrate ($MnS0_4.4H_20$)	0,05 g
Dipotassium hydrogen phosphate (K_2HPO_4)	2,0 g
Glucose ($C_6H_{12}O_6$)	20,0 g
Polyoxyethylene sorbitan monooleate (Tween 80)	1,08 g
Agar	12 g to18g (*)
Water	1000 ml

(*) Depending on the gel strength o1 the agar.

ISO 6222: 2001
Scheda 54 : Agar yeast extract

Yeast extract agar

Ttyptone (Peptone from Casein, pancr.)	6,0 g
Dehydrated yeast extract	3.0 g
Agar powdered or in pellets	10 g to 20 g (according to gel strength)
Water	1000 ml

Add the ingredients. or the complete dehydrated medium. to the water and dissolve by heating. Adjust the pH if
necessary so that after sterilization it will be 7,2 ± 0.2 at 25 °C.
Distribute volumes o1 15 ml to 20 ml in tubes, bottles or other containers. For storage in larger volumes, use
containers up to 500 ml capacity. Sterilise in the autoclave (5.1) at (121± 3)°C for (15 ± 1) minutes.
For use, melt Vie medium, allow to cool and maintain it at (45 ±1) °C using the water bath (5.5). It is recommended to store the medium no1 longer than 4 h at 45 °C, after which lime the medium shall be discarded.

ISO 7899:2003
Scheda 55 : Slanetz Bartley agar

Slanetz and Bartley medium

Basal medium

Tryptose	20,0 g
Yeast extract	5,0 g
Glucose	2,0 g
Dipotassium hydrogenphosphate (K_2HP0_4)	4,0 g
Sodium azide (NaN_3)	0,4 g
Agar	8 g to18 g (*)
Water	1000 ml

Dissolve the ingredients in boiling water.
Once dissolution is complete, heat for an additional 5 min.
Cool to 50 "C to 60 "C.

TTC solution

2,3,5-triphenyitetrazolium chloride	1,0 g
Water	100 ml

Dissolve the indicator in the water by stirring.
Sterilize by filtration (0,2 mm).
Protect the solution against the action o1 light, and discard it if a pink tinge develops.

Complete medium
Basal medium (6.3.1.1) 1 000 ml
lTC solution (6.3.1.2) 10 ml

Add the TTC solution to the basa1 medium cooled to 50 °C to 60 °C.
Adjust the pH if necessary so that after sterilization it is 7,2 $\pm$ 0,1 at 25 °C, with a solution of sodium carbonate
(100 g/l) or of sodium hydroxide (40 g/l) or of hydrochloric acid (36,5 g/l).
Pour 20 ml of medium into Petri dishes of 9 cm diameter (or an equivalent amount in a dish of another size) and
allow lo set on a cool. horizontal surface.
Poured plates can be stored in the dark for up to 2 weeks at 5 °C $\pm$ 3 °C.

ISO 21528-1:2004
Scheda 56 : Violet red bile glucose agar

Violet red bile glucose (VRBG) agar

Basal medium

Enzymatic digest of animal tissues	7,0 g
Yeast extract	3,0 g
Bile salts n. 3	1,5 g
Glucose	10,0 g
Sodium chloride	5,0 g
Neutral red	0,03 g
Crystal violet	0,002 g
Agar	9 g to 18 g (*)
Water	1000 ml

(*) Depending on the gel strength of the agar

Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.
Adjust the pH, l necessary. so that after boiling it is 7,4 ± 0,2 al 25 °C.
Dispense the culture medium into sterile tubes or flasks (6.5) of appropriate capacity.
Do not sterilize the medium.
Use the molten medium within 4 h of its preparation.

Preparation of agar plates

Immediately transfer approximately 15 ml of the culture medium, cooled to between 44 °C and 47 °C (6.4). to
Petri dishes (6.7) and allow lo solidify.
Just before use, dry the plates, preferably with the lids off and the agar surface downwards. in a drying cabinet
(6.3) unti1 the agar is dry.
If prepared in advance. the undried plates may be stored in conditions that do not change their composition for
up to 2 weeks at 5 °C ± 3 °C.

Performance testing for the quality assuance of the culture medium

For the definition of selectivity and productivity, refer to ISO/TS 11133-1. For the performance criteria, refer to
ISO/TS 11 133-2:2003. Table 0.1.

