ACETAMIDE BROTH

Dehydrated medium for the confirmation test of *Pseudomonas aeruginosa* in bottled water

TYPICAL FORMULA (g/L)

Acetamide	10.0
Sodium chloride	5.00
Dipotassium phosphate	1.39
Monopotasssium phosphate	0.73
Magnesium sulphate	0.5
Phenol red	0.012

Final pH 7.0 ± 0.2

DESCRIPTION

ACETAMIDE BROTH is a dehydrated medium used for the confirmation of *Pseudomonas aeruginosa* in bottled water.

PRINCIPLE

ACETAMIDE BROTH contains acetamide which, as a sole source of carbon in the medium, is used for the confirmation and identification of *Pseudomonas aeuruginosa.* It uses the ability of non-fermenting Gram-negative bacteria to deaminate the acetamide, the resulting alkalinization shown by a color change from orange-red to purple-red.
Acetamide deamination is accomplished by *P.aeruginosa* , *P.acidovorans,* Group III (*Achromobacter xylosoxidans*), and *Alcaligenes odorans.*
Acetamide is the single carbon source; the potassium salts have a high buffering capacity; sodium chloride maintains the osmotic balance and phenol red is the pH indicator.

PREPARATION

Suspend 17,2 g of powder in 1 litre of distilled or deionized water. If needed, heat gently to dissolve completely. Sterilize by filtration. Aseptically dispense into sterile test tubes.

TECHNIQUE

Inoculate with one or two loopfuls of growth from a presumptive fresh medium (ASPARAGINE ENRICHMENT BROTH code 610138) . Incubate at 36 +/- 1°C for 2-4 days.

INTERPRETATION OF RESULTS

A positive reaction is indicated by a color change of the tube from orange-red to an intense purple-red. The presence of *P.aeruginosa* is confirmed by a positive asparagine test and a positive acetamide test.

STORAGE

The powder is very hygroscopic: store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.
Store prepared media at 2-8°C.

WARNING and PRECAUTIONS

The product is classified as hazardous by current legislation. It is recommended that the Safety Data Sheet be consulted before use. The product must be used only by properly trained operators.

DISPOSAL of WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

- Kelly, N.M., C.T. Keans (1983). Acetamide broth for isolation of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J.Clin.Microbiol. **17**:159:163.
- CeNAN (1982) Técnicas para el Examen microbiològico de Alimentos y Bebidas. Madrid

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PRODUCT SPECIFICATIONS

NAME
ACETAMIDE BROTH

PRESENTATION
Dehydrated culture medium

STORAGE
10-30°C

PACKAGING

Code	Content	Packaging
610313	500 gr	500 gr of powder in plastic bottle
620313	100 gr	100 gr of powder in plastic bottle

pH OF THE MEDIUM
7.0 ± 0.2

USE
ACETAMIDE BROTH is a dehydrated medium used for the confirmation of *Pseudomonas aeruginosa* in bottled water.

TECHNIQUE
Refer to technical sheet of the product.

APPEARANCE of the MEDIUM
Dehydrated medium
Appearance: homogeneous.
Colour: beige
Prepared medium
Appearance: clear
Colour: orange-red

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Sterility control
   7 days at 25 ± 1°C, in aerobiosis
   7 days at 36 ± 1°C, in aerobiosis
3. Microbiological control
   Inoculum for productivity: 10-100 UFC/ml
   Inoculum for selectivity: 10^4-10^5 UFC/ml
   Inoculum for specificity: ≤ 10^4 UFC/ml
   Incubation conditions: up to 4 days at 36 +/- 1°C ,in aerobiosis

Microorganisms		Growth	Acetamide deamination
Escherichia coli	ATCC 25922	-	
Proteus mirabilis	ATCC 29906	-	
Pseudomonas aeruginosa	ATCC 9027	+	+
Pseudomonas aeruginosa	ATCC 27853	+	+

TABLE of SYMBOLS

Symbol	Meanings
REF	Catalogue number
	Manufacturer
	Temperature limitation
	Kit content
	Use by
LOT	Batch code
	Do not reuse
	Consult accompanying documents

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Agar

Purified agar for bacteriological use and culture media preparation

PHYSIC-CHEMICAL CHARACTERISTIC

Clarity (1.5% w/v)	8.2 NTU
pH at 25°C	6.75 ± 0.75
Gel Strength	950 g/cm2 maximum
Loss on Drying	12% maximum (9% on average)
Gelation Point	35°C
Melting Point	88°C
Divalent Cations	250 ppm
Heavy Metals (As, Pb)	< 10 mg/kg

DESCRIPTION

Agar is a solidifying agent used for culture media preparation, it is a purified agar from which the extraneous matter, pigmented portions and salts have been removed or reduced to a minimum. It is an hydrosoluble extract from red algae and can be used as a solidifying agent in bacteriological culture media or for determining motility and growth of anaerobes and microaerophiles.

PREPARATION

Agar is typically used in a final concentration of 1-2% for solidifying culture media. Smaller quantities (0.05-0.5%) are used in media for motility studies (0.5%w/v), growth of anaerobes (0.1%) and microaerophiles. 1.5% aqueous solution supplies solid gel at temperature of 35 °C because agar does not melt at temperature lower than 85 °C. The addition of such amounts of agar to liquid media permits all degrees of oxygen tension to exist, thus aids in the development of many fastidious aerobic and anaerobic organisms.

TECHNIQUE

Agar can be used as an ingredient of dehydrated culture media and need dissolution in distilled or deionized water and sterilization by autoclaving.

STORAGE

The powder is very hygroscopic, store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. Hitchens, A.P., and M.C.Leikind (1939) The introduction of agar-agar into bacteriology. J. Bacteriology 37:485-493
2. United States Pharmacopeia Convention (1995) The United States Pharmacopeia 23rd ed. Pharmacopeia Convention, Rockville, MD

PACKAGE

Code	Content	Packaging
611001	500 g	500 g of product in plastic bottle
621001	100 g	100 g of product in plastic bottle
6110015	5000 g	5000 g of product in plastic bottle

pH of THE MEDIUM

6.75 ± 0.75

SHELF LIFE

4 years

QUALITY CONTROL

Dehydrated powder
Appearance: free-flowing, homogeneous
Colour: light beige

TABLE OF SYMBOLS

LOT	Batch code		Consult instructions for use		Manufacturer		Contains sufficient for <n> tests
REF	Catalogue number		Temperature limitation		Use by		Keep away from heat sources

Yeast Extract Agar

Nutrient medium for the enumeration of microorganisms in water and materials of sanitary importance, according to ISO 6222.

DESCRIPTION

Yeast Extract Agar is a nutrient medium used for the determination of total microbial count in all types of water in accordance with the recommendations of ISO 6222.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	6.0
Yeast Extract	3.0
Agar	15.0
Final pH 7.2 ± 0.2 at 25°C	

METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 24 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in tubes/bottles	Melt the content of the tube/bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the tube/bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

1. Make dilutions of the test sample taking into account the level of pollution expected.
2. Inoculate the medium (two sets of plates for each sample) by pour plating or membrane filtration method.
3. Incubate one set of plates at 36 ± 2°C for 40-48 h and the other set at 22 ± 2°C for 64-72 h.

INTERPRETING RESULTS

Count colonies on each plate (reject any plate with confluent growth) and express the results as CFU/ml of sample allowing for dilution factors.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, beige.
Prepared medium: slightly opalescent, amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU
Incubation conditions: aerobically at 36 ± 2°C for 40-48 hours.

QC Table.

Microorganism		Growth
Escherichia coli	WDCM 00012	Good
Bacillus subtilis	WDCM 00003	Good

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 6222:2009. Water quality –Enumeration of culturable microorganisms – Colony count technique by inoculation in a nutrient agar culture medium.

PRESENTATION		Contents	Ref.
Yeast Extract Agar	60 mm ready-to-use plates	20 plates	163582
Yeast Extract Agar	Tubes	20 x 22 ml tubes	34074
Yeast Extract Agar	Tubes	100 x 22 ml tubes	26074
Yeast Extract Agar	Slant tubes	20 x 9 ml tubes	31102
Yeast Extract Agar	Bottles	6 x 200 ml bottles	412120
Yeast Extract Agar	Bottles	6 x 100 ml bottles	403120
Yeast Extract Agar	Dehydrated medium	500 g of powder	611016
Yeast Extract Agar	Dehydrated medium	100 g of powder	621016

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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Instructions For Use
ENGLISH

Nutrient Agar ISO 16266

Medium for cultivating non-fastidious organisms and confirming *Pseudomonas aeruginosa*, according to ISO 16266.

DESCRIPTION

Nutrient Agar ISO 16266 is a medium used for the cultivation of non-fastidious organisms from water samples.

This medium is formulated according to ISO 16266 for the detection and enumeration of *Pseudomonas aeruginosa* in water by the membrane filtration technique.

TYPICAL FORMULA*	(g/litre)
Peptone	5.0
Meat Extract	1.0
Yeast Extract	2.0
Sodium Chloride	5.0
Agar	15.0
Final pH 7.4 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Peptone and meat extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 28 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

According to ISO 16266, transfer the membrane and presumptive *Pseudomonas aeruginosa* to the plate medium.

Incubate aerobically at 36 ± 2°C for 20-24 hours.

Alternatively, the medium can be inoculated by spread plating or direct streaking of the sample over the agar surface.

INTERPRETING RESULTS

Observe for colony growth. Confirm *P. aeruginosa* by performing the oxidase test (ref. 88029).

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

Avoid quick temperature shifts of plated medium to prevent condensation.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Medium in slant tubes: 1 year.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, beige.

Appearance of Prepared Medium: Slightly opalescent, light amber.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Specification
Pseudomonas aeruginosa	WDCM 00025 (ATCC 27853, NCTC 12903)	50-100 CFU	20-24 h 36 ± 1°C	Good growth
Escherichia coli	WDCM 00013 (ATCC 25922, NCTC 12241)			

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Nutrient Agar ISO 16266	Plate 90 mm	20 plates	10044
Nutrient Agar ISO 16266	Slant tube	10 x 7 ml	30083
Nutrient Agar ISO 16266	Bottle	6 x 100 ml	402190
Nutrient Agar ISO 16266	Bottle	6 x 200 ml	412190
Nutrient Agar ISO 16266	Bottle	6 x 500 ml	470060
Nutrient Agar ISO 16266	Dehydrated media	100 g	620036
Nutrient Agar ISO 16266	Dehydrated media	500 g	610036
Nutrient Agar ISO 16266	Dehydrated media	5 kg	6100365

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

Tryptic Soy Agar

General purpose medium for the cultivation of a wide variety of organisms from clinical and nonclinical specimens, according to EN ISO 11133.

DESCRIPTION

Tryptic Soy Agar (TSA) is a non selective isolation medium used for the growth of bacteria which do not have specific nutritional requirements and for the preparation of reference strains with the aim of growth promotion tests of culture media.

This medium complies with EN ISO 11133 for microbiological examination of food, animal feed and water, where it is described as the main reference medium to carry out quantitative and qualitative testing of specific culture media.

Tryptic Soy Agar is also recommended in the harmonized chapters of the United States (USP), European (EP) and Japanese Pharmacopoeia (JP). For the usage in Pharmaceutical Industry, Liofilchem offers products having the same composition as TSA described in the ISO standard, but which are specifically controlled according to the Pharmacopoeial performance requirements. **See the IFU available for the product ref. number 10037S**.

TYPICAL FORMULA	(g/l)
Casein Peptone	15.0
Soy Peptone	5.0
Sodium Chloride	5.0
Agar	15.0

Final pH 7.3 ± 0.2 at 25°C

METHOD PRINCIPLE

Casein peptone and soy peptone provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Sodium chloride maintains osmotic balance in the medium. Agar is the solidifying agent.

The medium can be supplemented with blood for the growth of fastidious organisms and study of haemolytic reactions.

PREPARATION

Dehydrated medium	Suspend 40 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes. If desired, add appropriate volume of sterile defibrinated blood for preparing 5 to 10% blood agar.
Medium in tubes/ bottles	Melt the content of the tube/bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the tube/bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

Perform serial dilutions of the test sample in order to achieve a colony count of between 15 and 300 colonies per plate. Use a suitable diluent such as Buffered Peptone Water (ref. 24099) or Maximum Recovery Broth (ref. 20071).

Inoculate the medium by pour plating, spread/streak method or membrane filtration.

Incubation conditions may vary depending on the organisms under study. For a general aerobic count, incubate aerobically at 30°C for 72 hours.

For use as standard medium, refer to EN ISO 11133 for specific instructions.

INTERPRETING RESULTS

Observe colony growth.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: slightly opalescent, light amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 2 years.
Medium in slant tubes: 1 year.
Ready-to-use plates: 6 months.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU.
Incubation conditions: set according to EN ISO 11133 and shown on the quality control certificate that is available for each lot on liofilchem's website.

QC Table.

Microorganism		Growth
Listeria monocytogenes 4b	WDCM 00021	Good
Staphylococcus aureus	WDCM 00034	Good
Clostridium perfringens	WDCM 00007	Good
Bacillus cereus	WDCM 00001	Good
Escherichia coli	WDCM 00012	Good
Bacillus subtilis	WDCM 00003	Good
Pseudomonas aeruginosa	WDCM 00024	Good
Enterococcus faecalis	WDCM 00087	Good

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. United States Pharmacopoeia 41 NF 33 (2018) <61> Microbiological examination of non-sterile products: Microbial enumeration tests; <1116> Microbiological control and monitoring of aseptic processing environments.
3. European Pharmacopoeia 9.0 (2016) 2.6.12. Microbiological examination of non-sterile products: Microbial enumeration tests.
4. Japanese Pharmacopoeia 16th ed. (2011): 4.05 Microbial limit test.
5. Swanson, K.J., F.F. Busta, E.H. Peterson, and M.G. Johnson (1992). Colony Count Methods, p. 75-95.
6. Vanderzant C. and D.F. Splittstoesser (1992) Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington D.C.
7. Greenberg A.E, L.S. Clesceri and A.D. Eaton (1995) Standards methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington D.C.

PRESENTATION	Format	Packaging	Ref.
Tryptic Soy Agar	90 mm Plate	20 plates	10037
Tryptic Soy Agar	90 mm Plate	100 plates	10037*
Tryptic Soy Agar	60 mm Plate (membrane placement)	20 plates	163682 ◆
Tryptic Soy Agar	Slant tubes	10 x 9 ml tubes	30082
Tryptic Soy Agar	Slant tubes	20 x 9 ml tubes	31082
Tryptic Soy Agar	Tubes	100 x 20 ml tubes	26475
Tryptic Soy Agar	Bottles	6 x 500 ml bottles	470010
Tryptic Soy Agar	Bottles	6 x 225 ml bottles	414110 ◆
Tryptic Soy Agar	Bottles	6 x 200 ml bottles	432290
Tryptic Soy Agar	Bottles	25 x 200 ml bottles	452290
Tryptic Soy Agar	Bottles	6 x 100 ml bottles	442290
Tryptic Soy Agar	Dehydrated media	500 g of powder	610052
Tryptic Soy Agar	Dehydrated media	100 g of powder	620052
Tryptic Soy Agar	Dehydrated media	5 kg of powder	6100525

◆, not CE marked

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

Instructions For Use

ENGLISH

Sabouraud CAF Agar

Selective medium for the cultivation and isolation of pathogenic and nonpathogenic fungi.

DESCRIPTION

Sabouraud CAF Agar is a selective medium used for the cultivation and isolation of fungi from clinical and nonclinical specimens.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	5.0
Enzymatic Digest of Animal Tissue	5.0
Glucose	40.0
Chloramphenicol	0.5
Agar	15.0
Final pH 5.6 ± 0.2 at 25°C	

METHOD PRINCIPLE

Enzymatic digests of casein and enzymatic digest of animal tissue provide nitrogen and vitamins for the growth of fungi. The high glucose concentration along with the acid pH make this medium particularly well suited for cultivating fungi. Chloramphenicol is a broad-spectrum antibiotic inhibitory to a wide range of Gram-negative and Gram-positive bacteria. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 65.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shacking frequently until completely dissolved. Sterilize in autoclave at 118°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into final containers.

TEST PROCEDURE

Inoculate either plates or slant tubes by streaking directly the sample onto the agar surface. Streak the specimen as soon as possible after it is received in the laboratory. Incubate aerobically at 30°C for 2-7 days.

INTERPRETING RESULTS

Examine containers for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Transfer of growth from slants to plated media may be required in order to obtain pure cultures of fungi.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: slightly opalescent, amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Medium in tubes: 1 year.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 10-100 CFU/ml.
Inoculum for selectivity: 10^4-10^5 CFU/ml.
Incubation conditions: aerobically at 30±2°C for 2-7 days.

QC Table.

Microorganism		Growth
Aspergillus niger	ATCC® 16404	Good
Candida albicans	ATCC® 10231	Good
Saccharomyces cerevisiae	ATCC® 9763	Good
Trichophyton mentagrophytes	ATCC® 9533	Good
Escherichia coli	ATCC® 8739	Inhibited

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. Sabouraud. 1892. Ann. Dermatol. Syphil. 3:1061.
2. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.
3. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

PRESENTATION		Contents	Ref.
Sabouraud CAF Agar	90 mm ready-to-use plates	20 plates	11035
Sabouraud CAF Agar	90 mm ready-to-use plates	100 plates	11035*
Sabouraud CAF Agar	140 mm ready-to-use plates	10 plates	10242
Sabouraud CAF Agar	Slant tubes	10 x 10 ml tubes	30023
Sabouraud CAF Agar	Slant tubes	20 x 10 ml tubes	31023
Sabouraud CAF Agar	Bottles	6 x 200 ml bottles	412370
Sabouraud CAF Agar	Bottles	6 x 100 ml bottles	402370
Sabouraud CAF Agar	Dehydrated medium	500 g of powder	610203
Sabouraud CAF Agar	Dehydrated medium	100 g of powder	620203
Sabouraud CAF Agar	Dehydrated medium	5 kg of powder	6102035

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care	Keep away from sunlight
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse	

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CE IVD

ALKALINE PEPTONE WATER

Enrichment medium for *Vibrio spp* isolation from infectious materials.

TYPICAL FORMULA	(g/l)
Peptone	10.0
Sodium Chloride	10.0

Final pH= 8.5 ± 0.2 at 25°C.

DIRECTIONS

Suspend 20.0 g of powder in 1 liter of distilled or deionized water. Heat until completely dissolved. Dispense into final containers as appropriate. Sterilize in autoclave at 121 °C for 15 minutes.

DESCRIPTION

ALKALINE PEPTONE WATER is recommended by OMS and APHA as a tranport and enrichment medium for *Vibrio spp.* The enrichment in liquid medium is necessary for isolation of Vibrio from feces (after the first 48 hours of disease), for the control of carriers and for the examination of drinks , foods or sewage. The high value of pH slows down the development of common contaminants, so that after 6-7 hours of incubation, *vibrio,* if present, stand out on microscopical examination in dark field.

TECHNIQUE

The medium is inoculated with few drops or a loopful of material and incubated at 36 ± 1°C. After 12-24 hours, inoculate a loopful of the culture broth on a isolation medium. For confirming the diagnosis, submit the suspected colonies to serological and biochemical identification tests.

QUALITY CONTROL

Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: cream-white to light tan.
Prepared medium
Appearance: clear to very slight opalescent.
Color: light amber.
Incubation conditions: 36 ± 1°C for 24 hours.

Microorganism	ATCC	Growth
Vibrio cholerae	11218	good
Escerichia coli	25922	none
Staphylococcus aureus	25923	none

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared media at 2-8 °C.

REFERENCES

1. APHA (1985) – Standard Methods for the Examination of Water Wastewater , 16th ed.
2. Benenson, A.S., Islam M.R. & Greenoungh, W.B. (1964) – Rapid identification of Vibrio cholerae by darkfield microscopy. Bull, WHO, **30,** 827.

PRESENTATION

Product	REF	Σ
ALKALINE PEPTONE WATER (25.0 l)	610098	500g
ALKALINE PEPTONE WATER (5.0 l)	620098	100g

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests	IVD *In Vitro* Diagnostic Medical Device
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	Keep away from heat source

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CE IVD

Peptone Water

Liquid medium for the cultivation of non-fastidious microorganisms, indole testing and carbohydrate fermentation studies.

Instructions For Use
ENGLISH

DESCRIPTION

Peptone Water is a liquid medium used for the cultivation of non-fastidious microorganisms and indole testing. It is also a basal medium to which carbohydrates and indicator may be added for fermentation studies. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

TYPICAL FORMULA*	(g/litre)
Peptone	10.0
Sodium Chloride	5.0

Final pH 7.2 ± 0.2 at 25°C

*Adjusted and/or supplemented as required to meet performance criteria.

METHOD PRINCIPLE

Peptone provides carbon, nitrogen, vitamins and minerals and is rich in tryptophan content. Sodium chloride maintains the osmotic balance of the medium.

PREPARATION

Dehydrated medium: Suspend 15.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil and shake until completely dissolved. Dispense into final containers. Sterilize in autoclave at 121°C for 15 minutes.

TEST PROCEDURE

To perform indole test, inoculate a tube of Peptone Water preheated to 44°C, using a sampling loop. Incubate at 35 ± 2°C for 24-48 h. Add 2-3 drops of Kovac's Reagent (ref. 80271 / 87001) and examine soon after.

To study the fermentation ability of carbohydrates, add the sugar solution usually at 10% w/v concentration to the basal medium. Phenol red can be used as pH indicator and Durham tube to detect the gas production. Incubate at 35 ± 2°C for 18-24 h.

INTERPRETING RESULTS

Turbidity of the medium indicates microbial growth (compare to an uninoculated control).

The formation of a red to purple colour (“cherry-red ring”) in the reagent layer on top of the medium within 30 sec indicates a positive reaction for indole production. A negative reaction shows no colour change.

If phenol red has been included, the medium turns yellow in the case of acidic production, that is carbohydrate utilization.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes and bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Tubes/Bottles: 2 years.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, beige.

Appearance of Prepared Medium: Clear to very slightly opalescent, light amber.