ISO 21528-1:2004
Scheda 57 : Glucose agar

Glucose agar

Composition

Enzymatic digest of casein	10,0 g
Yeast extract	1,5 g
Glucose	10,0 g
Sodium chloride	5,0 g
Bromocresol purple	0,015 g
Agar	9 g to 18 g (*)
Water	1000 ml

(*) Depending on the gel strength of the agar

Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.
Adjust the pH. if necessary. so that after sterilization it is 7,0 $\pm$ 0,2 at 25 °C.
Dispense the culture medium in tubes (6.6) of appropriate capacity (e.g. 10 m1 of culture medium for tubes of 16mm x 160 mm).
Sterilize for 15 min in an autoclave (6.1) set at 121 °C. Leave the tubes in a vertical position.
The medium may be stored for up to 1 week at 5 °C $\pm$ 3 °C.
In order to remove oxygen. just before use. heat the medium in boiling water or flowing steam for 15 min, then
cool rapidly to the incubation temperature.

Scheda 58 : Campylobacter enrichment broth (Preston)

Brodo Preston

Composizione

TERRENO COMPLETO
1) terreno di base (nutrient broth n.2)
2) arricchimento (sangue lisato di cavallo)
3) supplemento di crescita
4) supplemento selettivo

1) TERRENO BASE (NUTRIENT BROTH n.2)

Estratto di carne	10 g/l
Peptone	10 g/l
Sodio cloruro	5 g/l

pH finale 73 $\pm$ 0,2

2) SANGUE LISATO DI CAVALLO
Sangue di cavallo sterile lisato con saponina

3) SUPPLEMENTO DI CRESCITA

Sodio piruvato	0,25 g/l
Sodio metabisolfito	0,25 g/l
Solfato ferroso	0,25 g/l

4) SUPPLEMENTO SELETTIVO

Polimixina B	5000 U.I.
Rifampicina	10 mg
Trimethoprim lattato	10 mg
Cicloeximide	100 mg

Preparazione

1) NUTRIENT BROTH n.2
Sospendere 12,5 g di Nutrient Broth n.2 in 950 ml di acqua distillata . Riscaldare a bagnomaria bollente fino a completa soluzione . Sterilizzare a 121°C per 15 minuti.
Raffreddare al di sotto di 50°C .
3) CAMPYLOBACTER supplemento di crescita.
Ricostituire asetticamente il contenuto di un flacone seguendo le modalità indicate dal produttore.
4) CAMPYLOBACTER supplemento selettivo.
Ricostituire asetticamente il contenuto di un flacone seguendo le modalità indicate dal produttore.
TERRENO COMPLETO
Aggiungere asetticamente a 950 ml di terreno di base, sterilizzato e raffreddato, 50 ml di sangue lisato di cavallo, il Campylobacter supplemento di crescita ed il Campylobacter supplemento selettivo .
Mescolare e distribuire aliquote di 90 ml in flaconi di vetro scuro da 100 ml .
E' importante che il volume d'aria sopra il liquido sia molto ridotto per assicurare le condizioni di microaerofilia .

Scheda 59 : Campylobacter Karmali agar

Karmali agar

Composizione

Columbia Blood agar Base	39 g/l (*)
Emina	32 mg/l
Sodio piruvato	100 mg/l
Cefoperazone	32rngll
Vancomicina	20rngll
Cicloexirnide	100rngIl

(*)	Peptone speciale	23	g/l
	Carbone attivato	4	g/l
	Amido	1	g/l
	Sodio cloruro	5	g/l
	Supplemento selettivo Agar	10	g/l

Preparazione

Sospendere i componenti di Campylobacter Agar Karmali in 1000 ml di acqua distillata. Riscaldare fino a completa soluzione. Sterilizzare in autoclave A 121 "C per 15 minuti. Raffreddare a 50°C. Aggiungere asetticamente il Campylobacter supplemento selettivo ricostituito come indicato dalla ditta produttrice. Mescolare con cura e versare in piastre sterili da 90/100 mm di diametro.

Scheda 60 : Preston agar

Preston agar

Composizione

1) terreno base (campylobacter agar base)
2) arricchimento (sangue lisato di cavallo)
3) supplemento di crescita e supplemento selettivo di antibiotici

1) campylobacter agar base

estratto di carne	10 g/l
peptone	10 g/l
sodio cloruro	5 g/l
agar	12 g/l

ph finale 7,5 $\pm$ 0,2

2) sangue di cavallo sterile lisato con saponina

3) campylobacter supplemento di crescita

sodio piruvato	0,25 g/l
sodio metabisolfito	0,25 g/l
solfato ferroso	0,25 g/l

4) campylobacter supplemento selettivo

polimixina b	5000 u.i./l
riiampicina	10 mg/l
trimethoprim lattato	10 mg/l
cicloeximide	100 mg/l

Preparazione

1) campylobacter agar base :
sospendere i componenti in 950 ml di acqua distillata . Riscaldare a bagnomaria bollente fino a completa soluzione . Sterilizzare a 121°C per 15 minuti. Raffreddare al di sotto di 50 °C .
3) campylobacter supplemento di crescita ricostituire asetticamente come indicato dal produttore.
4) campylobacter supplemento selettivo crescita ricostituire asetticamente come indicato dal produttore.

TERRENO COMPLETO
Aggiungere asetticamente a 950 ml di terreno di base, sterilizzato e raffreddato a 50°C , 50 ml di sangue lisato di cavallo, il campylobacter supplemento di crescita ed il campylobacter supplemento selettivo. Mescolare e distribuire in piastre da 90/100 mm di diametro.

ISO 10273:2003
Scheda 61 : Yersinia PSB Broth

Peptone, sorbitol and bile salts (PSB) broth

Composition

Enzymatic digest of casein	5 g
Sorbitol	10 g
Sodium chloride	5 g
Disodium hydrogen phosphate (Na_2HP0_4)	8,23 g
Sodium dihydrogen phosphate monohydrate ($NaH_2P0_4.H_20$)	1,2 g
Bile salts	1,5 g
Water	1000 ml

Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.
Adjust the pH, if necessary, so that after sterilization it is 7,6 $\pm$ 0,2 at 25 °C.
Dispense the medium into tubes or flasks of suitable capacity to obtain portions appropriate for the test samples (see 9.1.2).
Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

ISO 10273:2003
Scheda 62 : Yersinia CIN agar + supplemento

Cefsulodin, Irgasan™ and novoblocin (CIN) agar

B.3.1 Basic medium

Composition

Enzymatic digest of gelatin	17,0 g
Enzymatic digest of casein and animal tissues	3,0 g
Yeast extract	2,0 g
Mannitol	20,0 g
Sodium pyruvate	2,0 g
Sodium chloride	1,0 g
Magnesium sulfate heptahydrate ($MgS0_4.7H_20$)	0,01 g
Sodium desoxycholate	0,5 g
Neutral red	0,03 g
Crystal violet	0,001 g
Agar	9 to 18 g (*)
Water	1000 ml

Dissolve the components or the dehydrated basic medium in the water by boiling. Adjust the pH, if necessary, so that after sterilization it is 7,4 $\pm$ 0,2 at 25 °C. Dispense the medium into flasks (6.7) of suitable capacity.
Sterilize for 15 min in an autoclave (6.7) set at 121 °C.

B.3.2 Cefsulodin solution (15 mg/ml)

Cefsulodin	1,5 g
Water	100 ml

Dissolve the cefsulodin in the water. Sterilize by filtration.

B.3.3 Irgasan™ [5-chloro-2-(2,4-dichlorophenoxy)phenol], ethanolic solution (4 mg/ml)

Irgasan™	0,4 g
Ethanol, 95 % (by volume)	100 ml

Dissolve the Irgasan in the ethanol as and when required, or alternatively store the solution at about -20 °C
for not more than 4 weeks.

B.3.4 Novobiocin solution (2,5 mg/ml)

Novobiocin	0,25 g
Water	100 ml

Dissolve the novobiocin in the water. Sterilize by filtration.

B.3.5 Complete medium

Basic medium (B.3.1)	997 ml
Cefsulodin solution (B.3.2)	1 ml
IrgasanTM solution (B.3.3)	1 ml
Novobiocin solution (B.3.4)	1 ml

Add each antibiotic solution aseptically to the basic medium cooled to about 45 °C and mix.

B.3.5.3 Preparation of CIN agar plates

Pour approximately 15 ml of the complete medium into sterile Petri dishes (6.8). Leave to set.