Expected Cultural Response:

Strain		Inoculum	Incubation	Growth	Indole test	Lactose test
Escherichia coli	ATCC 25922	≤100 CFU	18-48 h 35 ± 2°C	Good	Positive	Positive
Salmonella Typhimurium	ATCC 14028				Negative	Negative

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Peptone Water	Tube	20 x 10 ml	24098
Peptone Water	Bottle	25 x 90 ml	452640
Peptone Water	Bottle	6 x 100 ml	402130
Peptone Water	Bottle (perforable cap)	6 x 100 ml	402530
Peptone Water	Bottle	6 x 500 ml	470320
Peptone Water	Dehydrated medium	500 g of powder	610038
Peptone Water	Dehydrated medium	100 g of powder	620038

This document is available from the online Support Center:

liofilchem.com/ifu-sds

Peptone Water

Liquid medium for the cultivation of non-fastidious microorganisms, indole testing and carbohydrate fermentation studies.

Instructions For Use
ENGLISH

DESCRIPTION

Peptone Water is a liquid medium used for the cultivation of non-fastidious microorganisms and indole testing. It is also a basal medium to which carbohydrates and indicator may be added for fermentation studies. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

TYPICAL FORMULA*	(g/litre)
Peptone	10.0
Sodium Chloride	5.0

Final pH 7.2 ± 0.2 at 25°C

*Adjusted and/or supplemented as required to meet performance criteria.

METHOD PRINCIPLE

Peptone provides carbon, nitrogen, vitamins and minerals and is rich in tryptophan content. Sodium chloride maintains the osmotic balance of the medium.

PREPARATION

Dehydrated medium: Suspend 15.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil and shake until completely dissolved. Dispense into final containers. Sterilize in autoclave at 121°C for 15 minutes.

TEST PROCEDURE

To perform indole test, inoculate a tube of Peptone Water preheated to 44°C, using a sampling loop. Incubate at 35 ± 2°C for 24-48 h. Add 2-3 drops of Kovac's Reagent (ref. 80271 / 87001) and examine soon after.

To study the fermentation ability of carbohydrates, add the sugar solution usually at 10% w/v concentration to the basal medium. Phenol red can be used as pH indicator and Durham tube to detect the gas production. Incubate at 35 ± 2°C for 18-24 h.

INTERPRETING RESULTS

Turbidity of the medium indicates microbial growth (compare to an uninoculated control).

The formation of a red to purple colour (“cherry-red ring”) in the reagent layer on top of the medium within 30 sec indicates a positive reaction for indole production. A negative reaction shows no colour change.

If phenol red has been included, the medium turns yellow in the case of acidic production, that is carbohydrate utilization.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes and bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Tubes/Bottles: 2 years.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, beige.

Appearance of Prepared Medium: Clear to very slightly opalescent, light amber.

Expected Cultural Response:

Strain		Inoculum	Incubation	Growth	Indole test	Lactose test
Escherichia coli	ATCC 25922	≤100 CFU	18-48 h 35 ± 2°C	Good	Positive	Positive
Salmonella Typhimurium	ATCC 14028				Negative	Negative

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Peptone Water	Tube	20 x 10 ml	24098
Peptone Water	Bottle	25 x 90 ml	452640
Peptone Water	Bottle	6 x 100 ml	402130
Peptone Water	Bottle (perforable cap)	6 x 100 ml	402530
Peptone Water	Bottle	6 x 500 ml	470320
Peptone Water	Dehydrated medium	500 g of powder	610038
Peptone Water	Dehydrated medium	100 g of powder	620038

This document is available from the online Support Center:

liofilchem.com/ifu-sds

Instructions For Use
ENGLISH

Buffered Peptone Water

Diluent and non-selective pre-enrichment liquid medium for microbiological examination of food, according to ISO 6887,11290, 21528 and 6579.

DESCRIPTION

Buffered Peptone Water (BPW) is a liquid medium recommended by ISO 6579 for increasing the recovery of injured *Salmonella* spp. from food and associated samples prior to selective enrichment and isolation.

According to ISO 21528, BPWis used for detection or enumeration of Enterobacteriaceae within foodstuffs.

Used as diluent, BPW complies with ISO 6887 and 11290 for the enumeration of organisms.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	10.0
Sodium Chloride	5.0
Disodium Hydrogen Phosphate	3.5*
Potassium Dihydrogen Phosphate	1.5

Final pH 7.0 ± 0.2 at 25°C

*Equivalent to 9.0 g of disodium hydrogen phosphate dodecahydrate.

METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon and minerals. Sodium chloride maintains the osmotic balance of the medium. Phosphates are the buffering agents.

PREPARATION

Dehydrated medium: Suspend 20.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shacking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 min.

TEST PROCEDURE

Suspend the sample in BPW to make dilutions as required.

For pre-enrichment, add sample to BPW at a ratio of 1:10 or 1:9 depending on the method being used. Incubate at 37 ± 1°C for 16-20 hours before transfer to selective enrichment media.

INTERPRETING RESULTS

Turbidity indicates microbial growth.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: clear, light amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Prepared medium: 2 years.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC tables.
Inoculum for use as diluent: 10^3-10^4 CFU.
Incubation conditions: 45 min - 1 h / 18-27°C.

QC Table 1.

Microorganism		Specification
Escherichia coli	WDCM 00012	± 30% colonies of original count
Staphylococcus aureus	WDCM 00034	± 30% colonies of original count
Listeria monocytogenes 4b	WDCM 00021	± 30% colonies of original count

Inoculum for productivity: ≤ 100 CFU.
Incubation conditions: 18 ± 2 h / 37 ± 1°C.

QC Table 2.

Microorganism		Specification
Salmonella typhimurium	WDCM 00031	Good growth/turbidity of the medium
Salmonella enteritidis	WDCM 00030	Good growth/turbidity of the medium
Escherichia coli	WDCM 00012	Good growth/turbidity of the medium

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 11290-2:2017. Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. – Part 2: Enumeration method.
3. ISO 21528-1:2017. Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 1: Detection of *Enterobacteriaceae*.
4. ISO 21528-2:2017. Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count technique.
5. ISO 6579-1:2017. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* spp. – Part 1: Detection of *Salmonella* spp.
6. Rose (2001) Isolation and identification of Salmonella from meat, poultry and egg products. In Microbiology laboratory guidebook, 3rd ed., Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C.
7. ISO 6887-1:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
8. Sadovski (1977) J. Food Technol. 12:85.
9. Edel and Kampelmacher (1973) Bull. W.H.O. 48:167.

PRESENTATION	Category	Packaging	Ref.
Buffered Peptone Water	Media in tubes	20 x 9 ml	24199
Buffered Peptone Water	Media in tubes	100 x 9 ml	26199
Buffered Peptone Water	Media in tubes	20 x 10 ml	24099
Buffered Peptone Water (Double Concentration	Media in tubes	20 x 9 ml	24463
Buffered Peptone Water	Media in bottles	6 x 90 ml	414030
Buffered Peptone Water	Media in bottles	25 x 90 ml	454030
Buffered Peptone Water	Media in bottles	6 x 200 ml	412090
Buffered Peptone Water	Media in bottles	6 x 225 ml	414020
Buffered Peptone Water	Media in bottles	25 x 225 ml	451402
Buffered Peptone Water - Bags	Media in bags	3 x 3 l	499030
Buffered Peptone Water - Bags	Media in bags	3 x 5 l	499035
Buffered Peptone Water	Dehydrated media	100 g	621014
Buffered Peptone Water	Dehydrated media	500 g	611014
Buffered Peptone Water	Dehydrated media	5 kg	6110145

TABLE OF SYMBOLS

LOT	Batch code		Keep away from sunlight		Manufacturer		Use by		Fragile, handle with care
REF	Catalogue number		Temperature limitation		Contains sufficient for <n> tests		Caution, consult Instruction For Use		Do not reuse

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Instructions For Use
ENGLISH

Brilliant Green Agar

Selective medium for isolation of *salmonellae* from
clinical specimens and other materials of sanitary importance.

DESCRIPTION

Brilliant Green Agar is a selective medium used for the isolation *Salmonella* spp, other than *S.* Typhi and *S.* Paratyphi from pathogen materials, stool, urine, environmental samples and food.

Brilliant Green Agar is recommended by APHA, FDA and USP.

TYPICAL FORMULA	(g/l)
Meat Peptone	5.0
Casein Peptone	5.0
Sodium Chloride	5.0
Yeast Extract	3.0
Lactose	10.0
Sucrose	10.0
Phenol Red	0.08
Brilliant Green	0.0125
Agar	20.0
Final pH 6.9 ± 0.2 at 25°C	

METHOD PRINCIPLE

Peptones provide amino acids, carbon, nitrogen, vitamins and minerals for organisms growth. Sodium chloride maintains the osmotic balance of the medium .Yeast extract is a source of vitamins, particularly of B-group. Lactose and sucrose are the fermentable carbohydrates. Lysine is the decarboxylase substrate. Phenol red is the pH indicator. Brilliant green is the selective agent inhibiting Gram-positive bacteria and most Gram-negative bacteria, other than *Salmonella* spp. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 58.1 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

Inoculate the plates by directly streaking the sample over the agar surface. Incubate aerobically at 35 ± 2°C for 18-24 hours.

INTERPRETING RESULTS

After incubation observe the color of the colonies and interpret the results as indicated in the ID Table.

ID Table.

Microorganism	Appearance of colonies
Salmonella spp (excepted *S.* Typhi and *S. Paratyphi*)	White to pink, with red zone
Escherichia coli, *Enterobacter*, *Klebsiella* spp	Yellow-green
Pseudomonas spp	Pink to red

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, pink.
Prepared medium: slightly opalescent, orange-brown.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU
Inoculum for selectivity: 10^4-10^6 CFU
Incubation conditions: aerobically at 35 ± 2°C for 18-24 hours.

QC Table.

Microorganism		Growth	Specification
Salmonella Typhimurium	ATCC® 14028	Good	White to red colonies with red zone
Salmonella Enteritidis	ATCC® 13076	Good	White to red colonies with red zone
Shigella flexneri	ATCC® 12022	Inhibited	---
Staphylococcus aureus	ATCC® 25923	Inhibited	---
Escherichia coli	ATCC® 25922	Poor	Yellow-green colonies

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. Kristensen M, V. Lester and A. Jurgens (1925) On the use of trypsinized casein, bromthymol blue, bromcresol purple, phenol red and brilliant green for bacteriological nutrient media. Br J Exp Pathol. 6:291.
2. Taylor W.J. (1965) Isolation of Shigellae I. Xylose lysine agars: new media for isolation of enteric pathogens. Am J Clin Pathol; 44:471-475.
3. United States Pharmacopeial Convention (1995) Microbial Limit Test. The United States Pharmacopoeia 23rd ed. The United States Pharmacopeial Convention, Rockville MD, USA.
4. US Food and Drug Administrations (1998) Bacteriological Analytical Manual 8th ed. AOAC International. Gaithersburg, MD, USA.

PRESENTATION		Contents	Ref.
Brilliant Green Agar	90 mm ready-to-use plates	20 plates	10022
Brilliant Green Agar	90 mm ready-to-use plates	100 plates	10022*
Brilliant Green Agar	Bottles	6 x 100 ml bottles	402330
Brilliant Green Agar	Dehydrated medium	500 g of powder	610009
Brilliant Green Agar	Dehydrated medium	100 g of powder	620009

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

BILE BACTERIOLOGICAL

Bacteriological bile obtained by bile purification.

USE

BILE BACTERIOLOGICAL is ox bile purified and dehydrated. It's a fine beige powder, easily soluble in water.
It contains a mix of biliary salts and is used in media for enterobacteria, as selective agent, and for identification of enterococci.

PHYSICO-CHEMICAL CHARACTERISTICS

	Standard	Method
Solubility in water at 5%	Complete	Eur.Ph.3rd ed.
pH of 5% solution	5.5-7.5	Eur.Ph.3rd ed.
Loss on drying	≤ 5.0%	Eur.Ph.3rd ed.
Bile acids	≥ 45.0%	Eur.Ph.10th ed.

TECHNIQUE

Bile Bacteriological can be used as an ingredient of dehydrated culture media and need dissolution in distilled or deionized water and sterilization by autoclaving.

QUALITY CONTROL

Dehydrated powder
Appearance: free-flowing, homogeneous.
Colour: beige.

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.

REFERENCES

1. Cowan, S.T., Steel, K.J. (1979) Manual for the identification of medical bacteria. Edi. Ermes.

PRESENTATION

Product	REF	Σ
BILE BACTERIOLOGICAL	**611367**	500 g
BILE BACTERIOLOGICAL	**621367**	100 g

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests	IVD *In Vitro* Diagnostic Medical Device
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	Keep away from heat source

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CE IVD

Bile Aesculin Azide Agar

Selective medium for detection and enumeration of enterococci in water and other materials, according to ISO 7899-2.

TYPICAL FORMULA	(g/l)
Tryptone	17.0
Peptone	3.0
Yeast Extract	5.0
Ox-bile	10.0
Sodium Chloride	5.0
Aesculin	1.0
Ferric Ammonium Citrate	0.5
Sodium Azide	0.15
Agar	15.0
Final pH 7.1 ± 0.1 at 25°C	

DESCRIPTION

Bile Aesculin Azide Agar is a selective medium used for isolating and enumerating enterococci from environmental samples.

This medium complies with ISO 7899-2 for rapid confirmation of typical colonies on the primary isolation Slanetz Bartley Agar.

PRINCIPLE

Tryptone and peptone provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Ox-bile inhibits the growth of numerous accompanying bacteria. Sodium chloride maintains the osmotic balance of the medium. The glycoside aesculin is hydrolyzed from enterococci to aesculetin and glucose. The aesculetin reacts with iron ions forming a dark brown or black complex. Sodium azide suppress the growth of Gram-negative bacteria. Agar is the solidifying agent.

PREPARATION

Suspend 56.7 g of powder in 1 liter of deionized or distilled water. Bring to boil and shake until completely dissolved. Mix well. Sterilize in autoclave at 121°C for 15 minutes. Cool up to 45-50°C. Pour in Petri dishes.

TECHNIQUE

ISO 7899-2 recommends to filter the water sample trough a filter membrane (0.45 µm pore diameter), transfer the membrane onto a Slanetz Bartley Agar plate (ref. 163462) and incubate aerobically at 36 ± 2°C for 40-48 h.
Confirm red-maroon-pink colonies by transferring the membrane and the colonies onto a plate of Aesculin Azide Bile Agar which as been preheated to 44°C. Incubate at 44 ± 0.5°C for 2 h.

Alternatively, sample can be inoculated by spread plating, pour plating or by direct streaking on the medium surface. Incubate at 35 ± 2°C for 18-24 h.

INTERPRETATION OF RESULTS

Enterococci typically produce colonies showing a tan-black color in the surrounding medium.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

- ISO 7899-2:2000. Water quality − Detection and enumeration of intestinal enterococci − Part 2: Membrane filtration method.
- Facklam R.R. and M. Moody (1970) Presumptive identification of group D streptococci: the bile-aesculin test. App. Microbiol. 20:245-250.
- Isenberg H.D. and D. Goldber (1970) Laboratory studies with a selective Enterococcus medium. Appl. Microbiol.20:433-436
- Slanetz L.W. and C.H. Bartley (1957) Numbers of enterococci in water, sewage and faeces determined by the membrane filtration technique with an improved medium. J. Bact. 74:591-595.

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PRODUCT SPECIFICATIONS

NAME
Bile Aesculin Azide Agar

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610001	500 g	500 g of powder in plastic bottle
620001	100 g	100 g of powder in plastic bottle
6100015	5 Kg	5 kg of powder in plastic bottle

pH OF THE MEDIUM
7.1 ± 0.1

USE
Bile Aesculin Azide Agar is a selective medium used for confirmation and enumeration of enterococci from water and other samples according to ISO 7899-2

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: beige
Ready-to-use medium
Appearance: slightly opalescent
Colour: dark amber to olive green

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Inoculum for selectivity: 10^4-10^6 CFU
   Incubation Conditions: 18-24 h at 35 ± 2°C, in aerobiosis

Microorganism		Growth	Specification
Enterococcus faecalis	ATCC® 19433	Good	Blackening
Enterococcus faecium	ATCC® 19434	Good	Blackening
Escherichia coli	ATCC® 25922	Inhibited	---
Streptococcus pyogenes	ATCC® 19615	Inhibited	---

TABLE OF SYMBOLS

LOT Batch code	Consult instructions for use	Manufacturer	Use by
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Keep away from sunlight

LIOFILCHEM® S.r.l.
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Brilliant Green Bile Broth 2%

Liquid medium for detection or confirmation of coliform bacteria in water and food, according to APHA, ISO 4831 and ISO 4832.

DESCRIPTION

Brilliant Green Bile Broth 2% is a liquid medium used for the detection or confirmation of coliform bacteria in water and wastewater, foods, dairy products and other materials of sanitary importance.

Brilliant Green Bile Broth 2% is formulated according to APHA, ISO 4831 and ISO 4832.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	10.0
Lactose	10.0
Ox Bile	20.0
Brilliant Green	0.0133

Final pH 7.2 ± 0.2 at 25°C

METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Lactose is the fermentable carbohydrate. Ox bile and brilliant green inhibit Gram-positive bacteria and many Gram-negative bacteria, other than coliforms.

PREPARATION

Dehydrated medium	Suspend 40.0* g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Distribute into 10 ml tubes with Durham gas collecting tube. Sterilize in autoclave at 121°C for 15 minutes. *Dissolve 80.0 g of the power in 1 liter of distilled or deionized water to make the double strength broth.
Medium in bottles	Aseptically, dispense the medium into tubes fitted with Durham tubes.

TEST PROCEDURE

1. For coliforms detection, inoculate tubes with 1 ml diluted or undiluted sample (use double strength broth for larger volume samples). For use in the confirmation of presumptive tests, subculture from Lauryl Sulphate Tryptose Broth (ref. 21453) or from typical coliform colonies on Violet Red Bile Lactose Agar (ref. 11183).
2. To indicate the presence of *Escherichia coli*, incubate at 44 ± 1°C for 48 hours.
   ISO 4831 and ISO 4832 recommend to incubate at 30°C or 37°C for 24-48 hours.
3. Examine tubes for gas formation.

INTERPRETING RESULTS

Turbidity and gas production indicate coli-aerogenes organisms.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, green-beige.
Prepared medium: clear, green.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 2 years.

QUALITY CONTROL

The tubes are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Inoculum for selectivity: >10^3 CFU.
Incubation conditions: 30 ± 1°C for 24-48 hours.

QC Table.

Microorganism		Growth	Gas
Escherichia coli	WDCM 00012	Good	+
Escherichia coli	WDCM 00013	Good	+
Citrobacter freundii	WDCM 00006	Good	+
Enterococcus faecalis	WDCM 00009	Partially to completely inhibited	–
Enterococcus faecalis	WDCM 00087	Partially to completely inhibited	–

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.ù
2. ISO 4831:2006. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Most probable number technique.
3. ISO 4832:2006. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony Count technique.
4. Clesceri, Greenberg and Eaton (1998) Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association (APHA), Washington, D.C.
5. Marshall (1993) Standard methods for the examination of dairy products, 16th ed. American Public Health Association (APHA), Washington, D.C.

PRESENTATION	Category	Packaging	Ref.
Brilliant Green Bile Broth 2%	Tubes/Bottles	10 x 10 ml tubes	20102
Brilliant Green Bile Broth 2%	Tubes/Bottles	20 x 10 ml tubes	24102
Brilliant Green Bile Broth 2%	Tubes/Bottles	100 x 10 ml tubes	26102
Brilliant Green Bile Broth 2%	Tubes/Bottles	6 x 100 ml bottles	402560
Brilliant Green Bile Broth 2%	Dehydrated medium	500 g of powder	610010
Brilliant Green Bile Broth 2%	Dehydrated medium	100 g of powder	620010
Brilliant Green Bile Broth 2%	Dehydrated medium	5 kg of powder	6100105

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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Instructions For Use
ENGLISH

Selenite Broth

Liquid medium for selective enrichment of *Salmonella* spp, from clinical and nonclinical samples, according to APHA.

DESCRIPTION

Selenite Broth is an enrichment medium used for the selective isolation of *Salmonella* and some species of *Shigella*.

This medium is prepared according to the original formula described as Selenite F Broth by Leifson and recommended by the American Public Health Association for the examination of food.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	5.0
Lactose	4.0
Sodium Phosphate	10.0
Sodium Selenite	4.0

Final pH 7.0 ± 0.2 at 25°C

METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Lactose is the fermentable carbohydrate. Sodium phosphate is the buffer. Sodium selenite is the selective agent inhibiting many species of Gram-positive and Gram-negative bacteria including enterococci and coliforms.

PREPARATION

Dehydrated medium: Suspend 23 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Dispense into suitable containers (bottles or tubes). A depth of at least 5 cm is recommended, as salmonellae survive better at low oxygen tensions. DO NOT AUTOCLAVE.

TEST PROCEDURE

Inoculate the tube with 1-2 g of stool specimen or other solid material (approximately 10-15% by volume) and emulsify in the broth. For urines, the broth should be used at double concentration and inoculated with its own volume of the specimen. Incubate at 35 ± 2°C for 12-24 hours (coliforms may overgrow the pathogens if incubated for longer than 24 hours).

INTERPRETING RESULTS

Turbidity indicates microbial growth.

Subculture to a selective and differential enteric plated medium, such as XLD Agar (ref. 10056), Hektoen Enteric Agar (ref. 10043) or MacConkey Agar (ref. 10029), streaking for isolation. Examine for typical colony morphology. Confirm with further biochemical tests.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, white to light beige.
Prepared medium: clear, very pale yellow.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and tubes at 2-8°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 1 year.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Inoculum for selectivity: >10^3 CFU.
Incubation conditions: aerobically at 35 ± 2°C for 18-24 hours.

QC Table.