ISO 10273:2003
Scheda 63 : Agar SS (Salmonella/Shigella)

Salmonella/Shigella agar with sodium desoxycholate and calcium chloride (SSDC)

Composition

Yeast extract	5,0 g
Meat extract	5,0 g
Enzymatic digest of animal tissues	5,0 g
Lactose	10,0 g
Bile salts	8,5 g
Sodium desoxycholate	10,0 g
Calcium chloride	1,0 g
Sodium citrate	10,0 g
Sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$)	8,5 g
Iron(III) citrate	1,0 g
Brilliant green	0,0003 g
Neutral red	0,025 g
Agar	9 g to 18 g (*)
Water	1000 ml

Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.
Adjust the pH, if necessary, so that it is 7,4 $\pm$ 0,2 at 25 °C.
Do not sterilize.

Preparation of SSDC agar plates

Pour approximately 20 ml of the medium, cooled to about 45 °C, into sterile Petri dishes (6.8). Leave to set.
If prepared in advance, the undried agar plates shall be kept in the dark for one week at 8 °C $\pm$ 2 °C in a
plastic bag. Do not refrigerate at 3 °C $\pm$ 2 °C as a precipitate forms in the medium and decreases its performance.

ISO 16266:2008
Scheda 64 : Pseudomonas CN Agar base

Pseudomonas Agar base/CN-agar

Gelatin peptone	16,0 g
Casein hydrolysate	10,0 g
Potassium sulfate (anhydrous) (K_2SO_4)	10,0 g
Magnesium chloride (anhydrous) ($MgCl_2$)	1,4 g
Glycerol	10,0 g
Agar	11 g to 18 g
Water (distilled or equivalent)	1000 ml

The amount of agar required is dependent on the gel strength. Follow the manufacture's instructions for the agar
used.

CN Supplement

Hexadecyltrimethyl ammonium bromide (cetrimide)	0,2 g
Nalidixic Acid	0,015 g

The final pH of the solidified medium should correspond to 7,1 $\pm$ 0,2 at 25°C.
Store prepared plates in the dark protected from dessication at 5 $\pm$ 3 °C and use within 1 months. Do not keep the agar molten for more than 4 h. Do not remelt the medium.

ISO 16266:2008
Scheda 65 : King's B Medium

King's B Medium

Peptone	20 g
Glycerol	10 ml
Di-potassium hydrogen phosphate (K2 - HP04)	1,5 g
Magnesium sulfate heptahydrate (MgS04 - 7H20)	1,5 g
Agar	15 g
Water (distilled or equivalent)	1000 ml

Preparation
Dissolve the ingredient in the water by heating. Cool down to (45 to 50) °C and adjust the pH corresponding to 7,2 ± 0,2 at °25C, using either hydrochloric acid or sodium hydroxide. Dispense the medium in 5 ml aliquots into culture tubes which are capped and autoclaved at 121 ± 3 °C for 15 min. Allow the tubes to cool and solidify in slants.
Store in the dark at 5 ± 3 °C and use within 3 months.

ISO 16266:2008
Scheda 66 : Acetamide Broth

Acetamide Broth

Solution A

Potassiurn di-hydragenphosphat'e (KH_2PO_4)	1 g
Magnesium sulfate arihydrous ($MgSO_4$)	0,2 g
Acetamide	2 g
Sodiurn Chloride (NaCl)	0,2 g
Water (distilled or equivalent, ammonia free)	900 ml

Dissolve the ingredients in water and then adjust the pH to correspond to 7,O l 0,5 at 25 "C with either hydrochloric acid or sodium hydroxide.

Solution B

Sodium molybdate ($Na_2MoO_4.2H_2O$)	0,2 g
Iron sulfate ($FeSO_4.7H_20$)	0,05 g
Water	100 ml

Preparation
To prepare the acetamide broth, add 1 ml of solution 6 to 900 ml of a freshly prepared solution A (5.3.2.1). Add water with constant stirring to a total volume of 1 l. Dispense this mixture in 5 ml aliquots to culture tubes which are then capped and sterilised in an autoclave at (121 $\pm$ 3) °C for 15 min. Store in the dark at (5 $\pm$ 3) °C and use within 3 rnonths.

ISO 6579:2008
Scheda 67: Rappaport Vassiliadis con soia (RVS broth)

Rappaport Vassiliadis medium con soia (RVS broth)

6.2.1 Solution A

Enzymatic digest of soya	5,0 g
Sodium chloride	8,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,4 g
Dipotassium hydrogen phosphate (K_2HPO_4)	0,2 g
Water	1000 ml

Dissolve the components in the water by heating to about 70 °C if necessary.
The solution shall be prepared on the day of preparation of the complete RVS medium.

B.2.2 Solution B

Magnesium chloride hexahydrate ($MgCl_2$ $6H_2O$)	400,0 g
Water	1000 ml

Dissolve the magnesium chloride in the water,
As this salt is very hygroscopic, it is advisable to dissolve the entire contents of $MgCl_2$ $6H_2O$ from a newly opened
container, according to the formula. For instance, 250 g of $MgCl_2$ $6H_2O$ is added to 625 ml of water. giving a
solution of total volume of 788 ml and a mass concentration of about 31,7 g per 100 ml of $MgCl_2$ $6H_2O$.
The solution may be kept in a dark glass bottle with tight stopper at room temperature for at least 2 years.

8.2.3 Solution C

Malachite green oxalate	0,4 g
Water	100 ml

Dissolve the malachite green oxalate in the water
The solution may be kept in a brown glass bottle at room temperature for at least 8 months.

8.2.4 Complete medium

Solution A (8.2.1)	1000 ml
Solution B (B.2.2)	100 ml
Solution C (8.2.3)	10 rnl

Add to 1000 ml of solution A, 100 ml of solution B and 10 ml of solution C.
Adjust the pH if necessary, so that after sterilization it is 5,2 $\pm$ 0,2 .
Before use, dispense into test tubes in 10 ml quantities.
Sterilize for 15 min. in the autoclave set at 115 °C .
Store the prepared medium at 3°C $\pm$ 2°C. Use the medium the day of its preparation.

NOTE: The final medium composition is: enzymatic digest of soya, 4.5 g/l: sodium chloride 7,2 g/l, potassium dihydrogen phosphate (KH_2PO_4 + K_2HPO_4), 1.44: g/l anhydrous magnesium chloride ($MgCl_2$). 13,4 g/l or magnesium chloride hexahydrate ($MgCl_2$ $6H_2O$), 28,5 g/l, malachite green oxalate. 0.036 g/l.

ISO 6579:2008
Scheda 68: Muller Kauffmann Tetrath. Novobiocin (MKTTn)

Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn)

8.3.1 Base medium

Meat extract	4,3 g
Enzymatic digest of casein	8,6 g
Sodium chloride (NaCl)	2,6 g
Calcium carbonate ($CaCO_3$)	38,7 g
Sodium thiosulfate pentahydrate ($Na_2S_2O_35H_20$)	47,8 g
Ox bile for bacteriological use	4,78 g
Brilliant preen	9,6 mg
Water	1000 ml

Dissolve the dehydrated basic components or the dehydrated complete medium in the water by boiling for 5 min.
Adjust the pH. if necessary, so that it is 8,2 $\pm$ 0,2 at 25 °C.
Thoroughly mix the medium.
The base medium may be stored for 4 weeks at 3 °C $\pm$ 2 °C.

B.3.2 lodine-iodide solution

lodine	20,0 g
Potassium iodide (KI)	25,0 g
Water	100 ml

Completely dissolve the potassium iodide in 10 ml of water, then add the iodine and dilute to 100 ml with sterile
water. Do not heat.
Store the prepared solution in the dark at ambient temperature in a tightly closed container.

B.3.3 Novobiocin solution

Novobiocin sodium salt	0,04 g
Water	5 ml

Dissolve the novobiocin sodium salt in the water and sterilize by filtration.
Store far up to 4 weeks at 3 °C $\pm$ 2 °C.

B.3.4 Complete medium

Base medium (B.3.1)	1000 ml
lodine-iodide solution (B.3.2)	20 ml
Novobiocin solution (B.3.3)	5 rnl

ISO 6579:2008
Scheda 69: Agar Xilosio Lisina Desossicolato (XLD)

Xylose lysine deoxycholate agar (XLD agar)

B.4.1 Base medium

Yeast extract powder	3,0 g
Sodium chloride (NaCl)	5,0 g
Xylose	3,75 g
Lactose	7,5 g
Sucrose	7,5 g
L-Lysine hydrochloride	5,0 g
Sodium thiosulfate	6,8 g
Imn(lll) ammonium citrate	0,8 g
Phenol red	0,08 g
Sodium deoxycholate	1,0 g
Agar	9 g to18 g
Water	1000 ml

Dissolve the dehydrated base components or the dehydrated complete base in the water by heating. with frequent
Agitation, until the medium starts to boil. Avoid overheating.
Adjust the pH. if necessary, so that after sterilization it is 7,4 $\pm$ 0,2 at 25 °C.
Pour the base to tubes or flasks (6.9) of appropriate capacity.
Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat.