Microorganism		Growth
Salmonella Typhimurium	ATCC® 14028	Good
Shigella sonnei	ATCC® 25931	Good
Escherichia coli	ATCC® 25922	Partially to completely inhibited

WARNING AND PRECAUTIONS

The product contains hazardous substances and is classified as dangerous. It is recommended to consult the safety data sheet for its correct use. The product is intended for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. Versalovic, J., K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry, and D.W. Warnock (2011) Manual of Clinical Microbiology. 10th ed. ASM Press, Washington, D.C.
2. Quality Control for Commercially Prepared Microbiological Media (2004) - 3rd ed. M22-A3. Clinical and Laboratory Standards Institute - CLSI (NCCLS), Wayne, PA.
3. Vanderzant, C., and D.F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. Leifson, E. (1939) New selenite selective enrichment medium for the isolation of typhoid and paratyphoid bacilli. Am. J. Hyg. 24:423-432.

PRESENTATION		Contents	Ref.
Selenite Broth	Tubes	20 x 10 ml tubes	24110
Selenite Broth	Tubes	20 x 5 ml tubes	24143
Selenite Broth	Bottles	6 x 100 ml bottles	402050
Selenite Broth	Bottles	6 x 200 ml bottles	412050
Selenite Broth (Double Concentration)	Bottles	6 x 200 ml bottles	432050
Selenite Broth	Bottles	6 x 500 ml bottles	470020
Selenite Broth	Bottles	6 x 1000 ml bottles	463130
Selenite Broth	Dehydrated medium	500 g of powder	610145
Selenite Broth	Dehydrated medium	100 g of powder	620145
Selenite Broth	Dehydrated medium	5 kg of powder	6101455

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

Instructions For Use
ENGLISH

Sabouraud Dextrose Broth

Liquid medium for the cultivation of yeasts and moulds from different materials, according to USP/EP/JP.

DESCRIPTION

Sabouraud Dextrose Broth (SDB) is a liquid medium recommended for use in qualitative procedures for isolation of yeasts and moulds and for the culture or subculture of fungi from clinical and nonclinical specimens.

This medium conforms to the requirements of the harmonized method in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) for the microbiological examination of non sterile products.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Dextrose	20.0
Final pH 5.6 ± 0.2 at 25°C	

METHOD PRINCIPLE

Pancreatic digest of casein and peptic digest of animal tissue provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Dextrose is an energy source. The high concentration of dextrose and the acidic pH of the medium permit selectivity of fungi.

The medium can be supplemented with chloramphenicol to increase bacterial inhibition and recovery of dermatophytes.

PREPARATION

Dehydrated medium — Suspend 30 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Dispense into appropriate containers. Sterilize in autoclave at 121°C for 15 minutes.

TEST PROCEDURE

For use in medical microbiology

Inoculate the specimen directly into the broth. Incubate aerobically at 25°C for 2-7 days (incubation conditions may vary according to the type of specimen and the microorganisms being tested for).

For use in industrial microbiology

To prepare the fungal test strains grow *C. albicans* or *A. brasiliensis* at 20-25°C for 48-72 hours or 5-7 days, respectively.

To test for *C. albicans*, inoculate the preparation of the product to be examined 1:100 in SDB and incubate at 30-35°C for 3-5 days. Subculture on a plate of Sabouraud Dextrose Agar (ref. 10035).

INTERPRETING RESULTS

Turbidity indicates microbial growth.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: clear, light amber, may have a slight precipitate.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes and bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles/tubes: 2 years.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Incubation conditions: 32.5 ± 2.5°C for 48-72 h (*C. albicans*) and at 22.5 ± 2.5°C for up to 5 days (all listed organisms), under aerobic atmosphere.

QC Table.

Microorganism		Growth
Candida albicans	ATCC® 10231	Good
Aspergillus brasiliensis	ATCC® 16404	Good
Saccharomyces cerevisiae	ATCC® 9763	Good

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. European Pharmacopoeia 6.5 (2009) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia 32 NF 27 (2009) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia 4.05 (2008) Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Sabouraud, R. (1892) Ann. Dermatol. Syphilol. 3:1061.

PRESENTATION		Contents	Ref.
Sabouraud Dextrose Broth	Tubes	20 x 10 ml tubes	24109
Sabouraud Dextrose Broth	Bottles	6 x 100 ml bottles	402040
Sabouraud Dextrose Broth	Bottles	25 x 100 ml bottles	452040
Sabouraud Dextrose Broth	Bottles	6 x 500 ml bottles	471070
Sabouraud Dextrose Broth	Dehydrated medium	500 g of powder	610104
Sabouraud Dextrose Broth	Dehydrated medium	100 g of powder	620104

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

Instructions For Use
ENGLISH

EC Broth

Selective liquid medium for detection and enumeration of coliform bacteria and *E. coli* in water and food, according to ISO 7251.

DESCRIPTION

EC Broth is a liquid medium used for the selective detection of coliform bacteria and *Escherichia coli* in water and wastewater, foods and other materials of sanitary importance, according to ISO 7251.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	20.0
Lactose	5.0
Bile Salts	1.5
Dipotassium Phosphate	4.0
Monopotassium Phosphate	1.5
Sodium Chloride	5.0

Final pH 6.9 ± 0.2 at 25°C

METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Lactose is the fermentable carbohydrate. Bile salts inhibit Gram-positive bacteria, especially enterococci. Phosphates act as buffer. Sodium chloride maintains the osmotic balance of the medium.

PREPARATION

Dehydrated medium: Suspend 37.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Distribute into 10 ml tubes with Durham gas collecting tube. Sterilize in autoclave at 121°C for 15 minutes.

TEST PROCEDURE

For detection and enumeration of *E. coli*, ISO 7251 recommends to first inoculate the initial suspension of the test sample into tubes of Lauryl Sulfate Tryptose Broth (LST), ref. 21453 and Lauryl Sulfate Tryptose Broth (LST) Double, ref. 21454. After incubation at 37°C for 24-48 h, tubes are examined for turbidity and gas production. Then, each positive test tube is subcultured to a EC Broth tube and incubated at 44°C for 24-48 h.

Alternatively, EC Broth can be directly inoculated with the sample and incubated for 24 ± 2 h and up to 48 h at 35 ± 2°C for detection of coliforms or at 44.5 ± 1°C for the isolation of *Escherichia coli*.

INTERPRETING RESULTS

Gas production is to be consider as a preliminary positive result. Indole test as well as other biochemical tests should be carried out for confirmation of *Escherichia coli* after subculturing on suitable media.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: clear, light amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes: 2 years.

QUALITY CONTROL

Tubes are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU
Inoculum for selectivity: >10^3 CFU
Incubation conditions: 44 ± 2°C for 24 ± 2 hours.

QC Table.

Microorganism		Growth	Gas
Escherichia coli	ATCC® 25922	Good	+
Escherichia coli	ATCC® 8739	Good	+
Enterococcus faecalis	ATCC® 29212	Inhibited	–

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. ISO 7251:2005. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive *Escherichia coli* – Most probable Number technique.
2. Clesceri, Greenberg and Eaton (1998) Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association (APHA), Washington, D.C.
3. Perry C.A. and A.A. Hajna (1944) Further evaluation of EC medium for the isolation of coliform bacteria and *Escherichia coli*. Am. J.Public Health 34:735-738.

PRESENTATION		Contents	Ref.
EC Broth	Tubes	10 x 10 ml tubes	20122
EC Broth	Tubes	20 x 10 ml tubes	24122
EC Broth	Tubes	100 x 10 ml tubes	26122
EC Broth	Dehydrated medium	500 g of powder	610063
EC Broth	Dehydrated medium	100 g of powder	620063

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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Instructions For Use
ENGLISH

Brain Heart Infusion Broth

Liquid medium for the cultivation of various fastidious organisms
and detection of staphylococci, according to ISO 6888.

DESCRIPTION

Brain Heart Infusion Broth is a liquid medium used for the cultivation of fastidious and nonfastidious microorganisms, including aerobic and anaerobic bacteria, from clinical specimens, food and environmental samples.

This medium is especially suited for the cultivation of coagulase-positive staphylococci for the plasma coagulase test according to ISO 6888.

Brain Heart Infusion Broth is recommended by the APHA for examination of water and wastewater and by the CLSI for preparing inocula used in antimicrobial susceptibility tests.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Animal Tissues	10.0
Dehydrated Calf Brain Infusion	12.5
Dehydrated Beef Heart Infusion	5.0
Glucose	2.0
Sodium Chloride	5.0
Disodium Hydrogen Phosphate, Anhydrous	2.5

Final pH 7.4 ± 0.2 at 25°C

METHOD PRINCIPLE

Enzymatic digest of animal tissues and brain-heart infusion provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Glucose is the carbohydrate source. Sodium chloride maintains the osmotic balance of the medium. Disodium phosphate is the buffering agent.

PREPARATION

Dehydrated medium: Suspend 37 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Distribute into final containers. Sterilize in autoclave at 121°C for 15 minutes.

TEST PROCEDURE

If material is being cultured directly from a swab, insert the swab into the broth after inoculation of plated media. For liquid specimens, transfer a loopful of the specimen into the broth medium using a sterile loop or aseptically pipette the specimen onto plated medium and into the broth. Examine for growth after 24-72 hours of incubation.

NB. It is recommended that liquid media for anaerobic incubation should be reduced prior to inoculation by placing tubes (with loosened caps) under anaerobic conditions for 18-24 hours. Alternatively, the media may be reduced by bringing the media up to 100°C in a boiling waterbath. Loosen screw caps slightly before heating, and tighten during cooling to room temperature.

To perform plasma coagulase tests, according to ISO 6888, inoculate tubes of Brain Heart Infusion Broth with selected colony from Baird Parker Agar plates (ref. 10020). Incubate at 37 ± 1°C for 24 ± 2 hours. Add 0.1 ml of each culture to 0.3 ml of the rabbit plasma. Examine after 4-6 hours incubation at 37°C for clotting of the plasma.

INTERPRETING RESULTS

Turbidity indicates microbial growth.

The coagulase test is considered positive if the clot volume is more than half of the original liquid volume.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: clear, amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 3 years.
Medium in tubes: 2 years.

QUALITY CONTROL

Tubes are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU
Incubation conditions: 37 ± 1°C for 24 ± 2 hours. 40-48 h under anaerobic atmosphere for *B. fragilis*.

QC Table.

Microorganism		Growth
Staphylococcus aureus	WDCM 00034	Good
Escherichia coli	ATCC® 25922	Good
Streptococcus pneumoniae	ATCC® 6305	Good
Bacteroides fragilis	ATCC® 25285	Good

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product Description	Format	Packaging	Ref.
Brain Heart Infusion Broth	Tube	20 x 2 ml tubes	24141
Brain Heart Infusion Broth	Tube	50 x 5 ml tubes	27502
Brain Heart Infusion Broth	Tube	20 x 9 ml tubes	24480
Brain Heart Infusion Broth	Tube	20 x 10 ml tubes	24104
Brain Heart Infusion Broth	Tube	100 x 10 ml tubes	26104
Brain Heart Infusion Broth	Bottle	6 x 200 ml bottles	412010
Brain Heart Infusion Broth	Dehydrated medium	100 g of powder	620008
Brain Heart Infusion Broth	Dehydrated medium	500 g of powder	610008
Brain Heart Infusion Broth	Dehydrated medium	5 kg of powder	6100085

This document is available from the online Support Center:
liofilchem.com/ifu-sds

COAGULASE TEST

Freeze-dried rabbit plasma for coagulase test.

TYPICAL FORMULA

Freeze-dried rabbit plasma with EDTA…….. 4.0 ml

DESCRIPTION

COAGULASE TEST is constituted by rabbit freeze-dried plasma with EDTA, for the determination of enzyme coagulase produced by *Staphylococcus aureus*.

PRINCIPLE

The test shows the coagulant activity of potentially pathogenic staphylococci on plasma. This activity is due to two factors at least: free coagulase (extracellular enzyme) and bound coagulase or clumping factor (antigen of cellular wall).
The enzyme is present in the most of biotypes belonging to the species of Staphylococcus aureus and in biotypes belonging to the species *S. intermedius* and *S. hycus,* pathogenic opportunist for animals, and is always absent in saprophyte and commensal species.

TECHNIQUE

1. Take a bottle of freeze-dried plasma from the kit and aseptically reconstitute with 4 ml of sterile physiological solution. Mix until completely dissolved, avoiding foam formation.
2. Prepare a culture broth in Brain Heart Infusion, taking one or more colonies from selective culture media for *Staphylococcus aureus* isolation, and incubate at 36+/-1°C for 4-6 hours.
3. Mix 0.5 ml of Coagulase Test with 0.5 ml of culture broth and incubate at 36+/-1°C for 1-2-4-8-24 hours.
4. Examine for the formation of coagulum, carefully inclining the tube to one side, without shaking. Do not incubate after 24 hours because cases of fibrinolysis may occur.

INTERPRETATION OF RESULTS

In the most or in the whole culture medium the presence of enzyme coagulase is shown by the formation of a well defined coagulum. The test determines both free and bound coagulase.

STORAGE

2-8°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

WARNING and PRECAUTIONS

The product contains dangerous substances according to directives 1999/45/CE and 2001/60/CE or for which exist recognized exposure limits.

DISPOSAL of WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. *W.E. Kloos and J.H. Jorgensen "Staphylococci" p. 143-153. In E.H. Lennette, A. Balows, W.J. Hausler, H.J. Shadomy (eds) Manual of Clinical Microbiology, 4th Edition, American Society for Microbiology, Washington, D.C. 1985.*

PRODUCT SPECIFICATIONS

NAME
COAGULASE TEST

PRESENTATION
Glass bottles containing 4 ml rabbit freeze-dried plasma with EDTA.

STORAGE
2-8°C

PACKAGING

Code	Content	Packaging
88030	5 bottles x 40 test	• 5 bottles in box

USE
COAGULASE TEST is constituted by rabbit freeze-dried plasma with EDTA, for the determination of enzyme coagulase produced by *Staphylococcus aureus*.

TECHNIQUE
Refer to technical sheet of the product.

APPEARANCE of the MEDIUM
Compact freeze-dried, beige to light pink.

SHELFLIFE
2 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Incubation conditions:12-24 h at 36 ± 1°C, in aerobiosis

Microorganisms		Coagulation
Escherichia coli	ATCC 25922	-
Staphylococcus aureus	ATCC 25923	+

TABLE OF SYMBOLS

Symbol	Meanings
REF	Catalogue number
IVD	*In vitro* Diagnostic Medical Device
	Manifacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult accompanying documents

CE IVD

Instructions For Use
ENGLISH

CLED Agar

Medium for the isolation, enumeration and differentiation of pathogenic bacteria in urine specimens.

DESCRIPTION

CLED Agar is a medium used for microbiological urine analysis since it supports the growth of all urinary potential pathogens providing good colony differentiation.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Gelatin	4.0
Enzymatic Digest of Casein	4.0
Beef Extract	3.0
Lactose	10.0
L-Cystine	0.128
Bromothymol Blue	0.02
Agar	15.0
Final pH 7.3 ± 0.2 at 25°C	

METHOD PRINCIPLE

Enzymatic digest of gelatin, enzymatic digest of casein and beef extract provide amino acids, nitrogen, carbon, vitamins and minerals required for organisms growth. Lactose is the fermentable carbohydrate. L-cystine is a growth supplement for cystine-dependent organisms. Bromothymol blue is the pH indicator changing color from green to yellow when lactose fermentation lowers the pH. Agar is the solidifying agent. Lack of electrolytes suppresses the swarming of *Proteus* and *Shigella* species.

PREPARATION

Dehydrated medium: Suspend 36.1 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

Medium in bottles: Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

Urine must be directly streaked over the agar surface no later than 2 h after collection or must be kept at 2-8°C (not longer than 24 h) to avoid microbial overgrowth. Use a calibrated loop (0.01 or 0.001 ml) to inoculate the medium with the undiluted, well-mixed urine sample. Incubate aerobically at 35 ± 2°C for 18-48 h.

RESULTS INTERPRETATION

Count the number of colonies on the plate. Each colony correspond to 100 or 1000 CFU/ml of urine, using a 0.01 ml or 0.001 ml loop, respectively. Observe the color and the morphology of the colonies for presumptive identification according to the ID table. Further tests should be performed for confirmation.

ID Table.

Microorganism	Appearance of the colonies
Escherichia coli	Yellow, opaque
Klebsiella, *Enterobacter* spp	Yellow to whitish-blue, mucoid
Proteus spp	Translucent blue
Salmonella spp	Flat blue
Pseudomonas aeruginosa	Green, matt surface and rough periphery
Enterococcus spp	Yellow, about 0.5 mm in diameter
Staphylococcus aureus	Deep yellow
Coagulase negative staphylococci	Pale yellow

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: slightly opalescent, green to blue-green.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Slides are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU
Inoculum for selectivity: 10^4-10^6 CFU
Incubation conditions: aerobically at 35 ± 2°C for 18-24 hours.

QC Table.

Microorganism		Growth	Specification
Escherichia coli	ATCC® 25922	Good	Yellow colonies
Klebsiella pneumoniae	ATCC® 13883	Good	Yellow to whitish-blue colonies
Proteus mirabilis	ATCC® 12453	Good	Blue colonies, no swarming
Salmonella Typhimurium	ATCC® 14028	Good	Blue colonies
Pseudomonas aeruginosa	ATCC® 27853	Good	Green colonies
Entercoccus faecalis	ATCC® 29212	Good	Yellow colonies
Staphylococcus aureus	ATCC® 25923	Good	Small yellow colonies

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for *in vitro* diagnostic use only and must be used by properly trained operators only.

DISPOSAL OF WASTE

Disposal of waste mast be carried out according to national and local regulation in force.

BIBLIOGRAPHY

1. Sandys G.H. (1960) A new method for preventing swarming of *Proteus* spp with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Technol. 17:224.
2. Mackey J.P. and G.H. Sandys (1966) Diagnosis of urinary tract infections. Br. Med. J. 1:1173.

PRESENTATION		Contents	Ref.
CLED Agar	90 mm ready-to-use plates	20 plates	10026
CLED Agar	90 mm ready-to-use plates	100 plates	10026*
CLED Agar	140 mm ready-to-use plates	10 plates	10236
CLED Agar	Bottles	6 x 100 ml bottles	402180
CLED Agar	Bottles	6 x 200 ml bottles	412180
CLED Agar	Bottles	6 x 500 ml bottles	470110
CLED Agar	Dehydrated medium	500 g of powder	610012
CLED Agar	Dehydrated medium	100 g of powder	620012
CLED Agar	Dehydrated medium	5 kg of powder	6100125

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

COLUMBIA AGAR (Horse Blood 5%)

Medium for the isolation and cultivation of nonfastidious and fastidious microorganisms from clinical and not clinical material.

TYPICAL FORMULA	(g/l)
Peptospecial	23.0
Sodium Chloride	5.0
Corn Starch	1.0
Agar	14.0
Horse Defibrinated Bood	50.0 ml
Final pH 7.3 ± 0.2	

DESCRIPTION

COLUMBIA AGAR (Horse Blood 5%) is a medium used for the isolation and cultivation of nonfastidious and fastidious microorganisms from clinical and not clinical material and for the detection of haemolytic reactions.

PRINCIPLE

Peptospecial provides nitrogen, carbon, amino acids and vitamins. Sodium chloride maintains the osmotic balance of the medium. Corn starch is included to absorb toxic by-products contained in the specimen and serves as energy source for organisms possessing alpha-amylases. Agar is the solidifying agent. Supplementation with blood provides additional growth factors for fastidious microorganisms and allows detection of haemolytic reactions.

TECHNIQUE

Inoculate by streaking the specimen onto the surface of the medium with a sterile loop in order to isolate pure cultures from specimens containing mixed flora. Then stab the agar several times in order to deposit β-hemolytic streptococci below the agar surface. Subsurface growth will show the haemolytic reactions due to the activity of both oxygen-stable and oxygen-liable streptolysins.
Incubate at 37°C for 18-48 hours, aerobically, anaerobically or under 5-10% CO_2 atmosphere, according to established laboratory procedures.

INTERPRETATION OF RESULTS

Examine plates for growth and hemolytic reactions. Four types of hemolysis on blood agar media can be described:

- α-hemolysis is the reduction of haemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish decolorization of the medium;
- β-hemolysis is the lysis of red blood cells, producing a clear zone surrounding the colony;
- γ-hemolysis indicates no destruction of red blood cells and no change in the color of the medium;
- ά-hemolysis indicates a partial lysis.

STORAGE AND TRANSPORT CONDITIONS

2-8°C away from light, until the expiry date on the label. However, our stability studies have shown that the transport at 18-25°C for 4 days, or at 35-39°C for 48 hours, does not alter in any way the performance of the product. Eliminate if signs of deterioration or contamination are evident.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. Ellner P.D., C.J. Stoessel, E. Drakeford, and F. Vasi (1966) A new culture medium for medical bacteriology. Am. J.Clin. Path. 45, 502-504.
2. Ruoff K.L. (1995) Streptococcus, In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (eds) Manual of Clinical Microbiology 6th ed. American Society for Microbiology, Washington, D.C.
3. Isenberg and Garcia (eds.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed., American Society for Microbiology, Washington, D.C.

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CE IVD

PRODUCT SPECIFICATIONS

NAME
COLUMBIA AGAR (Horse Blood 5%)

PRESENTATION
Ready to use plates (90 mm) containing 22+/−1 ml of medium

STORAGE
2-8°C

PACKAGING

Ref.	Content	Packaging
10025	20 plates	• 10 plates in thermally soldered film • 2 x 10 plates in cardboard box
10025*	100 plates	• 10 plates in thermally soldered film • 10 piles (10 x 10 plates) in cardboard box

pH OF THE MEDIUM
7.3 ± 0.2

USE
COLUMBIA AGAR (Horse Blood 5%) is a medium used for the isolation and cultivation of nonfastidious and fastidious microorganisms from clinical and not clinical material and for the detection of haemolytic reactions

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Cherry red medium, opaque

SHELFLIFE
60 days

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Sterility control
   7 days at 22 ± 2°C, in aerobiosis
   7 days at 35 ± 2°C, in aerobiosis
3. Microbiological control
   Inoculum for productivity: 10-100 CFU/ml
   Incubation Conditions: 18-24 hours at 35 ± 2°C, in 10% CO_2 atmosphere

Microorganism		Growth	Hemolysis
Streptococcus pyogenes	ATCC® 19615	Good	β-hemolysis
Enterococcus faecalis	ATCC® 19433	Good	None
Escherichia coli	ATCC® 25922	Good	None
Staphylococcus aureus	ATCC® 25923	Good	β-hemolysis

TABLE OF SYMBOLS

LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	Do not reuse

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CE IVD

COLUMBIA AGAR BASE

Medium for fastidious microorganisms isolation from clinical samples.