B.4.2 Preparation of the agar plates

Transfer immediately to a water bath (6.5) at 61 °C to 47 °C, agitate and pour into plates. Allow to solidify.
Immediately before use. dry the agar plates carefully (preferably with the lids off and the agar surface downwards)
in the oven (6.2) set between 37 °C and 55 °C until the surface of the agar is dry.
Store the poured plates for up to 5 days at 3 °C $\pm$ 2 °C.

ISO 6579:2008
Scheda 70: Triple sugar/iron agar (TSI agar)

Triple sugar/iron agar (TSI agar)

composizione	
Meat extract	3,0 g
Yeast extract	3,0 g
Peptone	20,0 g
Sodium chloride (NaCl)	5,0 g
Lactose	10,0 g
Sucrose	10,0 g
Glucose	1,0 g
Iron(III) citrate	0,3 g
Sodium thiosulfate	0,3 g
Phenol red	0,024 g
Agar	9 g to18 g
Water	1000 ml

Scheda 71: Rose Bengala agar

Rose Bengala Chloramphenicol-Agar (RBC)

Peptone micologico	5	g/l
Destrosio	10	g/l
Potassio fosfato monoacido	1	g/l
Magnesio solfato	0,5	g/l
Rose bengal	0,05	g/l
Agar	15,5	g/l

pH finale 7,2

Supplemento

Chloramphenicol	100 mg/l

istruzione per la preparazione

Sospendere gli ingredienti in 1000ml di acqua distillata; riscaldare delicatamente fino a completa dissoluzione. Aggiungere il Chloramphenicol ricostituito come indicato dal produttore. Sterilizzare in autoclave a 121 °C per 15 minuti. Lasciare raffreddare a 50°C. Distribuire in piastre sterili da 90/100 mm.

ISO 4833 :2004
Scheda 72: Plate Count Skim Milk Agar

Plate Count Skim Milk Agar

Composizione

Digerito enzimatico di caseina	5,0 g
Estratto di lievito	2,5 g
Skim milk powder (no inhibitors)	1,0 g
Glucosio anidro ($C_6H_{12}O_6$)	1,0 g
Agar	10,5 g
Acqua	1000 ml

pH: 7.0 ± 0.2 at 25°C.

The reconstituted culture medium is more or less opalescent. According to DIN it can be stored for up to 3 months in the refrigerator, the temperature should not exceed 5°C.

ISO 9308-1:2002

Scheda 73 : Triptofano brodo

Triptofano brodo

triptone	g 10
L-triptofano	g 1
sodio cloruro	g 5
acqua distillata	ml 1000

pH 7,5 $\pm$ 0,1

ISO 16266:2008
Scheda 74: Pseudomonas CFC selective supplement

Pseudomonas CFC selective supplement

Cefalotina	50 mg/l
Acido fusidico	10 mg/l
Cetrimide	10 mg/l

03 - Agar gelisato

gelisato al peptone di gelatina	g	5
agar	g	15
acqua distillata	ml	1000

pH 7

Scheda 76 : Sospensione per elutriato

Sospensione per elutriato

K_2HPO_4	g/l	3
KH_2PO_4	g/l	1
NaCl	g/l	8,5
acqua distillata	ml	1000

pH 7,2 $\pm$ 0,2

Scheda 77: Cromogeno per isolamento salmonella

Cromogeno per isolamento salmonella

Peptone mix	g/l	18
Miscela cromogenica	g/l	0,32
Fattore di crescita	g/l	10
Agar	g/l	14

pH finale 7,3 $\pm$ 0,2

D.M. 13.01.1993 (G.U. n.14 del 19.01.93)
Scheda 78 : Mossel e Martin agar

Mossel e Martin agar

Triptosio)	g/l	10
Estratto di lievito	g/l	1,50
Sodio cloruro	g/l	5
Mannitolo	g/l	10
Bromocresoloporpora	g/l	0,015
Agar	g/l	5
Acqua distillata	ml	1000

pH finale 7,4