TYPICAL FORMULA	(g/l)
Peptospecial	23.0
Starch	1.0
Sodium Chloride	5.0
Agar	14.0

Final pH = 7.3 ± 0.2 at 25 °C.

DIRECTIONS

Suspend 43.0 g of powder in 1 liter of distilled or deionized water. Heat to boiling until completely dissolved. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and aseptically add 5% defibrinated sterile sheep blood. Mix well. Dispense in petri dishes.
Columbia Agar Base can be also enriched in various way:
· with 2 vials of CNA (Staf / Strep) supplement (colistin sulphate 5 mg/vial, nalidixic acid 8 mg/vial, code 81048), each one reconstituted with 5 ml of sterile distilled water; final medium will contain colistin sulphate 10 mg/l and nalidixic acid 16 mg/l.
· with 2 vials of *Gardnerella vaginalis* supplement (gentamicin 3 mg/vial, amphotericin B 1mg/vial, nalidixic acid 15 mg/vial, code 81040), each one reconstituted with 5 ml of a 1:1 solution of ethyl alcohol and sterile distilled water; final medium will contain gentamicin 6 mg/l, amphotericin B 2 mg/l and nalidixic acid 30 mg/l.

DESCRIPTION

COLUMBIA AGAR BASE, enriched with sterile sheep blood (5%), is suitable for isolation and growth of fastidious microorganisms such as streptococci, staphylococci, pneumococci and listeriae from clinical samples.

TECHNIQUE

Inoculate the medium with the specimen streaking by a sterile loop and incubate at 36 ± 1 °C for 18-48 hours aerobically, anaerobically or under conditions of increased CO_2 (5-10%), in accordance with established laboratory procedures.
Examine plates for growth and hemolytic reactions. Four types of hemolysis on blood agar media can be described:
1. α-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.
2. β-hemolysis is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. γ-hemolysis indicates no destruction of red blood cells and no change in the color of the medium.
4. ά-hemolysis indicates a partial lysis.

QUALITY CONTROL

Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: beige.
Prepared medium
Appearance: opaque.
Color: cherry red.
Incubation conditions: 36 ± 1°C for 18-48 hours at 5-10% CO_2.

Microorganism	ATCC	Growth	Characteristics
Streptococcus pyogenes	19615	good	β-hemolysis
Streptococcus pneumoniae	6303	good	α -hemolysis
Staphylococcus aureus	25923	good	β-hemolysis
Gardnerella vaginalis	14018	good	β-hemolysis

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CE IVD

PERFORMANCE AND LIMITATIONS

When this medium is enriched with 10% sterile sheep blood, heated at 80 °C for 10 minutes until a chocolate color is obtained, and an antibiotic mixture is added (vancomycin, colimycin, trimethoprim, amphoterycin B) it is suitable for the selective isolation of the pathogens neisseria. If used without the addition of blood, the medium is suitable for growing of *Brucella abortus, Yersinia pestis, Clostridium perfrigens and enterobacteria.* Hemolytic reactions of some strains of Group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta –hemolytic on horse and rabbit blood agar and alpha-hemolytic on sheep blood agar.

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.
Store prepared plates at 2-8 °C.

REFERENCES

1. Ellner, P.D., C.J. Stoessel., E. Drakeford, and F. Vasi (1966). A new culture medium for medical bacteriology. Am. J.Clin. Path. **45**, 502-504.
2. Isenberg, H.D. (ed.) (1992). Clinical microbiology procedures handbook, vol. 1 American Society for Microbiolgy, Washington, DC.

PRESENTATION

Product	REF	Σ
COLUMBIA AGAR BASE (11.6 l)	610013	500 g
COLUMBIA AGAR BASE (2.3 l)	620013	100 g
COLUMBIA AGAR BASE (116.2 l)	6100135	5 Kg
SHEEP BLOOD DEFIBRINATED	83296	50 ml
CNA (Staf / Strep) supplement	81048	10 vials
Gardnerella vaginalis supplement	81040	10 vials

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests	IVD *In Vitro* Diagnostic Medical Device
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	Keep away from heat source

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CE IVD

Columbia Agar (Sheep blood 5%)

Medium for the cultivation of fastidious and non-fastidious microorganisms from clinical specimens.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Casein	12.0
Starch	1.0
Peptic Digest of Animal Tissue	5.0
Sodium Chloride	5.0
Yeast Extract	3.0
Beef Extract	3.0
Sheep Blood Defibrinated	50.0 ml
Agar	14.0
Final pH 7.3 ± 0.2	

DESCRIPTION

Columbia Agar (Sheep blood 5%) is a medium used for the growth of fastidious and non-fastidious organisms and determination of haemolytic reactions

PRINCIPLE

Pancreatic digest of casein, beef extract and peptic digest of animal tissue and yeast extract provide nitrogen, carbon, sulfur and other essential growth factors. Sodium chloride maintains the osmotic balance of the medium. Sheep blood defibrinated supplies additional growth factors for fastidious microorganisms and allows to evidence the haemolytic reactions. Agar is the solidifying agent.

TECHNIQUE

Inoculate the plates by streaking the sample to examine onto the surface of the medium using a sterile loop in order to isolate single colonies. Then stab the agar several times to deposit beta-haemolytic streptococci below the agar surface. Incubate plates at 36 ± 1°C for 18-48 hours, in aerobic, anaerobic or microaerophilic atmosphere.

INTERPRETATION OF RESULTS

Observe for growth and haemolytic reactions. Four different kinds of haemolysis can be distinguished:

1. alfa haemolysis: haemoglobin is reduced to metahaemoglobin in the medium that surrounds the colony with a consequent greenish decolouring of the medium;
2. beta haemolysis: it is the lysis of erythrocytes which is evident in a light zone around the colony;
3. gamma haemolysis: it does not occur any destruction of erythrocytes or any change in the medium;
4. alfa-prime haemolysis: it's evident a small zone of complete haemolysis surrounded by an area of partial lysis

STORAGE AND TRANSPORT CONDITIONS

2-8°C away from light, until the expiry date on the label. However, our stability studies have shown that the transport at 18-25°C for 4 days, or at 35-39°C for 48 hours, does not alter in any way the performance of the product. Eliminate if signs of deterioration or contamination are evident.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. Isenberg, H.D. (ed.) (1992). Clinical microbiology procedures handbook, vol. 1 American Society for Microbiolgy, Washington, DC.
2. Ellner, P.D., C.J. Stoessel., E. Drakeford, and F. Vasi (1966). A new culture medium for medical bacteriology. Am. J.Clin. Path. 45, 502-504.

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CE IVD

PRODUCT SPECIFICATIONS

NAME
Columbia Agar (Sheep blood 5%)

PRESENTATION
Ready to use plates (90 mm) containing 22 ± 1 ml of medium

STORAGE
2-8°C

PACKAGING

Ref.	Content	Packaging
11025	20 plates	• 10 plates in thermally soldered film • 2 x 10 plates in cardboard box
11025*	100 plates	• 10 plates in thermally soldered film • 10 piles (10 x 10 plates) in cardboard box

pH OF THE MEDIUM
7.3 ± 0.2

USE
Columbia Agar (Sheep blood 5%) is a medium used for the cultivation of fastidious microorganisms from clinical specimens

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Cherry red, opaque

SHELFLIFE
90 days

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Sterility control
   7 days at 22 ± 2°C, in aerobiosis
   7 days at 35 ± 2°C, in aerobiosis
3. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Incubation Conditions: 18-24 hours at 36 ± 1°C

Microorganism		Growth	Haemolysis
Staphylococcus aureus	ATCC® 25923	Good	β-haemolysis
Streptococcus pneumoniae	ATCC® 6305	Good	α-haemolysis
Streptococcus pyogenes	ATCC® 19615	Good	β-haemolysis
Escherichia coli	ATCC® 25922	Good	None

TABLE OF SYMBOLS

LOT	Batch code	IVD	*In vitro* Diagnostic Medical Device		Manufacturer		Use by		Fragile, handle with care
REF	Catalogue number		Temperature limitation	Σ	Contains sufficient for <n> tests		Caution, consult instructions for use		Do not reuse

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CE IVD

D/E Neutralizing Broth

Liquid medium for testing antiseptics and disinfectants.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	5.0
Yeast Extract	2.5
Dextrose	10.0
Sodium Thioglycollate	1.0
Sodium Thiosulfate	6.0
Sodium Biosulfite	2.5
Lecithin	7.0
Bromcresol Purple	0.02
Final pH 7.6 ± 0.2 at 25°C	

DESCRIPTION

D/E Neutralizing Broth is a liquid medium used for neutralizing disinfectants in qualitative procedures for environmental sampling, allowing to distinguish between bacteriostatic and bactericidal activity.

PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, minerals, vitamins and other nutrients which support the growth of microorganism. Yeast extract is a source of vitamins, particularly of B-group. Dextrose is the fermentable carbohydrate. Sodium thioglycollate neutralizes mercurial compounds. Sodium thiosulfate neutralizes iodine and chlorine. Sodium biosulfite neutralizes aldehydes. Lecithin neutralizes quaternary ammonium compounds. Bromcresol purple is the pH indicator.

Supplementation with Tween 80 (ref. 80031), a non-ionic surface-active agent, serves to neutralize phenoilics.

PREPARATION

Suspend 34.0 g of powder in 1 liter of deionized or distilled water. Add 5 ml of Tween 80 Supplement (ref. 80031). Mix well. Sterilize by autoclaving at 121°C for 15 minutes. Distribute into final containers.

TECHNIQUE

Add 1 ml of disinfectant to a tube containing 9 ml of D/E Neutralizing Broth. Inoculate the tube with a overnight microbial culture and incubate at 35 ± 2°C for 24-48 hours.

To determine whether viable organisms are present in a “bacteriostatic” or “bactericidal” solution, inoculate samples from the broth onto D/E Neutralzing Agar (ref. 610086) or other suitable media. Incubate plates at 35 ± 2°C for 48 hours.

INTERPRETATION OF RESULTS

Either the color change of the medium from purple to yellow or the formation of a pellicle on the surface of the broth indicate microbial growth.

Growth on the plates from negative broth tubes indicates a bacteriostatic substance. No growth on the plates from negative broth tubes indicates a bactericidal substance. All positive broth tubes should be positive on the plates.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared tubes at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

- Dey B.P. and F.B. Engley Jr. (1995) Comparison of Dey and Engly (D/E) Neutralizing Medium to Letheen Medium and Standard Methods Medium recovery of Staphylococcus aureus from sanitized surfaces. J. Ind. Microbiol. 14:21-25
- Dey B.P. and F.B. Engley Jr. (1994) Neutralization of antimicrobial chemicals by recovery media. J. Microbiol. Methods. 19:51-58.
- Dey B.P. and F.B. Engley Jr. (1983) Methodology for recovery of chemical treated Staphylococcus aureus with neutralizing medium. Appl. Environ. Microbiol. 45:1533-1537.
- Engely F.B. Jr. and B.P. Dey (1970) A universal neutralizing medium for antimicrobial chemicals. Chem. Spec. Manuf. Assoc. Proc. Mid-Year Meet. p. 100-106.

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PRODUCT SPECIFICATIONS

NAME
D/E Neutralizing Broth

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610088	500 g	500 g of powder in plastic bottle
620088	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.6 ± 0.2

USE
D/E Neutralizing Broth is a liquid medium used for neutralizing disinfectants in qualitative procedures for environmental sampling, allowing to distinguish between bacteriostatic and bactericidal activity

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: bluish-gray
Ready-to-use medium
Appearance: opaque
Colour: purple

SHELFLIFE
2 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Tubes are prepared with and without the addition of disinfectants.
   Inoculum: 10^3-10^4 CFU
   Incubation conditions: 24-48 h at 35 ± 2°C

Microorganism		Growth
Bacillus subtilis	ATCC® 6633	Good
Escherichia coli	ATCC® 8739	Good
Pseudomonas aeruginosa	ATCC® 27853	Good
Salmonella typhimurium	ATCC® 14028	Good
Staphylococcus aureus	ATCC® 6538	Good

TABLE OF SYMBOLS

LOT Batch code	Do not reuse	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	

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Egg Yolk Emulsion

Liquid supplement for the identification of lecithinase-producing bacteria (*Bacillus* and *Clostridium* species).

Instructions For Use
ENGLISH

DESCRIPTION

A sterile, stabilized egg yolk emulsion (50%) for use in the preparation of various culture media, including the followings:

Polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA) and Mannitol egg yolk polymixin agar (MYP) for detection of *Bacillus cereus,* according to ISO 7932, ISO 21871 and FDA-BAM;

Tryptose sulfite cycloserine agar (TSC) and Shahidi-Ferguson perfringens agar (SFP) for detection of *Clostridium perfringens*.

TYPICAL COMPOSITION (Per Litre)

Egg Yolk	500 ml
Saline Solution (0.85% NaCl)	500 ml

METHOD PRINCIPLE

When added to a nutrient medium, egg yolk emulsion permits the detection of microbial lecithinase activity.

PREPARATION

Shake the bottle well to suspend any sediment. Aseptically add to the culture medium, sterilised and cooled to 45-50°C. Mix well and pour into sterile Petri dishes.

See details in the Technical Sheet of the specific medium:

- Bacillus cereus Agar Base (Mossel), ref. numbers 610114, 620114 and 402710;
- Bacillus cereus Agar Base (Pemba), ref. numbers 610136 and 620136;
- Clostridium perfringens Agar Base, ref. numbers 610207 and 620207.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical sheet of the medium being prepared.

STORAGE

Store at 2-8°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

1 year.

QUALITY CONTROL

Appearance: Yellowish opaque emulsion. May contain a precipitate that can be resuspended.

Microbiological Performance:

Control strains

Bacillus cereus WDCM 00001 (ATCC® 11778, NCTC 10320)

Escherichia coli WDCM 00012 (ATCC® 8739, NCTC 12923)

Bacillus subtilis WDCM 00003 (ATCC® 6633, NCTC 10400)

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For *In Vitro* Diagnostic use. For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

Product	Format	Packaging	Ref.
Egg Yolk Emulsion	Bottle	4 x 50 ml	80219
Egg Yolk Emulsion	Bottle	6 x 100 ml	80124

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

Egg Yolk Emulsion

Supplemento liquido per l'identificazione dei batteri produttori di lecitinasi (*Bacillus* e *Clostridium* spp).

Istruzioni per l'uso

ITALIANO

DESCRIZIONE

Un'emulsione sterile stabilizzata di tuorlo d'uovo (50%) da utilizzare nella preparazione di vari terreni di coltura, incluso i seguenti:

Polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA) e Mannitol egg yolk polymixin agar (MYP) per la ricerca di *Bacillus cereus,* secondo ISO 7932, ISO 21871 e FDA-BAM;

Tryptose sulfite cycloserine agar (TSC) e Shahidi-Ferguson perfringens agar (SFP) per la ricerca di *Clostridium perfringens*.

COMPOSIZIONE TIPICA (Per Litro)

Tuorlo d'Uovo	500 ml
Soluzione Salina (85% NaCl)	500 ml

PRINCIPIO DEL METODO

Aggiunto al terreno nutritivo, l'emulsione di tuorlo d'uovo consente di rilevare l'attività della lecitinasi microbica.

PREPARAZIONE

Agitare bene il flacone per sospendere eventuali sedimenti. Aggiungere asetticamente al terreno di coltura, sterilizzato e raffreddato a 45-50°C. Mescolare bene e versare in piastre Petri sterili.

Vedere i dettagli nella Scheda Tecnica del terreno specifico:

- Bacillus cereus Agar Base (Mossel), numeri di catalogo (ref.) 610114, 620114 e 402710;
- Bacillus cereus Agar Base (Pemba), numeri di catalogo (ref.) 610136 e 620136;
- Clostridium perfringens Agar Base, numeri di catalogo (ref.) 610207 e 620207.

TECNICA ED INTERPRETAZIONE DEI RISULTATI

Fare riferimento alla scheda tecnica del terreno in preparazione.

E.M.B. LEVINE AGAR

Terreno selettivo per l'isolamento di enterobatteri gram-negativi (armonizzato Farmacopea SU)

FORMULA TIPICA	(g/l)
Peptone	10.0
Lattosio	10.0
Fosfato dipotassico	2.0
Eosina Y	0.4
Blu di Metilene	0.065
Agar	15.0
pH finale 7.2 ± 0.2 a 25°C	

DESCRIZIONE

E.M.B. LEVINE AGAR è un terreno selettivo per l'isolamento di enterobatteri gram-negativi conforme con le specifiche della Famacopea degli Stati Uniti (USP). E.M.B. LEVINE AGAR è utilizzato per l'analisi sia di campioni clinici che alimentari come i prodotti caseari principalmente per l'individuazione e la conferma dei coliformi.

PRINCIPIO

Il peptone è la fonte di azoto, il lattosio è il carboidrato fermentabile ed il fosfato dipotassico è il tampone. Eosina Y e blu di metilene sono gli indicatori. Questi coloranti permettono anche di differenziare i microrganismi che fermentano il lattosio da i non fermentanti sulla base del loro assorbimento all'interno delle colonie batteriche. Il blu di metilene agisce anche come agente selettivo in grado di inibire parzialmente i batteri gram-positivi.

PREPARAZIONE

Sospendere 37.5 g di polvere in 1 litro di acqua distillata. Scaldare fino a completo scioglimento. Autoclavare a 121°C per 15 minuti. Raffreddare a 45-50°C. Mescolare accuratamente. Distribuire in piastre petri.

TECNICA

Utilizzare le procedure appropriate per ottenere colonie isolate dai campioni in esame. Si dovrebbe seminare anche un terreno non selettivo per aumentare la probabilità di recupero quando la popolazione di batteri gram-negativi è bassa e per avere inoltre una indicazione degli altri organismi presenti nel campione. Incubare le piastre, al riparo dalla luce, a 35±2 per 18-24 ore. Se dopo 24 ore non si verifica nessuna crescita, reincubare per altre 24 ore.

INTERPRETAZIONE DEI RISULTATI

I microrganismi che fermentano il lattosio, come i coliformi, mostrano colonie blu-nere, mentre le colonie dei lattosio-non fermentanti come *Salmonella* spp e *Shigella* spp, appaiono incolori, trasparenti o color ambra. Alcuni batteri gram-positivi, come gli streptococchi fecali, stafilococchi e lieviti, crescono su questo terreno formando di solito colonie puntiformi. Diversi batteri gram-negativi non patogeni e che non fermentano il lattosio sono in grado di crescere su questo terreno ma possono essere distinti dai ceppi patogeni tramite analisi biochimiche.

CONSERVAZIONE

La polvere è molto igroscopica, conservare a 10-30°C in ambiente asciutto nel suo contenitore originale chiuso ermeticamente. Utilizzare prima della data di scadenza apposta sull'etichetta o finché non sono evidenti segni di deterioramento o contaminazione. Conservare le piastre pronte a 2-8°C al riparo dalla luce.

AVVERTENZE E PRECAUZIONI

Il prodotto non contiene sostanze nocive in concentrazioni superiori ai limiti stabiliti dalla legislazione corrente e perciò non è classificato come pericoloso. Comunque per un uso corretto del prodotto si raccomanda di consultare la scheda di sicurezza. Il prodotto è progettato esclusivamente per uso diagnostico *in vitro* e deve essere utilizzato da parte di personale qualificato.

SMALTIMENTO DEI RIFIUTI

Smaltimento dei rifiuti deve essere effettuato secondo le normative nazionali e locali vigenti.

BIBLIOGRAFIA

1. Holf-Harris and Tongue (1916) J. infect. Dis. 18:596.
2. Levine (1918) J. Infect. Dis. 23:43.
3. Marshall ed. (1993) Standard methods for the examination of dairy products, 16th ed. American Public Health Association , Washington, D.C.
4. Downes and Ito ed. (2001) Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association , Washington, D.C.
5. United States Pharmacopeial Convention, Inc. (2001) The United States Pharmacopeia 25/The National formulary 20 – 2002. The United States Pharmacopeial Convention, Rockville, Md.

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CE IVD

SPECIFICHE DI PRODOTTO

DENOMINAZIONE
E.M.B. LEVINE AGAR

PRESENTAZIONE
Terreno disidratato in polvere

CONSERVAZIONE
10-30°C

CONFEZIONAMENTO

Ref.	Contenuto	Confezionamento
610019	500 g	500 g di polvere in contenitore di plastica
620019	100 g	100 g d i polvere in contenitore di plastica

pH DEL TERRENO
7.2 ± 0.2

IMPIEGO
E.M.B. LEVINE AGAR è un terreno selettivo per l'isolamento di enterobatteri gram-negativi conforme con le specifiche della Famacopea degli Stati Uniti (USP)

TECNICA
Far riferimento alla scheda tecnica del prodotto

ASPETTO DEL TERRENO
Terreno disidratato
Aspetto: omogeneo
Colore: rosso chiaro-porpora
Terreno in piastra
Aspetto: opaco
Colore: rosso scuro blu-porpora

VALIDITA' DALLA DATA DI PRODUZIONE
4 anni

CONTROLLO DI QUALITÀ
1. Controllo caratteristiche generali, etichettatura e stampa
2. Controllo microbiologico
   Inoculo per produttività: 10-100 UFC/ml
   Inoculo per selettività: 104-105 UFC/ml
   Inoculo per specificità: ≤104 UFC/ml
   Condizioni di incubazione: 18-24 ore a 36 ± 1°C

Microrganismo	ATCC	Crescita	Caratteristiche
Escherichia coli	25922	Buona	Colonie verdi con riflessi metallici
Klebsiella pneumoniae	13883	Buona	Colonie rosa
Proteus mirabilis	25933	Buona	Colonie Incolori
Pseudomonas aeruginosa	27853	Buona	Colonie Incolori
Salmonella typhimurium	14028	Buona	Colonie Incolori
Streptococcus faecalis	19433	Inibizione	---

TABELLA DEI SIMBOLI

LOT Numero di lotto	IVD Per uso diagnostico *in vitro*	Fabbricante	Data di scadenza	Tenere lontano da fonti di calore
REF Numero di catalogo	Limiti di temperatura	Contenuto sufficiente per <n> test	Attenzione, consultare le istruzioni per l'uso	

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CE IVD

Fluid Thioglycollate Medium

Liquid medium for sterility test and cultivation of fastidious anaerobic and aerobic microorganisms, according to Harmonized USP/EP/JP and ISO 7937.

Instructions For Use
ENGLISH

DESCRIPTION

Fluid Thioglycollate Medium is a general purpose liquid enrichment medium used for sterility control of pharmaceutical products and for cultivation and isolation of fastidious anaerobic and aerobic microorganisms.

The composition is in accordance with the requirements of the Harmonized US, European and Japanese Pharmacopoeia as well as with ISO 7937 for isolation of *Clostridium perfringens*.

TYPICAL FORMULA*	(g/l)
Enzymatic Digest of Casein	15.0
Yeast Extract	5.0
Glucose	5.5
Sodium Chloride	2.5
Sodium Thioglycollate	0.5
L-Cystine	0.5
Resazurin	0.001
Agar	0.75

Final pH 7.1 ± 0.2 at 25°C

*Formula may be adjusted and/or supplemented as required to meet performance specifications;
Grams per litre of purified water.

METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Glucose is a source of energy. Sodium chloride maintains the osmotic balance of the medium. Sodium thioglycollate and L-cystine are included to reduce the redox potential of the medium and create an anaerobic atmosphere. These reducing agents also neutralize the bacteriostatic effects of mercury and other heavy metal compounds in the preparation to be tested for sterility. Resazurin is an oxidation-reduction indicator being pink when oxidized ad colorless when reduced. The small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintaining anaerobiosis in the lower depths of the medium.

PREPARATION

Dehydrated medium	Suspend 29.8 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Dispense into appropriate containers. Sterilize in autoclave at 121°C for 15 minutes.
Medium in tubes/bottles	If the medium exhibits more than 20% pink color (due to oxidation), the medium may be reheated once for 5 minutes with cap slightly loosened in steam or boiling water in order to expel the oxygen.

TEST PROCEDURE

The medium can be directly inoculated with the test sample (the amount of the inoculated sample material should not be exceed 10% volume of the medium). Incubate at 30-35°C for up to 14 days. Growth of strictly aerobic bacteria can be improved by slightly loosening the cap.

According to ISO 7937 for confirmation of *Clostridium perfringens* incoculate each black colony from Sulfite Cycloserine Agar (ref. 402700) into Fluid Thioglycollate Medium. Incubate at 37 ± 1°C for 18-24 hours. Subsequently, transfer 5 drops of the enrichment culture into Lactose Sulfite Medium (ref. 610358) and incubate at 46 ± 1°C for 18-24 hours.

INTERPRETING RESULTS

Turbidity of the medium indicates microbial growth. Obligate anaerobic microorganisms such as *Clostridium sporogenes* are growing in the lower, yellowish part of the broth medium. The growth of facultative anaerobic microorganisms such as *Staphylococcus aureus* is distributed throughout all the medium. Aerobic microorganisms such as *Pseudomonas aeruginosa* are able to grow in the upper slightly pink layer (oxidized part) of the medium.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles or tubes: 2 years.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, light beige.

Appearance of Prepared Medium: Slightly opalescent, light amber (20% or less of upper layer may be pink).

Expected Cultural Response:

Control strain		Inoculum	Incubation	Specification
Bacillus subtilis[a]	WDCM 00003 (ATCC 6633; NCTC 10400)	≤100 CFU	24 h 32.5 ± 2.5°C	Visible turbidity
Clostridium sporogenes[a]	WDCM 0008 (ATCC 19404; NCTC 532)			
Escherichia coli[a]	WDCM 00012 (ATCC 8739; NCTC 12923)			
Pseudomonas aeruginosa[a]	WDCM 00026 (ATCC 9027; NCTC 12924)			
Staphylococcus aureus[a]	WDCM 00032 (ATCC 6538; NCTC 10788)			
Clostridium perfringens[b]	WDCM 00007 (ATCC 13124; NCTC 8237)		18-24 h 37 ± 1°C	
Candida albicans[a]	WDCM 00054 (ATCC 10231; NCPF 3179)		48 h 22.5 ± 2.5°C	
Aspergillus brasiliensis[a]	WDCM 00053 (ATCC 16404; NCPF 2275)		72 h 22.5 ± 2.5°C	Slight to good turbidity

[a] Pharmacopoeia Growth Promotion Test

[b] EN ISO 11133

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For *in-vitro* diagnostic use (see the product list the next page). **For professional use only**. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed on the next page. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.	
Fluid Thioglycollate Medium	Tube	100 x 9 ml	26493	•
Fluid Thioglycollate Medium	Tube	20 x 10 ml	24124	
Fluid Thioglycollate Medium	Tube	100 x 10 ml	26124	•
Fluid Thioglycollate Medium	Tube	10 x 20 ml	21241	•
Fluid Thioglycollate Medium	Tube	20 x 20 ml	24241	•
Fluid Thioglycollate Medium	Bottle (screw cap)	25 x 90 ml	452110	
Fluid Thioglycollate Medium	Bottle (screw cap)	6 x 100 ml	452060	•
Fluid Thioglycollate Medium	Bottle (screw cap)	25 x 100 ml	453060	
Fluid Thioglycollate Medium	Bottle (flip-off cap)	6 x 100 ml	400020	
Fluid Thioglycollate Medium	Bottle (flip-off cap)	25 x 100 ml	453020	
Fluid Thioglycollate Medium	Bottle (crimp cap)	6 x 100 ml	495020	
Fluid Thioglycollate Medium	Bottle (perforable cap)	6 x 100 ml	493000	
Fluid Thioglycollate Medium	Bottle (wide neck)	6 x 500 ml	470300	
Fluid Thioglycollate Medium	Bottle (screw cap)	6 x 900 ml	463100	
Fluid Thioglycollate Medium	Dehydrated medium	100 g	620050	
Fluid Thioglycollate Medium	Dehydrated medium	500 g	610050	
Fluid Thioglycollate Medium	Dehydrated medium	5 kg	6100505	

• CE-marked product for *in-vitro* diagnostic use

All the other above listed products are not CE-IVD marked.

This IFU document and the SDS are available from the online Support Center:
liofilchem.com/ifu-sds

GIOLITTI-CANTONI BROTH BASE

Basal medium for *Staphylococcus aureus* enrichment from foods, recommended by ISO 6888-3: 2003.

TYPICAL FORMULA	(g/l)
Tryptone	10.0
Beef Extract	5.0
Yeast Extract	5.0
D-Mannitol	20.0
Lithium Chloride	5.0
Sodium Chloride	5.0
Glycine	1.2
Sodium Pyruvate	3.0

Final pH = 6.9 ± 0.2 at 25 °C.

DIRECTIONS

Suspend 54.2 g of powder in 1 liter of distilled or deionized water. Heat gently and shake until completely dissolved. Distribute into tubes in amounts of 19 ml. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C. Aseptically add 0.3 ml of Potassium Tellurite 3.5% supplement (code 80291) to each tube or 0.03 ml when testing meat products or quality control organisms. Mix well.

DESCRIPTION

GIOLITTI-CANTONI BROTH BASE is used with Potassium Tellurite 3.5% supplement in enriching *Staphylococcus aureus* from foods during isolation procedures, as recommended by ISO 6888-3: 2003.

TECHNIQUE

Inoculate 1 g or 1 ml of test sample (0.1 g or 0.1 ml when testing meat or meat products) and 1 ml of each of a suitable decimal dilution series of the test sample into duplicate tubes.
Overlay each tube with 5 ml of sterile molten paraffin wax to an approximate height of 2 cm. Incubate at 36 ± 1 °C for 40-48 hours. Examine daily. Read tubes for blackening of the medium (a positive reaction) or no blackening (a negative reaction). If blackening occurs subculture on Baird Parker Agar, it confirms the isolation of *S. aureus.*

QUALITY CONTROL

Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: tan.
Prepared medium
Appearance: clear without significant precipitate.
Color: medium amber.
Incubation conditions: 36 ± 1 °C for 40-48 hours.

Microorganism	ATCC	Growth	Characteristics
Escherichia coli	25922	inhibited	no blackening
Micrococcus luteus	10240	inhibited	no blackening
Staphylococcus aureus	25923	good	blackening
Staphylococcus aureus	6538	good	blackening

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STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared tubes at 2-8 °C.

REFERENCES

1. Giolitti, G., and C. Cantoni. (1996). A medium for the isolation of staphylococci from foodstuffs. J. Appl. Bacteriol. **29**: 395-398.
2. International Dairy Federation. (1978). IDF Standard 60A.
3. ISO 6888-3: 2003. Microbiology of food and animal feeding stuffs- Horizontal medium for the enumeration of coagulase – positive staphylococci (*Staphylococcus aureus* and other species).

PRESENTATION

Product	REF	Σ
GIOLITTI CANTONI BROTH BASE (9.2 l)	610100	500 g
GIOLITTI CANTONI BROTH BASE (1.8 l)	620163	100 g
POTASSIUM TELLURITE 3.5% supplement	80291	5 x 10 ml

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests	Keep away from heat source
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	

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Plate Count Agar

Medium for the enumeration of bacteria in food, water and other materials, according to APHA and ISO 4833.

DESCRIPTION

Plate Count Agar is a medium used for the determination of the total microbial content from food and animal feed, water and other materials.

This medium, also known as Tryptone Glucose Yeast Agar or Casein-Peptone Dextrose Yeast Agar, complies with the specifications given by the American Public Health Association and ISO 4833.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	5.0
Yeast Extract	2.5
Glucose	1.0
Agar	15.0

Final pH 7.0 ± 0.2 at 25°C

METHOD PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Glucose is the fermentable carbohydrate. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 23.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes. Note: ISO 4833 recommends to add 1.0 g of skimmed milk powder per liter of medium when dairy products are examined.
Medium in tubes/bottles	Melt the content of the tube/bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the tube/bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

1. Perform serial dilutions of the test sample in order to achieve a colony count of between 15 and 300 colonies per plate. Use a suitable diluent such as Buffered Peptone Water (ref. 24099) or Maximum Recovery Broth (ref. 20071).
2. Inoculate the medium by pour plating, spread plating or membrane filtration method.
3. Incubation conditions may vary depending on the organisms under study. For a general aerobic count, incubate aerobically at 30°C for 72 hours.

INTERPRETING RESULTS

Count colonies on all plates containing 15-300 colonies. Report the count as CFU per ml of sample allowing for dilution factors.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: slightly opalescent, light amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 2 years.
Medium in slant tubes: 1 year.
Ready-to-use plates: 6 months.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU.
Incubation conditions: aerobically at 30 ± 1°C for 72 ± 3 hours.

QC Table.

Microorganism		Growth
Bacillus subtilis	WDCM 00003	Good
Enterococcus faecalis	WDCM 00009	Good
Escherichia coli	WDCM 00012	Good
Staphylococcus aureus	WDCM 00034	Good
Pseudomonas aeruginosa	WDCM 00024	Good

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 4833 (2003) Microbiology of food and animal feeing stuffs – Horizontal method for the enumeration of microorganisms – Colony count technique at 30°C.
3. Davidson, Roth, and Gambrel-Lenarz (2004) In Wehr and Frank (ed.) Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
4. Kornacki and Johnson (2001) In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington D.C.
5. Greenberg A.E, L.S. Clesceri and A.D. Eaton (1992) Standards methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington D.C.

PRESENTATION		Contents	Ref.
Plate Count Agar	90 mm ready-to-use plates	20 plates	10032
Plate Count Agar	90 mm ready-to-use plates	100 plates	10032*
Plate Count Agar	140 mm ready-to-use plates	10 plates	10232
Plate Count Agar	55 mm ready-to-use RODAC plates	20 plates	15325
Plate Count Agar	60 mm ready-to-use plates	20 plates	163452
Plate Count Agar	Tubes	20 x 22 ml tubes	31073
Plate Count Agar	Tubes	10 x 22 ml tubes	34073
Plate Count Agar	Slant tubes	10 x 9 ml tubes	33070
Plate Count Agar	Bottles	6 x 500 ml bottles	470180
Plate Count Agar	Bottles	6 x 200 ml bottles	412260
Plate Count Agar	Bottles	6 x 150 ml bottles	401940
Plate Count Agar	Bottles	6 x 100 ml bottles	402260
Plate Count Agar	Dehydrated medium	500 g of powder	610040
Plate Count Agar	Dehydrated medium	100 g of powder	620040
Plate Count Agar	Dehydrated medium	5 kg of powder	6100405

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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KLIGLER IRON AGAR

Differential medium for enterobacteria identification.

TYPICAL FORMULA	(g/l)
Proteose Peptone	20.0
Sodium Chloride	5.0
Yeast Extract	3.0
Meat Extract	3.0
Ferrous Sulfate	0.2
Sodium Thiosulphate	0.3
Lactose	10.0
Glucose	1.0
Phenol Red	0.024
Agar	11.0

Final pH = 7.4 ± 0.2 at 25 °C.

DIRECTIONS

Suspend 53.5 g of powder in 1 liter of distilled or deionized water. Heat to boiling until completely dissolved. Dispense into final tubes. Sterilize in autoclave at 121°C for 15 minutes. Cool in a slanting position.

DESCRIPTION

KLIGLER IRON AGAR is a solid medium used to distinguish between *Enterobacteriaceae* on the basis of their ability to ferment lactose and / or glucose and to produce hydrogen sulphide.

TECHNIQUE

Inoculate by stabbing the butt and abundantly streaking the slope. Incubate at 36 ± 1°C for 18-24 hours and check the color of the medium both in the butt and at the slope. Also check for the presence of gas in the butt and the presence of the black precipitate (H_2S).

QUALITY CONTROL

Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: pinkish beige.
Prepared medium
Appearance: slightly opalescent, slight precipitate.
Color: slightly orange-red.
Incubation conditions: 36 ± 1°C for 18-24 hours.

Microorganism	ATCC	Growth	Slant/butt	Gas	H_2S
Citrobacter freundii	8090	good	acid/acid	+	+
Escherichia coli	25922	good	acid/acid	+	-
Proteus vulgaris	6380	good	alkaline/acid	-	+

PERFORMANCE AND LIMITATIONS

A pure culture is essential when inoculating Kligler Iron Agar. If inoculated with a mixed culture, irregular observations may occur.

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.
Store prepared tubes at 2-8°C.

REFERENCES

1. MacFaddin, J.F. (1976). Biochemical tests for identification of medical bacteria.
2. Kligler, I.J. (1918). J. Exp. Med. **28**: 319-322.

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CE IVD

PRESENTATION

Product	REF	Σ
KLIGLER IRON AGAR (9.3 l)	610023	500 g
KLIGLER IRON AGAR (1.8 l)	620023	100 g

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests	IVD *In Vitro* Diagnostic Medical Device
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	Keep away from heat source

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CE IVD

LAURYL TRYPTOSE BROTH (LAURYL SULPHATE BROTH)

Selective medium for coliforms detection in water and wastewater.

TYPICAL FORMULA	(g/l)
Tryptose	20.0
Lactose	5.0
Sodium Chloride	5.0
Sodium Lauryl Sulphate	0.1
Dipotassium Phosphate	2.75
Monopotassium Phosphate	2.75
Final pH 6.8 ± 0.2 at 25°C	

DESCRIPTION

LAURYL TRYPTOSE BROTH provides a selective medium which is used for the detection of coliform organisms in water and wastewater, according to the formula of the American Public Health Association.

PRINCIPLE

Tryptose provides the nitrogen and vitamins required for organism growth. Lactose is the fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Sodium lauryl sulphate is the selective agent used to inhibit organism other than coliforms. Potassium phosphates are the buffering agents.

PREPARATION

Suspend 35.6 g of powder in 1 liter of distilled or deionized water. Heat until completely dissolved. Dispense into final containers provided with Durham tubes. Autoclave at 121°C for 15 minutes.

TECHNIQUE

Inoculate 1 ml of the sample (or of its serial tenfold diluitions) into a tube of LAURYL TRYPTOSE BROTH. Invert once the tube to permit the coming out of air from the Durham tube. Incubate for 24-48 hours at 36±1°C.

INTERPRETATION OF RESULTS

Turbidity of the medium and formation of gas is a positive presumptive test for the presence of coliforms. Perform indole test directly in the tubes for confirmation.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. Christen, G.L., P.M. Davidson, J.S. McAllister,.and L.A. Roth (1992). Coliforms an other indicator bacteria, p. 247-267.
2. Eaton , A.D., L.S. Clesceri, and A.E. Greenberg (ed.) Standard methods for the examination of water and wastewater, 19th ed.
3. Association of Official Analytical Chemist (1995). Bacteriological analytical manual 8th ed.
4. American Public Health Association (1980) Standard methods for the examination of water and wastewater. 15th ed. APHA.
5. ISO Standard 11866-2 Milk and milk products-Enumeration of presumptive *Escherichia coli*.

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PRODUCT SPECIFICATIONS

NAME
LAURYL TRYPTOSE BROTH (LAURYL SULPHATE BROTH)

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGE

Ref.	Content	Packaging
610085	500 g	500 g of powder in plastic bottle
620085	100 g	100 g of powder in plastic bottle
6100855	5000 g	5 kg of powder in plastic container

pH OF THE MEDIUM
6.8 ± 0.2

USE
LAURYL TRYPTOSE BROTH provides a selective medium which is used for the detection of coliform organisms in water and wastewater, according to the formula of the American Public Health Association.

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Dehydrated medium
Appearance: free-flowing, homogeneous
Colour: beige
Prepared medium
Appearance: clear to very slightly opalescent
Colour: light amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print

2. Microbiological control
   Inoculum for productivity: 10-100 CFU/ml
   Inoculum for selectivity: 10^4-10^5 UFC/ml
   Inoculum for specificity: ≤ 10^4 UFC/ml
   Incubation conditions: 48 h at 30 ± 1°C

Microorganism	ATCC®	Growth	Gas
Escherichia coli	25922	Good	+
Salmonella thphimurium	14028	Good	-
Staphylococcus aureus	25923	Inhibited	-
Klebsiella pneumoniae	13883	Good	+

TABLE OF SYMBOLS

LOT Batch code	Keep away from heat sources	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Consult instructions for use	

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Instructions For Use
ENGLISH

MacConkey Agar

Selective and differential medium for detection of Enterobacteriaceae from clinical samples and other materials, according to USP/EP/JP.

DESCRIPTION

MacConkey Agar is a slightly selective medium giving excellent differentiation between lactose-fermenting and lactose-nonfermenting Gram-negative enteric bacilli, from faeces, urine, foodstuffs, waste water and other materials of sanitary importance.

This medium is prepared according to recommendations of the harmonized USP/EP/JP method for the detection of *E. coli* in non sterile pharmaceutical products.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Gelatin	17.0
Peptone from Meat	1.5
Peptone from Casein	1.5
Lactose	10.0
Sodium Chloride	5.0
Bile Salts	1.5
Agar	15.0*
Neutral Red	0.03
Crystal Violet	0.001
Final pH 7.1 ± 0.2 at 25°C	

* Adjusted according to gel strength to meet performance specifications.

METHOD PRINCIPLE

Pancreatic digest of gelatin and peptones from meat and casein provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Lactose is the fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Bile salts and crystal violet are the selective agents, inhibiting Gram-positive organisms and allowing Gram-negative bacteria to grow. Agar is the solidifying agent. Neutral red is the pH indicator.

PREPARATION

Dehydrated medium	Suspend 51.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil for 1 minute shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

Inoculate the plates by directly streaking the specimen on the agar surface or spread the sample from an enrichment culture. Incubate aerobically at 35 ± 2°C for 18-24 h

To isolate *E. coli* in pharmaceutical products, the Harmonized USP/EP/JP method recommends to carry out a two steps enrichment procedure by inoculating the sample in Tryptic Soy Broth (ref. 452080) and afterwards in MacConkey Broth (ref. 494000). Subculture on a plate of MacConley Agar and incubate aerobically at 30-35°C for 18-72 hours.

INTERPRETING RESULTS

Lactose-nonfermenting organisms, such as *Salmonella, Shigella* and *Proteus* spp, form colorless or clear colonies.

Lactose-fermenting organisms, such as *E. coli* and *Klebsiella* spp, grow as pink to red colonies with or without a zone of precipitated bile.

Enterococci, Staphylococci and other Gram-positive bacteria are partially or completely inhibited.

APPEARANCE OF THE MEDIUM

Dehydrated medium: free-flowing, homogeneous, beige-pink.
Prepared medium: slightly opalescent, pinkish-red.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU.
Inoculum for selectivity: 10^4-10^6 CFU.
Incubation conditions: aerobically at 35 ± 2°C for 18-24 hours.
18-72 h at 30-35°C for *E. coli* (Pharmacopoeia growth promotion).

QC Table.

Microorganism		Growth	Specification
Salmonella Typhimurium	ATCC® 14028	Good	Colorless colonies
Shigella flexneri	ATCC® 12022	Good	Colorless colonies
Proteus mirabilis	ATCC® 12453	Good	Colorless colonies
Escherichia coli	ATCC® 8739	Good	Pink colonies with bile ppt
Klebsiella pneumoniae	ATCC® 13883	Good	Pink colonies
Enterococcus faecalis	ATCC® 29212	Partially to completely inhibited	Very small, opaque colonies
Staphylococcus aureus	ATCC® 25923	Inhibited	---

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. European Pharmacopoeia 6.5 (2009). 2.6.13 Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia 32 NF 27 (2009). <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia 4.05 (2008). Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Murray, Baron, Jorgensen, Landry and Pfaller ed. (2007) Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. MacConkey A. (1905) Lactose-fermenting bacteria in faeces. J. Hygiene 8:333-379.

PRESENTATION		Contents	Ref.
MacConkey Agar	90 mm ready-to-use plates	20 plates	10029
MacConkey Agar	90 mm ready-to-use plates	100 plates	10029*
MacConkey Agar	Bottles	6 x 500 ml bottles	470090
MacConkey Agar	Bottles	6 x 200 ml bottles	412240
MacConkey Agar	Bottles	6 x 100 ml bottles	402240
MacConkey Agar	Dehydrated medium	500 g of powder	610028
MacConkey Agar	Dehydrated medium	100 g of powder	620028
MacConkey Agar	Dehydrated medium	5 kg of powder	6100285

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Medical Diagnostic Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

Istruzioni per l'uso
ITALIANO

MacConkey Agar

Terreno selettivo e differenziale per la ricerca delle Enterobacteriaceae
da campioni clinici ed altri materiali, secondo USP/EP/JP.

DESCRIZIONE

MacConkey Agar è un terreno leggermente selettivo utilizzato per l'isolamento dei bacilli enterici Gram negativi dalle feci, urine, alimenti, acque di scarico ed altri materiali di importanza sanitaria e la differenziazione dei microrganismi che fermentano il lattosio dai non fermentanti.

Questo terreno è conforme alle raccomandazioni del metodo armonizzato delle Farmacopee Statunitense (USP) Europea (EP) e Giapponese (JP) per la ricerca di E. coli nei prodotti non sterili.

FORMULA TIPICA	(g/l)
Digerito Pancreatico di Gelatina	17.0
Peptone da Carne	1.5
Peptone da Caseina	1.5
Lattosio	10.0
Sodio Cloruro	5.0
Sali di Bile	1.5
Agar	15.0*
Rosso Neutro	0.03
Cristal Violetto	0.001
pH Finale 7.1 ± 0.2 a 25°C	

* Modificato in base alla forza del gel per soddisfare le specifiche di performance.

PRINCIPIO DEL METODO

Il digerito pancreatico di gelatina ed i peptoni da carne e caseina forniscono aminoacidi, azoto, carbonio, vitamine e minerali per la crescita dei microrganismi. Il lattosio è il carboidrato fermentabile. Il sodio cloruro mantiene il bilancio osmotico del terreno. I sali di bile ed il cristal violetto sono gli agenti selettivi che inibiscono i microrganismi Gram positivi e consentono la crescita dei batteri Gram negativi. L'agar è l'agente solidificante. Il rosso neutro è l'indicatore di pH.

PREPARAZIONE

Terreno disidratato	Sospendere 51.5 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire per 1 minuto per ottenere la completa dissoluzione. Sterilizzare in autoclave a 121°C per 15 minuti.
Terreno in flaconi	Sciogliere il contenuto di un flacone in bagnomaria a 100°C (con il tappo leggermente svitato) fino a completa dissoluzione del terreno. Verificare, una volta fuso, la buona omogeneità del terreno capovolgendo il flacone dopo averne avvitato il tappo. Raffreddare a 45-50°C, mescolare bene senza formazione di bolle. Versare in piastre Petri in condizioni di asepsi.

PROCEDURA DEL TEST

Inoculare le piastre strisciando il campione clinico direttamente sulla superficie dell'agar o spatolare il materiale proveniente da una coltura di arricchimento. Incubare in atmosfera aerobica a 35 ± 2°C per 18-24 ore.

Per l'isolamento di *E. coli* da prodotti farmaceutici, il metodo armonizzato USP/EP/JP raccomanda di seguire una procedura di arricchimento in due passaggi consecutivi. Ovvero, inoculare il campione in Tryptic Soy Broth (ref. 452080) ed utilizzare la coltura ottenuta per inoculare MacConkey Broth (ref. 494000). Quindi subcoltivare su una piastra di MacConkey Agar ed incubare a 30-35°C per 18-72 ore.

INTERPRETAZIONE DEI RISULTATI

I microrganismi che non fermentano il lattosio, come *Salmonella*, *Shigella* e *Proteus* spp, formano colonie incolori o chiare.

I microrganismi fermentanti il lattosio, come *E. coli* e *Klebsiella* spp, crescono come colonie di colore da rosa a rosso con o senza un alone di precipitati di bile.

Enterococchi, Stafilococchi ed altri batteri Gram positivi risultano parzialmente o completamente inibiti.

ASPETTO

Terreno disidratato: omogeneo, fine granulometria, beige-rosa.
Terreno preparato: rosastro-rosso, leggermente opalescente.

CONSERVAZIONE

La polvere è fortemente igroscopica, conservare a 10-30°C, in ambiente asciutto, nel suo contenitore originale chiuso ermeticamente. Conservare i flaconi e le piastre pronte a 10-25°C al riparo dalla luce. Non usare il prodotto dopo la sua data di scadenza indicata sull'etichetta o se il prodotto mostra segni di contaminazione o deterioramento.

VALIDITÀ

Terreno disidratato: 4 anni.
Terreno in flaconi: 2 anni.
Piastre pronte all'uso: 6 mesi.

CONTROLLO DI QUALITÀ

Le piastre vengono inoculate con i ceppi microbici indicati nella tabella CQ.
Inoculo per produttività: 50-100 UFC.
Inoculo per selettività: 10^4-10^6 UFC.
Condizioni di incubazione: ambiente aerobico a 35 ± 2°C per 18-24 ore.
18-72 ore a 30-35°C per *E. coli* (Pharmacopoeia growth promotion).

Tabella CQ.

Microrganismo		Crescita	Specifiche
Salmonella Typhimurium	ATCC® 14028	Buona	Colonie incolori
Shigella flexneri	ATCC® 12022	Buona	Colonie incolori
Proteus mirabilis	ATCC® 12453	Buona	Colonie incolori
Escherichia coli	ATCC® 8739	Buona	Colonie rosa con precipitati di bile
Klebsiella pneumoniae	ATCC® 13883	Buona	Colonie rosa
Enterococcus faecalis	ATCC® 29212	Da parzialmente a completamente inibita	Colonie molto piccole ed opache
Staphylococcus aureus	ATCC® 25923	Inibita	---

AVVERTENZE E PRECAUZIONI

Il prodotto non contiene sostanza nocive in concentrazioni superiori ai limiti fissati dall'attuale legislazione e perciò non è classificato come pericoloso. Ciononostante si raccomanda di consultare la scheda di sicurezza per il suo corretto uso. Il prodotto è da intendersi per uso diagnostico *in vitro* e deve essere utilizzato esclusivamente da operatori adeguatamente addestrati.

SMALTIMENTO DEI RIFIUTI

Lo smaltimento dei rifiuti deve essere effettuato in conformità alle normative nazionali e locali in vigore.

BIBLIOGRAFIA

1. European Pharmacopoeia 6.5 (2009). 2.6.13 Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia 32 NF 27 (2009). <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia 4.05 (2008). Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Murray, Baron, Jorgensen, Landry and Pfaller ed. (2007) Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. MacConkey A. (1905) Lactose-fermenting bacteria in faeces. J. Hygiene 8:333-379.

PRESENTAZIONE		Contenuto	Ref.
MacConkey Agar	Piastre da 90 mm pronte all'uso	20 piastre	10029
MacConkey Agar	Piastre da 90 mm pronte all'uso	100 piastre	10029*
MacConkey Agar	Flaconi	Flaconi 6 x 500 ml	470090
MacConkey Agar	Flaconi	Flaconi 6 x 200 ml	412240
MacConkey Agar	Flaconi	Flaconi 6 x 100 ml	402240
MacConkey Agar	Terreno disidratato	500 g di polvere	610028
MacConkey Agar	Terreno disidratato	100 g di polvere	620028
MacConkey Agar	Terreno disidratato	5 kg di polvere	6100285

TABELLA DEI SIMBOLI

LOT Codice del lotto	**IVD** *In vitro* Diagnostic Medical Device	Fabbricante	Utilizzare entro	Fragile, maneggiare con cura
REF Numero di catalogo	Limiti di temperatura	Contenuto sufficiente per <n> saggi	Attenzione, Consultare le istruzioni per l'uso	Non riutilizzare

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CE IVD

Instrucciones de Uso
ESPAÑOL

MacConkey Agar

Medio selectivo y diferencial para la detección de Enterobacteriaceae
a partir de muestras clínicas y otros materiales de acuerdo a USP/EP/JP.

DESCRIPCIÓN

MacConkey Agar es un medio ligeramente selectivo que proporciona una excelente diferenciación entre bacilos entéricos Gram-negativos lactosa-fermentantes y no fermentantes, de heces, orina, alimentos, aguas residuales y otros materiales de importancia sanitaria.

Este medio sigue las recomendaciones del método armonizado del la Farmacopea de los Estados Unidos (USP), Farmacopea Europea (EP) y Farmacopea Japonesa (JP) para la detección de *E. coli* en productos no estériles.

FÓRMULA	(g/l)
Digerido Pancreático de Gelatina	17.0
Peptona de Carne	1.5
Peptona de Caseína	1.5
Lactosa	10.0
Cloruro Sódico	5.0
Sales Biliares	1.5
Agar	15.0*
Rojo Neutro	0.03
Violeta de Cristal	0.001
pH final 7.1 ± 0.2 a 25°C	

* Ajustado según la consistencia del gel para alcanzar las especificaciones de actuación.

PRINCIPIO DEL MÉTODO

El digerido pancreático de gelatina, y las peptonas suministran los aminoácidos, nitrógeno, carbono, vitaminas y minerales necesarios para el crecimiento de los microorganismos. La lactosa es el carbohidrato fermentable. El cloruro sódico mantiene el equilibrio osmótico del medio. Las sales biliares y el violeta de cristal son agentes selectivos que inhiben a los organismos Gram-positivos, permitiendo el crecimiento de los Gram-negativos. El agar es el agente solidificante. El rojo neutron es el indicador de pH.

PREPARACIÓN

Medio deshidratado	Suspender 51.5 g del polvo deshidratado en 1 litro de agua destilada o desionizada. Mezclar bien. Calentar hasta la ebullición removiendo frecuentemente hasta la completa disolución. Esterilizar en autoclave a 121°C durante 15 minutos.
Medio en botellas	Disolver el contenido de la botella en un baño con agua a 100°C (con el tapón ligeramente desenrroscado) hasta su completa disolución. Comprobar la homogeneidad del medio disuelto, girar la botella si es necesario para ayudar a la homogeneización. Enfriar a 45-50°C, mezclar bien evitando la formación de burbujas y distribuir en placas Petri de forma aséptica.

PROCEDIMIENTO DEL TEST

Inocular las placas directamente por estriación en el agar o extender la muestra procedente de un medio pre-enriquecido. Incubar en aerobiosis a 35 ± 2°C durante 18-24 h

Para aislar *E. coli* en productos farmacëuticos, los métodos armonizados de las farmacopeas USP/EP/JP recomiendan realizar antes dos pasos de enriquecimiento inoculando la muestra en Tryptic Soy Broth (ref. 452080) y posteriormente en MacConkey Broth (ref. 494000). Realizar un subcultivo en una placa de MacConley Agar e incubar aerobicamente a 30-35°C durante 18-72 horas.

INTERPRETACIÓN DE LOS RESULTADOS

Los organismos no fermentantes de la lactosa, como *Salmonella, Shigella* y *Proteus* spp, forman colonias claras o incoloras.

Los organismos fermentantes de la lactosa, como *E. coli* y *Klebsiella* spp, forman colonias rojo-rosáceas con o sin zona de precipitado de bilis.

Los Enterococci, Staphylococci y otras bacterias Gram-positivas están inhibidas parcial o completamente.

ASPECTO

Medio deshidratado: suelto, homogéneo, beige rosáceo.
Medio preparado: ligeramente opalescente, rojo - rosáceo.

ALMACENAMIENTO

El polvo deshidratado es muy higroscópico, almacenar a 10-30°C, en un entorno seco, en su frasco original correctamente cerrado. Almacenar las botellas y las placas preparadas a 10-25°C fuera del contacto de la luz. No utilizar el producto fuera de la fecha de caducidad descrita en la etiqueta o si el producto presenta alguna muestra de deterioro o contaminación.

VIDA ÚTIL

Medio deshidratado: 4 años.
Medio en botellas: 2 años.
Placas preparadas: 6 meses.

CONTROL DE CALIDAD

Las placas se inoculan con las cepas indicadas en la siguiente tabla.
Inóculo para productividad: 50-100 CFU.
Inóculo para selectividad: 10^4-10^6 CFU.
Condiciones de incubación: aeróbicas a 35 ± 2°C durante 18-24 horas.
18-72 h a 30-35°C para *E. coli* (según Farmacopea).

Tabla CC

Microorganismo		Crecimiento	Apariencia
Salmonella Typhimurium	ATCC® 14028	Bueno	Colonias incoloras
Shigella flexneri	ATCC® 12022	Bueno	Colonias incoloras
Proteus mirabilis	ATCC® 12453	Bueno	Colonias incoloras
Escherichia coli	ATCC® 8739	Bueno	Colonias rosáceas con precipitado de bilis
Klebsiella pneumoniae	ATCC® 13883	Bueno	Colonias rosáceas
Enterococcus faecalis	ATCC® 29212	Inhibición parcial o completa	Colonias pequeñas opacas
Staphylococcus aureus	ATCC® 25923	Inhibición	---

ADVERTENCIAS Y PRECAUCIONES

Este producto no contiene sustancias peligrosas en concentraciones que excedan los límites fijados por la legislación actual y no está clasificado como peligroso. Se recomienda de todas formas las lectura de la hoja de seguridad para el uso apropiado. El producto está pensado para un uso exclusivo de diagnóstico in vitro y debe ser utilizado sólo por operadores debidamente adiestrados.

DESECHO DE RESÍDUOS

El desecho de los resíduos debe realizarse según la regulación nacional y local vigente.

BIBLIOGRAFÍA

1. European Pharmacopoeia 6.5 (2009). 2.6.13 Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia 32 NF 27 (2009). <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia 4.05 (2008). Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Murray, Baron, Jorgensen, Landry and Pfaller ed. (2007) Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. MacConkey A. (1905) Lactose-fermenting bacteria in faeces. J. Hygiene 8:333-379.

PRESENTACIÓN		Contenido	Ref.
MacConkey Agar	Placas de 90 mm listas para su uso	20 placas	10029
MacConkey Agar	Placas de 90 mm listas para su uso	100 placas	10029*
MacConkey Agar	Botellas	6 x 500 ml botellas	470090
MacConkey Agar	Botellas	6 x 200 ml botellas	412240
MacConkey Agar	Botellas	6 x 100 ml botellas	402240
MacConkey Agar	Medio deshidratado	500 g de polvo deshidratado	610028
MacConkey Agar	Medio deshidratado	100 g de polvo deshidratado	620028
MacConkey Agar	Medio deshidratado	5 kg de polvo deshidratado	6100285

TABLA DE SÍMBOLOS

LOT Código de lote	**IVD** Sistema medico para el Diagnóstico *In vitro*	Fabricante	Utilizar antes de	Frágil, manipular con cuidado
REF Número de catálogo	Límites de temperatura	Contenido suficiente para <n> análisis	Atención, consultar el documento adjunto	No reutilizar

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CE IVD

MRS Agar

Medium for isolation and enumeration of mesophilic lactic acid bacteria, according to 15214.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	10.0
Meat Extract	10.0
Yeast Extract	4.0
Triammonium Citrate	2.0
Sodium Acetate	5.0
Magnesium Sulfate Heptaydrate	0.2
Manganese Sulfate Tetrahydrate	0.05
Dipotassium Hydrogen Phosphate	2.0
Glucose	20.0
Agar	15.0
Final pH 5.7 ± 0.1 at 25°C	

DESCRIPTION

MRS Agar is a medium used with supplements for the cultivation of *Lactobacillus* spp from different types of material. It may also support the growth of *Pediococcus* and *Leuconostoc* species as well as other secondary bacteria.

The complete medium complies with the recommendations of ISO 15214 and APHA.

PRINCIPLE

Enzymatic digest of casein and meat extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Ammonium citrate and sodium acetate are the selective agents effective against streptococci and moulds. The low pH is also inhibitory for most organisms other than lactobacilli. Magnesium and manganese sulfates are sources of ions and sulfate acting as growth stimulants. Dipotassium phosphate is the buffer. Glucose is the fermentable carbohydrate. Agar is the solidifying agent.

Supplementation with Tween 80 Supplement (ref. 80031) provides a mixture of oleic esters and fatty acids essential for the growth of lactic acid bacteria.

PREPARATION

Suspend 68.3 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Add 1 ml of Tween 80 Supplement. Sterilize in autoclave at 121°C for 15 minutes.

Note. According to ISO 15214, 1.4 g of Sorbic Acid (dissolved in about 10 ml of a 1 mol/l solution of sodium hydroxide) can be added to 1 liter of sterilized medium if extensive yeast contamination is suspected.

TECHNIQUE

1. Use a suitable diluent such as Buffered Peptone Water (ref. 24099) to perform serial dilutions of the test sample in order to achieve a colony count of between 15 and 300 colonies per plate.
2. Inoculate each plate with 1 ml of sample suspension by pour plating. Overlays may be used if required.
3. Incubate at 30°C for 72 hours.

INTERPRETATION OF RESULTS

Count colonies on all plates containing 15-300 colonies. Report the count as CFU/ml of sample allowing for dilution factors.

It may be necessary in some cases and for some products to confirm the colonies by simple techniques such as Gram staining, or the test for catalase.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5th edition. American Public Health Association, Washington, D.C.
2. EN ISO 11133:2014. Microbiology of food, animal feed and water − Preparation, production, storage and performance testing of culture media.
3. Schillinger U and Holzapfel WH (2012) Culture media for Lactic Acid Bacteria. In: Handbook of Culture Media for Food and Water Microbiology. (Corry JEL, Curtis GDW and Baird RM eds), pp 174-186. Royal Society of Chemistry, Cambridge, UK.
4. ISO 15214:1998. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count technique at 30°C.
5. De Man JD, Rogosa M, and Sharpe ME (1960): A Medium for the cultivation of Lactobacilli. J. Appl. Bact. 23: 130-135.

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PRODUCT SPECIFICATIONS

NAME
MRS Agar

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610024	500 g	500 g of powder in plastic bottle
620024	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
5.7 ± 0.1

USE
MRS Agar is a medium used with supplements for the cultivation of mesophilic lactic acid bacteria, according to 15214

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: beige
Ready-to-use medium
Appearance: slightly opalescent
Colour: amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Inoculum for selectivity: 10^4-10^6 CFU
   Incubation Conditions: 72 ± 3 h at 30 ± 1°C, in microaerobiosis

Microorganism		Growth
Lactobacillus sakei	WDCM 00015	Good
Lactobacillus lactis	WDCM 00016	Good
Escherichia coli	WDCM 00012	Inhibited
Bacillus cereus	WDCM 00001	Inhibited

TABLE OF SYMBOLS

LOT Batch code	Consult instructions for use	Manufacturer	Use by
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Keep away from sunlight

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Instructions For Use
ENGLISH

EE Broth-Mossel

Liquid medium for the cultivation and selective enrichment of
Enterobacteriaceae from different types of samples, according to USP/EP/JP.

DESCRIPTION

Enterobacteriaceae Enrichment Broth-Mossel is a selective medium used for the detection of bile-tolerant Gram-negative bacteria in food and other materials of sanitary importance.

This medium complies with the recommendations of the harmonized method in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) for the microbiological examination of nonsterile products.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Gelatin	10.0
Glucose Monohydrate	5.0
Dehydrated Ox Bile	20.0
Potassium Dihydrogen Phosphate	2.0
Disodium Hydrogen Phosphate, Anhydrous	6.4*
Brilliant Green	0.015

Final pH 7.2 ± 0.2 at 25°C

* Equivalent to 8.0 g of Disodium Hydrogen Phosphate Dihydrate.

METHOD PRINCIPLE

Pancreatic digest of gelatin provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Glucose is the fermentable carbohydrate. Ox bile and brilliant green are selective agents effective against Gram-positive cocci. Potassium phosphate and sodium phosphate act as buffer.

PREPARATION

Dehydrated medium — Suspend 43.4 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.

TEST PROCEDURE

As in the Pharmacopoeia, prepare the sample using a 1 in 10 dilution of not less than 1 g of the product to be examined by choosing Tryptic Soy Broth (ref. 24513 or 452080) as diluent and incubate at 20-25°C for 2-5 hour to resuscitate bacteria.

For qualitative test (test for absence), transfer the volume of the pre-enrichment broth corresponding to 1 g of the product to be examined to EE Broth-Mossel.

For quantitative test, enumerate Enterobacteriaceae found per milliliter or per gram of test sample by using the Most Probable Number (MPN) technique. Use the volume of the pre-enrichment broth containing 0.1 g, 0.01 g and 0.001 g (or 0.1 ml, 0.01 ml and 0.001 ml) of the product to be examined to inoculate EE Broth-Mossel.

For both types of test incubate EE Broth-Mossel at 30-35°C for 24-48 h and continue analysis by subculturing on Violet Red Bile Glucose Agar (ref. 11184). Incubate plates aerobically at 30-35°C for 18-24 hours.

INTERPRETING RESULTS

Turbidity of EE Broth-Mossel indicates microbial growth; acid production causes a color change of the medium to yellow.

No growth of colonies on Violet Red Bile Glucose Agar is reported as absence of bile-tolerant Gram-negative bacteria. Growth of colonies constitutes a positive result and the probable number of bacteria is determined from the table below.

MPN Table.

Results for each quantity of product			Probable number of bacteria
0.1 g or 0.1 ml	0.01 g or 0.01 ml	0.001 g or 0.001 ml	per gram or per milliliter of product
+	+	+	$>10^3$
+	+	–	10^3 - 10^2
+	–	–	10^2 - 10
–	–	–	<10

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige to light green.
Prepared medium: clear, green.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 1 year.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Inoculum for selectivity: >100 CFU.
Incubation conditions: 18-24 h at 30-35°C (Pharmacopoeia growth promotion).

QC Table.

Microorganism		Specification
Escherichia coli	ATCC® 8739	Good growth
Pseudomonas aeruginosa	ATCC® 9027	Good growth
Staphylococcus aureus	ATCC® 6538	Inhibition

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. European Pharmacopoeia 6.5 (2009) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
3. United States Pharmacopoeia 32 NF 27 (2009) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Japanese Pharmacopoeia 4.05 (2008) Microbiological examination of non-sterile products: Test for specified microorganisms.
5. ISO 21528-1:2004. Microbiology of food and animal feeing stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Detection and enumeration by MPN technique with pre-enrichment.
6. ISO 21528-2:2004. Microbiology of food and animal feeing stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Colony count method.
7. Davidson, Roth, and Gambrel-Lenarz (2004) In Wehr and Frank (ed.) Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
8. Kornacki and Johnson (2001) In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington D.C.
9. Mossel, Vissar, and Cornellisen (1963) J. Appl. Bacteriol. 26:444.

PRESENTATION		Contents	Ref.
EE Broth-Mossel	Tubes	20 x 10 ml tubes	24096
EE Broth-Mossel	Bottles (screw cap)	6 x 100 ml bottles	402480
EE Broth-Mossel	Bottles (flip-off cap)	25 x 100 ml bottles	453080
EE Broth-Mossel	Bottles (perforable cap)	6 x 100 ml bottles	495000
EE Broth-Mossel	Dehydrated medium	500 g of powder	610017
EE Broth-Mossel	Dehydrated medium	100 g of powder	620017

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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Instructions For Use
ENGLISH

Mannitol Salt Agar

Selective medium for isolation and enumeration of staphylococci
from clinical samples and other materials, according to USP/EP/JP.

DESCRIPTION

Mannitol Salt Agar is a selective medium used for isolating pathogenic staphylococci from clinical samples, food and other materials of sanitary importance.

This medium is prepared according to recommendations of the harmonized USP/EP/JP method for the detection of *S. aureus* in non sterile pharmaceutical products.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Beef Extract	1.0
D-Mannitol	10.0
Sodium Chloride	75.0
Phenol Red	0.025
Agar	15.0
Final pH 7.4 ± 0.2 at 25°C	

METHOD PRINCIPLE

Pancreatic digest of casein, peptic digest of animal tissue and beef extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Mannitol is the fermentable carbohydrate. The high salt content of 7.5% inhibits most bacteria other than staphylococci. Phenol red is the pH indicator. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 111 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil for 1 minute shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

Inoculate plates by the direct streaking of the material to be examined over the agar surface. Incubate aerobically at 35 ± 2°C for 24-48 hours.

Harmonized USP/EP/JP method for microbiological examination of non sterile products recommends to inoculate the sample in Tryptic Soy Broth (ref. 24444). Subculture on a plate of Mannitol Salt Agar and incubate at 30-35°C for 18-72 hours.

INTERPRETING RESULTS

S. *aureus* cultivates with yellow or white colonies surrounded by a yellow zone. Confirm by identification tests*. Coagulase-negative Staphylococci form small colorless to red colonies with no color change to the medium

*Suspect colonies can be subcultured to a moderately selective medium such as Baird Parker RPF Agar (ref. 10521, 402210) for the determination of coagulase activity (ISO 6888-2).

APPEARANCE OF THE MEDIUM

Dehydrated medium: free-flowing, homogeneous, beige-pink.
Prepared medium: slightly opalescent, pinkish-red.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 10-100 CFU
Inoculum for selectivity: 10^4-10^6 CFU
Incubation conditions: aerobically at 35 ± 2°C for 24-48 hours.
*30-35°C for 18-72 h (USP/EP/JP Growth Promotion Testing).

QC Table.

Microorganism		Growth	Specification
Staphylococcus aureus	ATCC® 25923	Good	Yellow colonies with yellow zone
*Staphylococcus aureus**	ATCC® 6538	Good	Yellow colonies with yellow zone
Staphylococcus epidermidis	ATCC® 12228	Good	Red colonies
Escherichia coli	ATCC® 25922	Inhibited	---
*Escherichia coli**	ATCC® 8739	Inhibited	---

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. European Pharmacopoeia 6.5 (2009). 2.6.13 Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia 32 NF 27 (2009). <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia 4.05 (2008). Microbiological examination of non-sterile products: Test for specified microorganisms.
4. ISO 6888-2:1999 + A1:2003. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Part 2: Technique using rabbit plasma fibrinogen agar medium.
5. Kloos, W.E., and T.L. Bannerman (1995) Staphylococcus and Micrococcus. In Manual of clinical microbiology, 6th ed.
6. Chapman, G.H. (1945) The significance of sodium chloride in studies of staphylococci. J. Bacteriol. 50:201-203.

PRESENTATION		Contents	Ref.
Mannitol Salt Agar	90 mm ready-to-use plates	20 plates	10030
Mannitol Salt Agar	90 mm ready-to-use plates	100 plates	10030*
Mannitol Salt Agar	Bottles	6 x 500 ml bottles	470080
Mannitol Salt Agar	Bottles	6 x 200 ml bottles	412290
Mannitol Salt Agar	Bottles	6 x 100 ml bottles	402290
Mannitol Salt Agar	Dehydrated medium	500 g of powder	610029
Mannitol Salt Agar	Dehydrated medium	100 g of powder	620029
Mannitol Salt Agar	Dehydrated medium	5 kg of powder	6100295

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Medical Diagnostic Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

Yersinia Selective Agar Base

Differential and selective medium for the isolation of *Y. enterocolitica* from clinical and nonclinical specimens, according to ISO 10273.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Gelatin	17.0
Enzymatic Digest of Casein and Animal Tissues	3.0
Yeast Extract	2.0
Sodium Chloride	1.0
Sodium Pyruvate	2.0
Magnesium Sulfate	0.01
Mannitol	20.0
Sodium Deoxycholate	0.5
Crystal Violet	0.001
Neutral Red	0.03
Agar	14.0
Final pH 7.4 ± 0.2 at 25°C	

DESCRIPTION

Yersinia Selective Agar Base is a medium used with supplements for the selective isolation and differentiation of *Yersinia enterocolitica*

The complete medium (CIN agar) is recommended by ISO 10273 for the examination of food and animal feed stuffs as well as environmental samples in the area of food production and food handling.

PRINCIPLE

Enzymatic digest of gelatin and enzymatic digest of casein and animal tissues provide amino acids, nitrogen, carbon, minerals, vitamins and other nutrients which support the growth of microorganisms. Yeast extract is a source of vitamins, particularly of B-group. Sodium chloride maintains the osmotic balance of the medium. Sodium pyruvate and magnesium sulfate stimulate organisms growth. Mannitol is the carbohydrate which allows to differentiate between mannitol fermenting and non-fermenting bacteria. Sodium deoxycholate and crystal violet inhibit Gram-positive bacteria. Neutral red is the pH indicator. Agar is the solidifying agent.

Supplementation with Yersinia Supplement (ref. 81039), containing cefsulodin, irgasan (triclosan) and novobiocin, inhibits the growth of most Gram-negative enteric bacteria.

PREPARATION

Suspend 59.6 g of powder in 1 liter of deionized or distilled water. Bring to boil and shake until completely dissolved. Sterilize at 121°C for 15 minutes. Cool up to 45-50°C. Aseptically, add the contents of 2 vials (6 ml) of Yersinia Supplement reconstituted as directed in the instructions for use that accompany the product. Pour in Petri dishes.

TECHNIQUE

Inoculate the specimen onto the medium by either direct plating or pour plating (*). Incubate aerobically at 30 ± 1°C for 18-24 h.

(*) The ISO method for the detection of presumptive pathogenic *Yersinia enterocolitica* recommends to first perform enrichment in Peptone, Sorbitol and Bile Salts (PSB) Broth for 48-72 hours at 22-25°C with agitation, or 5 days without agitation.

INTERPRETATION OF RESULTS

Organisms capable of fermenting mannitol cause a localized pH reduction, forming colonies with red centre surrounded by a transparent border (characteristic “bull’s-eye” colony). Organisms that do not ferment mannitol form colorless, translucent colonies. Some strains of *Serratia*, *Citrobacter* and *Enterobacter* may give a colonial morphology resembling *Yersinia enterocolitica.* Final identification should be confirmed by standard biochemical tests.

STORAGE AND TRANSPORT CONDITIONS

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

- EN ISO 11133:2014. Microbiology of food, animal feed and water − Preparation, production, storage and performance testing of culture media.
- ISO 10273:2003. Microbiology of food and animal feeding stuffs − Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*.
- Schieman, D.A. (1979) Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can. J. Microbiol. 25:1298-1304.
- Schieman, D.A. (1980) *Yersinia enterocolitica*: Observation on some growth characteristics and response to selective agents. Can. J. Microbiol. 43:14-27.
- Devenish, J.A., and D.A. Schieman (1981) An abbreviated scheme for identification of *Yersinia enterocolitica* isolated from food enrichments on CIN (cefsulodin-irgasan-novobiocin) agar. Can. J. Microbiol. 27:937-941.

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CE IVD

PRODUCT SPECIFICATIONS

NAME
Yersinia Selective Agar Base

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610111	500 g	500 g of powder in plastic bottle
620111	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.4 ± 0.2

USE
Yersinia Selective Agar Base is a differential and selective medium used with supplements for the isolation of *Yersinia enterocolitica* from clinical specimens and other types of samples, according to ISO 10273

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: pink-beige to beige
Ready-to-use medium
Appearance: slightly opalescent
Colour: reddish-orange

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Inoculum for selectivity: 10^4-10^6 CFU
   Incubation Conditions: 18-24 h at 30 ± 1°C, in aerobiosis

Microorganism		Growth	Colony Appearance
Yersinia enterocolitica	WDCM 00038	Good	Colonies with red center
Escherichia coli	WDCM 00012	Partially to totally inhibited	---
Staphylococcus aureus	WDCM 00034	Inhibited	---

TABLE OF SYMBOLS

LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	Do not reuse

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CE IVD

ENGLISH

Yersinia Supplement

Selective supplement for the isolation of *Yersinia enterocolitica*.

DESCRIPTION

Yersinia Supplement is a selective lyophilized supplement used for the preparation of Yersinia Selective Agar Base (ref. 610111, 620111).
The complete medium is used for the isolation of *Yersinia enterocolitica* from clinical and nonclinical specimens.

KIT CONTENTS

Each kit contains:

- 10 vials of freeze-dried Yersinia Supplement
- 1 instruction sheet

PRINCIPLE OF THE METHOD

Yersinia Supplement is an antibiotic mix that inhibits the growth of most Gram-negative enteric bacteria.

COMPOSITION

Antibiotic	Content / vial	Content / liter of medium
Cefsulodin	7.5 mg	15.0 mg
Irgasan	2.0 mg	4.0 mg
Novobiocin	1.25 mg	2.5 mg

PROCEDURE FOR USE

1. Aseptically reconstitute the content of one vial of Yersinia Supplement with 2 ml sterile distilled water and 1 ml ethanol(*).
2. Mix to complete dissolution and add to 500 ml Yersinia Selective Agar Base, autoclaved and cooled to 45-50°C.
3. Mix well and dispense in Petri dishes.

(*) Ethanol 50% purity minimum.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical documentation of the medium being prepared.

QUALITY CONTROL

1. Control of the appearance: freeze-dried white in colour.
2. Microbiological control.
   Prepare the medium per label directions. Inoculate the plates with the strains indicated below and incubate at 30 ± 1°C for 18-24 hours.

Control strains		Growth
Yersinia enterocolitica	WDCM 00038	Good
Escherichia coli	WDCM 00012	Partially to totally inhibited
Staphylococcus aureus	WDCM 00034	Inhibited

PRECAUTIONS

Yersinia Supplement is classifiable as hazardous under current legislation; it is recommended that the Safety Data Sheet be consulted on its use.
The product is a selective supplement to be used only for *in vitro* diagnostic use. It is intended for use in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

2-8°C in its original packaging. Keep away from sources of heat and avoid excessive changes of temperature. Use until the expiry date indicated on the label. Eliminate without using if there are signs of deterioration. Once reconstituted , the product can be stored for a maximum duration of 30 days at -20°C, shielded from light.

REFERENCES

- EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
- ISO 10273:2003. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*.
- Schieman, D.A. (1979) Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can. J. Microbiol. 25:1298-1304.
- Schieman, D.A. (1980) *Yersinia enterocolitica*: Observation on some growth characteristics and response to selective agents. Can. J. Microbiol. 43:14-27.
- Devenish, J.A., and D.A. Schieman (1981) An abbreviated scheme for identification of *Yersinia enterocolitica* isolated from food enrichments on CIN (cefsulodin-irgasan-novobiocin) agar. Can. J. Microbiol. 27:937-941.

PRESENTATION

Product	Ref.	Contents
Yersinia Supplement	81039	10 vials

One vial is sufficient to prepare 500 ml of medium.

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	LOT Batch code

LIOFILCHEM® S.r.l.
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CE IVD Rev.3 / 18.09.2015

Cronobacter Selective Broth

Selective liquid medium for detection of *Cronobacter* spp, according to ISO 22964.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Animal Tissues	10.0
Meat Extract	3.0
Sodium Chloride	5.0
Bromocresol Purple	0.04
Sucrose	10.0
Final pH 7.4 ± 0.2 at 25°C	

DESCRIPTION

Cronobacter Selective Broth (CSB) is a liquid medium used with supplements for the selective enrichment of *Cronobacter* spp (formerly *Enterobacter sakazakii*) in food, animal feed end environmental samples.

The complete CSB is formulated according to ISO 22964:2017 and is used in conjunction with Chromatic™ Cronobacter Isolation (CCI) Agar.

PRINCIPLE

Enzymatic digest of animal tissues and meat extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Sodium chloride maintains the osmotic balance of the medium. Bromocresol purple is the pH indicator. Sucrose is the fermentable carbohydrate.

Supplementation with Vancomycin (5mg) Supplement (ref. 81064), along with a high incubation temperature, inhibit most contaminant bacteria.

PREPARATION

Suspend 28.04 g of powder in 1 liter of deionized or distilled water. Bring to boil and shake until completely dissolved. Sterilize at 121°C for 15 minutes. Cool up to 45-50°C. Aseptically, add rehydrated content of 2 vials (10 ml) of Vancomycin (5mg) Supplement. Mix well and dispense into tubes.

TECHNIQUE

1. To prepare the initial suspension, add 10 g or 10 ml of the test sample to 90 ml of Buffered Peptone Water (ref. 414030).
2. Incubate the inoculated pre-enrichment medium at 36 ± 2°C for 18 ± 2 h.
3. Mix well and transfer 0.1 ml of the pre-enrichment culture to a tube of CSB.
4. Incubate the inoculated enrichment medium at 41.5°C for 24 ± 2 h.

INTERPRETATION OF RESULTS

A yellow coloration in the CSB (sucrose-positive) indicates presumptive *Cronobacter* spp. If the medium remains purple, the sample is considered negative.

Using a loop, take 10 µl the enrichment culture from a positive tube and streak onto a plate of CCI (ref. 11636). For the continuation of the detection procedure, refer to the instructions of the CCI agar.

STORAGE CONDITIONS

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared tubes at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. ISO 22964:2017. Microbiology of the food chain − Horizontal method for the detection of Cronobacter spp.
2. EN ISO 11133:2014. Microbiology of food, animal feed and water − Preparation, production, storage and performance testing of culture media.
3. Guillame-Gentil O., Sonnard V., Kandahai M.C., Mauragg J.D., and Jootsen H.A. (2005) A simple Rapid Cultural Method for Detection of Enterobacter sakazakii in environmental samples. J Food Prot. 68 (1): 64-69.

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PRODUCT SPECIFICATIONS

NAME
Cronobacter Selective Broth

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610389	500 g	500 g of powder in plastic bottle
620389	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.4 ± 0.2

USE
Cronobacter Selective Broth is a liquid medium used with supplements for the detection of *Cronobacter* spp from food and environmental samples

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: yellowish
Ready-to-use medium
Appearance: clear
Colour: purple

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: ≤100 CFU
   Inoculum for selectivity: >10^3 CFU
   Incubation Conditions: 41.5 ± 1°C for 24 ± 2 h

Microorganism		Growth	Specification
Cronobacter sakazakii	WDCM 00214	Good	Positive acid reaction (+), yellow medium, >10 colonies on CCI
+ *Staphylococcus aureus*	WDCM 00032		
Staphylococcus aureus	WDCM 00032	Totally or partially inhibited	Negative acid reaction (−), purple medium, ≤100 colonies on TSA

TABLE OF SYMBOLS

LOT Batch code	Keep away from Sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	Do not reuse

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ENGLISH

Vancomycin (5 mg) Supplement

Selective supplement for the isolation of *Enterobacter sakazakii*.

DESCRIPTION

Vancomycin (5 mg) Supplement is a selective lyophilized supplement consisting of vancomycin and used for the preparation of Modified Lauryl Sulfate Tryptose Broth (Ref. 610247). The complete medium is used for the selective enrichment of *Enterobacter sakazakii* from powder milk and milk products.

KIT CONTENTS

Each kit contains:

- 10 vials of lyophilized Vancomycin (5 mg) Supplement
- 1 instruction sheet
-

PRINCIPLE OF THE METHOD

Vancomycin is a bactericide antibiotic which inhibits the synthesis of the cell-walls. It is usually active against Gram-positive cocci and bacilli, included (with rare exceptions) *Staphylococcus aureus* and coagulase-negative staphylococci, which are resistant to cephalosporin and penicillin. Vancomycin is bacteriostatic against enterococci even if many strains of *E. faecium* are resistant. All Gram-negative microorganisms are resistant to vancomycin.

COMPOSITION

	Content / vial	Content / liter of medium
Vancomycin	5.0 mg	10.0 mg

PROCEDURE FOR USE

1. Reconstitute aseptically the content of one vial of Vancomycin (5 mg) Supplement with 5 ml of sterile distilled water
2. Mix to complete dissolution and add aseptically to 500 ml of Modified Lauryl Sulfate Tryptose Broth autoclaved and cooled at 45-50°C.
3. Mix with care and pour into 10 ml tubes in aseptic conditions.
4.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical sheet of the medium being prepared.

QUALITY CONTROL

1. Visual inspection: whitish button, limpid solution once reconstituted.
2. Microbiological control.
   Prepare the medium per label directions. Inoculate the tubes with the microbial strains indicated below and incubate at 44±0.5°C for 18-24 h.

Control strains		Growth
Escherichia coli	ATCC® 25922	Good
Enterobacter sakazakii	ATCC® 29544	Good
Staphylococcus aureus	ATCC® 25923	Inhibited

WARNING AND PRECAUTIONS

The product contains substances classified as hazardous by current legislation. It is recommended that the Safety Data Sheet be consulted before using. The product is intended for professional use only and must be used by properly trained operators.

STORAGE AND TRANSPORT CONDITIONS

2-8°C away from light, until the expiry date on the label. However, our stability studies have shown that the storage or transport at 18-25°C for 4 days, or at 35-39°C for 48 hours, do not alter in any way the performance of the product. Eliminate if signs of deterioration or contamination are evident.

REFERENCES

- ISO 22964: 2006. Milk and milk products detection of *Enterobacter sakazakii*.
- Guillame-Gentil O., Sonnard V., Kandahai M.C., Muragg J.D., and Jootsen H.A. (2005) A simple Rapid Cultural Method for Detection of Enterobacter sakazakii in environmental samples. J Food Prot. 68,1:64-69.
- ISO 6887-1: 1999. Microbiology of food and animal feeing stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

PRESENTATION

Product	Ref.	Content
Vancomycin (5 mg) Supplement	81064	10 vials

One vial is sufficient to prepare 500 ml of medium.

TABLE OF SYMBOLS

LOT Batch code	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	

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Rev.0 / 29.07.2014

LISTERIA PALCAM AGAR

Selective medium for the isolation and enumeration of *Listeria monocytogenes* according to ISO 11290-1.

TYPICAL FORMULA	(g/ l)
Peptone	23.0
Starch	1.0
Sodium Chloride	5.0
Yeast Extract	3.0
Glucose	0.5
Mannitol	10.0
Esculin	0.8
Ferric Ammonium Citrate	0.5
Lithium Chloride	15.0
Phenol Red	0.08
Agar	12.0

Final pH = 7.2 ± 0.2 at 25 °C.

DESCRIPTION

The complete LISTERIA PALCAM AGAR, prepared by adding Listeria Palcam supplement to the medium base, is a selective and differential medium, formulated by Van Netten and other, and according to ISO 11290, for isolation and enumeration of *Listeria monocytogenes* from foods.
The medium is also recommended by:

1. AFNOR for the research of *L. monocytogenes i*n foods.
2. IDF as an additional plating medium for the detection of *Listeria spp* in milk and milk products.
3. Health Canada for the detection of *L. monocytogenes* in food and environmental samples.

Aesculin and mannitol, present in the medium, yield a presumptive differentiation of Listeria from other aesculin positive bacteria, such as faecal streptococci.

PRINCIPLE

The peptones favour the excellent growth of Listeria, glucose and starch are energy sources, esculin is hydrolysed by Listeria strains to glucose and esculetin, the latter compound forming a black complex with ferric ions. The competitive flora is inhibited by lithium chloride and by the antimicrobials of the selective supplement: ceftazidim, polymixin B, acriflavine. The fermentation of mannitol by contaminating bacteria that may grow causes phenol red to turn yellow.

PREPARATION

Suspend 35.4 g of powder in 500 ml of distilled or deionized water. Heat until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50°C. Aseptically add 1 vial of Listeria Palcam Supplement (code 81026). Mix well. Dispense into Petri dishes.

TECHNIQUE

Streak a loopful of the suitable enriched broth, inoculated with the sample to analyze, onto the surface of the medium. Incubate at 36+/-1°C for 24-48 hours.

INTERPRETATION OF RESULTS

Listeria monocytogenes cultivates with grey-green colonies surrounded by a black zone (aesculin hydrolysis) with medium's turning to red for missed mannitol fermentation. Possible contaminants such as staphylococci and enterococci, ferment mannitol and cultivate with yellow colonies surrounded by a yellow zone. The suspected colonies must be submitted to Gram colouring, catalase test, mobility examination and identification biochemical tests (Listeria System 18R cod. 71640).

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.
Store prepared media at 2-8°C.

WARNING and PRECAUTIONS

The product is classifiable as hazardous under current legislation; it is recommended that the Safety Data Sheet be consulted on its use.
The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. ISO 11290- 1/2 Microbiology of food and animal feeding stuffs- Horizontal method for the detection and enumeration of *Listeria monocytogenes*; Part 1 Detection method – Part 2: enumeration method
2. Normalisation Française, AFNOR (1993) V08-55.
3. Manuel suisse des denrées alimentaires, Chapitre 56, E21, juillet 2000,
4. Rapporto ISTISAN 96/35 Istituto Superiore di Sanità; ISSN 1123- 3117
5. Van Netten, P. et al. (1989) Int. J. Food Microbiol. 8, 299-316.

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PRODUCTION SPECIFICATIONS

NAME
LISTERIA PALCAM AGAR

PRESENTATION
Dehydrated culture medium.

STORAGE
10-30°C.

PACKAGING

Code	Content	Packaging
610168	500 g	500 g of powder in plastic bottle
620168	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.2 ± 0.2

USE
The complete LISTERIA PALCAM AGAR, prepared by adding Listeria Palcam supplement to the medium base, is a selective and differential medium, formulated by Van Netten and other, and according to ISO 11290, for isolation and enumeration of *Listeria monocytogenes* from foods.

TECHNIQUE
Refer to technical sheet of the product.

APPEARANCE of the MEDIUM
Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: pink.
Prepared medium
Appearance: slightly opalescent.
Color: red.

SHELFLIFE
4 years.

QUALITY CONTROL

1. Control of general characteristics, label and print
2. Sterility control
   7 days at 25 ± 1°C, in aerobiosis
   7 days at 36 ± 1°C, in aerobiosis

   Microbiological control
   Inoculum for productivity: 10-100 UFC/ml
   Inoculum for selectivity: 10^4-10^5 UFC/ml
   Inoculum for specificity: ≤ 10^4 UFC/ml
   Incubation conditions: 37± 1°C for 24-48 hours.

Microorganism	ATCC	Growth	Characteristics
Listeria monocytogenes	19111	good	Gray colonies/ black halo
Listeria monocytogenes	13932	good	Gray colonies/ black halo
Escherichia coli	25922	inhibited	
Enterococcus faecalis	29212	inhibited	
Candida albicans	10231	inhibited	

TABLE OF SYMBOLS

LOT Batch code	Temperature limitation	Manufacturer	Contains sufficient for <n> tests	IVD *In vitro* Diagnostic Medical Device
REF Catalogue number	Keep away from heat	Use by	Caution, consult accompanying documents	

CE IVD

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ENGLISH

LISTERIA PALCAM *Supplement*

Selective supplement for the isolation of *Listeria monocytogenes.*

DESCRIPTION

LISTERIA PALCAM *Supplement* is a selective supplemento made of a freeze-dried mixture of Polymyxin B, Ceftazidime and Acriflavine to use as supplement of the culture medium LISTERIA PALCAM AGAR code 610168 or 620168 for the isolation of *Listeria monocytogenes*.

KIT CONTENTS

Each kit contains:

- 10 vials of freeze-dried LISTERIA PALCAM *Supplement*
- 1 instructions sheet

PRINCIPLE OF THE METHOD

Polymyxin B, Ceftazidime and Acriflavine contribute to the finale medium selectivity by inhibiting the growth of most of common bacterial species non-*Listeria spp* frequently found in food. Polymyxin B acts against Gram-negative bacteria, Ceftazidime is active against Gram-positive and enterobacteria, Acriflavine inhibits many Gram-positive bacteria.

COMPOSITION

LISTERIA PALCAM *Supplement*		
	Content / vial	*Content / l of medium*
Polymyxin B	5.0 mg	10.0 mg
Ceftazidime	10.0 mg	20.0 mg
Acriflavina HCl	2.5 mg	5.0 mg

PROCEDURE FOR USE

1. Reconstitute aseptically the content of one vial of LISTERIA PALCAM *Supplement* with 5 ml of sterile distilled water. Shake until completely dissolved, avoiding foam formation.
2. Add aseptically the entire content of one vial (5 ml) to 500 ml of medium LISTERIA PALCAM AGAR code 610168 or 620168 autoclaved and cooled at 45-50°C.
3. Mix with care.
4. Distribute into Petri dishes.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical documentation for LISTERIA PALCAM AGAR code 610168 or 620168.

QUALITY CONTROL

1. Control of the appearance: freeze-dried product, yellow colour .
2. Microbiological control.

Prepare the plates using as base the medium LISTERIA PALCAM AGAR code 610168 or 620168 added with LISTERIA PALCAM *Supplement* (1 vial in 500 ml of medium). The plates are seeded with the strains indicated in the microbiological control table.
Incubation condition: 24-48 h at 36 ± 1 °C.
Microbiological control:

	Control strains	**Growth**
Listeria monocytogenes	ATCC 19111	Good
Listeria monocytogenes	ATCC 13932	Good
Escherichia coli	ATCC 25922	Inhibited
Enterococcus faecalis	ATCC 29212	Inhibited

PRECAUTIONS

The product LISTERIA PALCAM *Supplement* is classifiable as hazardous under current legislation; it is recommended that the Safety Data Sheet be consulted on its use.
LISTERIA PALCAM *Supplement* is a selective supplement to be used only for *in vitro* diagnostic use. It is intended for use in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store LISTERIA PALCAM *Supplement* at 2-8 °C in its original packaging. In such condit ions LISTERIA PALCAM *Supplement* will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

REFERENCES

- Van Netten, P. et al.,(1989). J. of Food Microbiol. 8:299-317.
- AFNOR. (1993). Food Microbiology – "Detection of Listeria monocytogenes". IDF Provisional International Standard n° 143. International Dairy Federation, Brussels.

PRESENTATION

Product	REF	Σ
LISTERIA PALCAM *Supplement*	81026	10 vials

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	LOT Batch code

LIOFILCHEM Bacteriology Products
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CE IVD

Rev.0 / 23.10.2005

Muller Kauffmann Tetrathionate Broth Base

Basal medium for detection of *Salmonella* spp from foodstuffs and environmental samples, according to ISO 6579.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Casein	8.6
Meat Extract	4.3
Sodium Chloride	2.6
Calcium Carbonate	38.7
Sodium Thiosulfate anhydrous	30.5*
Ox Bile	4.78
Brilliant Green	0.096
Final pH 8.2 ± 0.2 at 25°C	

*Equivalent to 47.8 g of sodium thiosulfate pentahydrate.

DESCRIPTION

Muller Kauffmann Tetrathionate Broth Base is used with supplements for the selective enrichment of Salmonellae in food and environmental samples. The medium is formulated in compliance with ISO 6579 requirements.

PRINCIPLE

Enzymatic digest of casein and meat extract provide amino acids, nitrogen, carbon, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Calcium carbonate is the buffer. Sodium thiosulfate is included to produce tetrathionate after adding iodine to the medium. Organisms-reducing tetrathionate, such as Salmonella, grow luxuriant while most faecal organisms are inhibited. Bile promotes the growth of Salmonella while inhibiting the contaminant bacterial flora. Brilliant green suppresses primarily Gram-positive bacteria. Novobiocin is added to inhibit Gram-positive bacteria.

PREPARATION

Suspend 89.5 g of powder in 1 liter of deionized or distilled water. Heat with frequent agitation and boil for 5 minutes to completely dissolved the powder. DO NOT AUTOCLAVE. Cool up to 45-50°C. Aseptically, add the contents of 2 tubes (20 ml) of Iodine MKTT Solution (ref. 80009). Also add the contents of 2 vials of Novobiocin MKTT Supplement (ref. 81073) each reconstituted with 5 ml sterile distilled water. Mix well. Dispense into sterile containers.

TECHNIQUE

For pre-enrichment, add the sample to Buffered Peptone Water (ref. 414020) at a ratio of 1:9 (e.g. 25 g per 225 ml), homogenize well and incubate at 37 ± 1°C for 16-20 h.

Transfer 1 ml of the pre-enrichment culture to 10 ml of Muller Kauffmann Tetrathionate Broth. Incubate at 37 ± 1°C for 18-24 h.

INTERPRETATION OF RESULTS

Turbidity indicates microbial growth.

Presumptive identification is achieved by subculture onto XLD Agar (ref. 10056) and a second *Salmonella* agar of choice such as Chromatic™ Salmonella (ref. 11614). Characteristic presumptive *Salmonella* colonies should be confirmed with biochemical and serological tests.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

1. ISO 6579:2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.
2. DeSmedit J.M., R. Bolderdijk, H. Rappold and D. Lautenschlaeger (1986) Rapid Salmonella detection in food by motility enrichment on a modified semi-solid Rappaport-Vassiliadis Medium. J. Food Prot. 49:510-514.
3. Vassiliadis P., D. Trichopoulos, A. Kalandidi and E. Xirouchaki (1978) Isolation of salmonellae from sewage with a new procedure of enrichment. J. Appl. Bacteriol 44:233-239.
4. Rappaport F., N. Konforti and B Navon (1956) A new enrichment medium for certain salmonellae. J. Clin. Pathol. 9:261-266.

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PRODUCT SPECIFICATIONS

NAME
Muller Kauffmann Tetrathionate Broth Base

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610239	500 g	500 g of powder in plastic bottle
620239	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
8.2 ± 0.2

USE
Muller Kauffmann Tetrathionate Broth Base is used with supplements for the selective enrichment of Salmonellae in food and environmental samples. The medium is formulated in compliance with ISO 6579 requirements

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: pale green
Ready-to-use medium
Appearance: opaque
Colour: very pale green

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Incubation conditions: 18-24 hours at 37 ± 1°C

   Inoculum for productivity: ≤100 CFU

Microorganism		Growth	Specification
Salmonella Typhimurium + *Escherichia coli* + *Pseudomonas aeruginosa*	WDCM 00031 WDCM 00013 WDCM 00025	Good	>10 colonies on XLD agar or other medium of choice

   Inoculum for selectivity: $>10^3$ CFU

Microorganism		Growth	Specification
Escherichia coli	WDCM 00013	Partially inhibited	≤100 colonies on TSA
Enterococcus faecalis	WDCM 00009	Partially to completely inhibited	<10 colonies on TSA

TABLE OF SYMBOLS

LOT Batch code	Do not reuse	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	

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ENGLISH

NOVOBIOCIN MKTT Supplement

Selective supplement for the detection of *Salmonella spp*.

DESCRIPTION

NOVOBIOCIN MKTT Supplement is a selective supplement for the detection of *Salmonella spp,* used for enrichment of MULLER KAUFFMANN BROTH BASE cod. 610239 or 620239.

KIT CONTENTS

Each kit contains:
- 10 bottles of NOVOBIOCIN MKTTSupplement freeze-dried
- 1 Instruction sheet

PRINCIPLE OF THE METHOD

Novobiocin is an antibiotic effective against both Gram-negative and Gram-positive bacteria.

COMPOSITION

NOVOBIOCIN MKTT Supplement		
	Contents / bottle	*Contents / l of medium*
Novobiocin	20.0 mg	40.0 mg

PROCEDURE FOR USE

1. Reconstitute the contents of a bottle of NOVOBIOCIN MKTT Supplement aseptically with 5 ml of sterile distilled water. Shake until completely dissolved, avoiding foam formation.
2. Add the entire contents of a bottle (5 ml) aseptically to 500 ml of MULLER KAUFFMANN BROTH BASE cod. 610239 or 620239, boiled, cooled to 45-50°C and added with IODINE MKTT SOLUTION.
3. Mix with care.
4. Distribute into sterile tubes.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical documentation of MULLER KAUFFMANN BROTH BASE cod. 610239 or 620239.

QUALITY CONTROL

1. Control of the appearance: a white freeze-dried product.
2. Microbiological control.

Prepare the tubes using as base MULLER KAUFFMANN BROTH BASE cod. 610239 or 620239 enriched with NOVOBIOCIN MKTT Supplement (1 bottle in 500 ml of medium) and IODINE MKTT SOLUTION. The tubes are seeded with the strains indicated in the microbiological control table.
Incubation conditions: 24 ± 3 h at 37 ± 1 °C

Microbiological control

Control strains		**Growth**
Salmonella typhimurium	ATCC 14028	Good
Escherichia coli	ATCC 25922	Inhibited
Salmonella seftenberg	ATCC 10384	Good

PRECAUTIONS

The product NOVOBIOCIN MKTT Supplement is classifiable as hazardous under current legislation; it is recommended that the Safety Data Sheet be consulted on its use.
NOVOBIOCIN MKTT Supplement is a selective supplement to be used in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store NOVOBIOCIN MKTT Supplement at 2-8°C in its original packaging. Keep away from sources of heat and avoid excessive changes of temperature. In such conditions NOVOBIOCIN MKTT Supplement maintains its validity until the expiry date indicated on the label. Eliminate without using if there are signs of deterioration.

REFERENCES

- DeSmedt, Bolderdijk, Rappold and Lautenschlaeger. 1986. J. Food Prot. *49*:510.
- Dusch and Altwegg. 1995. J. Clin. Microbiol. *33*:802.
- Aspinall, Hindle and Hutchinson. 1992. Eur. J. Clin. Microbiol. Infect. Dis. *11*:936.

PRESENTATION

Product	REF	Σ
NOVOBIOCIN MKTT Supplement	**81073**	**10 bottles**

One bottle is sufficient to prepare 500 ml of medium

TABLE OF SYMBOLS

LOT Batch code	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	

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Rev.0/ 07.07.2009

UREA AGAR BASE

Medium for urease test, recommended by ISO 6785 and IDF 93.

TYPICAL FORMULA	(g/l)
Peptone	1.0
Glucose	1.0
Sodium Chloride	5.0
Monopotassium Phosphate	2.0
Phenol Red	0.012
Agar	15.0
Final pH 6.8 ± 0.2 at 25°C	

DESCRIPTION

UREA AGAR BASE is a medium used for urease test, recommended by ISO 6785 and IDF 93.

PRINCIPLE

Peptone provides nitrogen, carbon, and amino acids required for organism growth. Glucose is an energy source. Sodium chloride maintains the osmotic balance of the medium. Monopotassium phosphate is the buffer. Phenol red is the pH indicator. Agar is the solidifying agent. Urea is added to the medium as substrate for urease enzyme. The splitting of urea by urease causes the release of ammonia, increasing pH of the medium to the alkaline side. This is indicated by a color change of the pH indicator.

PREPARATION

Suspend 24.0 g of powder in 950 ml of distilled or deionized water. Heat until completely dissolved. Autoclave at 121°C for 15 minutes. Cool to 45-50°C. Aseptically add 50 ml of Urea 40% Supplement (ref. 80292). Dispense into sterile tubes and allow to solidify in a slanting position.

TECHNIQUE

Use a heavy inoculum of the growth from a pure 18-24 hours culture. Inoculate by streaking back and forth over the entire slant surface. Do not stab the butt because it serves as color control. Incubate the tubes with the caps loosened at 36 ± 1°C for 6-24 hours. Longer period of incubation may not be necessary.

INTERPRETATION OF RESULTS

The production of urease is a positive reaction, indicated by an intense red or pink color on the slant.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for *in vitro* diagnostic use and must be used by properly trained operators only.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. Christensen, W.B. (1946) J. Bact. 52:461-466.
2. Maslen, L.G.C. (1952) Brit. Med. J. 2:545-546.
3. ISO 6785:2001. IDF 93:2001.

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CE IVD

PRODUCT SPECIFICATIONS

NAME
UREA AGAR BASE

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGE

Ref.	Content	Packaging
610107	500 g	500 g of powder in plastic bottle
620107	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
6.8 ± 0.2

USE
UREA AGAR BASE is a medium used for urease test, recommended by ISO 6785 and IDF 93

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Dehydrated medium
Appearance: free-flowing, homogeneous
Colour: orange
Prepared medium
Appearance: slightly opalescent
Colour: reddish-orange

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print

2. Microbiological control
   Inoculum for productivity: 10-100 CFU/ml
   Incubation conditions: 6-24 h at 36 ± 1°C

Microorganism	ATCC®	Urease Production
Proteus vulgaris	13315	+
Escherichia coli	25922	-

TABLE OF SYMBOLS

LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Consult instructions for use	Keep away from heat sources

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CE IVD

ENGLISH

UREA 40% *Supplement*

Supplement for the detection of urease activity of bacteria

DESCRIPTION

UREA 40% *Supplement* is a supplement per la detection of urease activity of bacteria and it is made of a 40% urea aqueous solution for microbiological use. UREA 40% *Supplement* is used for the enrichment of medium Urea Agar Base cod. 610107 or 620107.

KIT CONTENTS

Each kit contains:
- 10 bottles each containing 5 ml of UREA 40% *Supplement*.
- 1 Instruction sheet

PRINCIPLE OF THE METHOD

The utilization of urea by microorganisms provided of urease causes the alkalinization of medium and consequently the colour turning of indicator red phenol from amber to pink colour.

COMPOSITION

UREA 40% *Supplement*		
Contents / bottle		*Contents / l of medium*
Urea	2.0 g	20.0 g

PROCEDURE FOR USE

1. Aseptically take the content of one bottle of UREA 40% *Supplement* and add it to 95 ml of Urea Agar Base cod. 610107 or 620107 autoclaved and cooled to 45-50 °C.
2. Mix with care avoiding the formation of foam.
3. Distribute into the final containers.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical documentation for medium Urea Agar Base cod. 610107 or 620107.

QUALITY CONTROL

1. Control of the appearance: clear, colourless solution.
2. Microbiological control:

prepare the plastes using as base the medium Urea Agar Base cod. 610107 or 620107 added with UREA 40% *Supplement*.
The plates are inoculated with the strains indicated in the table of microbiological control.
Conditions of incubation: 6-24 h at 36 ± 1 °C.
Microbiological control:

Control strains		**Ureasic activity**
Proteus vulgaris	ATCC 13315	Positive / pink medium
Escherichia coli	ATCC 25922	Negative / no change in colour

PRECAUTIONS

The product UREA 40% *Supplement* is classified as irritant under current legislation;; it is recommended that the Safety Data Sheet be consulted on its correct use.
UREA 40% *Supplement* is a supplement to be used only for *in vitro* diagnostic use. It is intended for use in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store *UREA 40% Supplement* at 2-8°C in its original packaging. In such conditions *UREA 40% Supplement* maintains its validity until the expiry date indicated on the label. Non utilizzare oltre questa data. Eliminate without using if there are signs of deterioration.

REFERENCES

- Christensen, W.B. (1946). J. Bact. **52**: 461-466.
- Maslen, L.G.C. (1952). Brit. Med. J. **2**: 545-546.

PRESENTATION

product	REF	Σ
UREA 40% *Supplement*	**80292**	**10 bottles**

One bottle is sufficient to prepare 100 ml of medium

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	LOT Batch code

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CE IVD *Rev.0 / 06.04.2005*

BORDET GENGOU AGAR

Medium for *Bordetella spp* isolation.

TYPICAL FORMULA	(g/l)
Potato, Infusion from	4.5
Peptone	5.0
Tryptone	5.0
Sodium Chloride	5.5
Agar	16.0

Final pH = 6.7 ± 0.2 at 25 °C.

DIRECTIONS

Suspend 36.0 g of powder in 1 liter of distilled or deionized water. Add 10 ml of glycerol. Heat to boiling until completely dissolved. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and aseptically add 15-20% sterile defibrinated sheep blood and 2 vials of Bordetella supplement (cephalexin 20 mg/vial) (code 81013) rehydrated with 5 ml of sterile distilled water. Mix well. Dispense in petri dishes. Final medium will contain 40 mg/l of cephalexin.

DESCRIPTION

BORDET GENGOU AGAR is used with added blood and Bordetella supplement, for isolating *Bordetella pertussis* and other *Bordetella* species.

TECHNIQUE

Inoculate the medium with the specimen to examine spreading with a swab and incubate at 36 ± 1 °C for 3-5 days for isolating *Bordetella pertussis*. Other Bordetella species can appear in 1-3 days. Otherwise allow the patient to cough directly on the plate at a distance of about 10 cm from mouth. The colonies of *Bordetella pertussis* appear small, dropshaped, transparent, shiny and surrounded by a characteristic zone of hemolysis that is not sharply defined but merges diffusely into the medium.

QUALITY CONTROL

Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: beige.
Prepared medium
Appearance: opaque.
Color: cherry red.
Incubation conditions: 36 ± 1°C for 48-72 hours.

Microorganism	ATCC	Growth
Bordetella pertussis	8467	good
Bordetella parapertussis MDH	32472	good

PERFORMANCE AND LIMITATIONS

Since nutritional requirements of organisms vary, some strains of Bordetella may be encountered that grow poorly or fail to grow on this medium.

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.
Store prepared plates at 2-8 °C.

REFERENCES

1. Bordet, J., and D. Gengou. 1906. Le microbe de la coqueluche. Ann. Inst. Pasteur **20**: 731.
2. Isenberg, H.D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society of Microbiology, Washington, D.C.

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CE IVD

PRESENTATION

Product	REF	Σ
BORDET GENGOU AGAR (13.8 l)	610006	500 g
BORDET GENGOU AGAR (2.7 l)	620006	100 g
BORDETELLA supplement Cephalexin 20 mg / vial	81013	10 vials
SHEEP BLOOD DEFIBRINATED	83296	50 ml

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests	IVD *In Vitro* Diagnostic Medical Device
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	Keep away from heat source

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CE IVD

BORDETELLA *Supplement*

ENGLISH

Selective supplement for the enrichment of BORDET GENGOU AGAR BASE medium
for the isolation of *Bordetella spp.*

DESCRIPTION

BORDETELLA *Supplement* is a selective supplement for the isolation of *Bordetella pertussis* and other species of Bordetella, made of Cephalexin freeze-dried. BORDETELLA *Supplement* is used for the selective enrichment of Bordet Gengou Agar Base medium code 610006 or 620006.

KIT CONTENTS

Each kit contains:

- 10 bottles of freeze-dried BORDETELLA *Supplement*
- 1 Instruction sheet

PRINCIPLE OF THE METHOD

La Cephalexin is a cephalosporins is a broad-spectrum antibiotic, active against both Gram-positive bacteria and Gram-negative bacteria.

COMPOSITION

BORDETELLA *Supplement*		
	Contents / bottle	*Contents / l of medium*
Cephalexin	20.0 mg	40.0 mg

PROCEDURE FOR USE

1. Aseptically reconstitute the contents of one bottle of BORDETELLA *Supplement* with 5 ml of sterile distilled water. Shake until completely dissolved, avoiding foam formation.
2. Aseptically add the entire contents of one bottle (5 ml) to 500 ml of Bordet Gengou Agar Base medium code 610006-620006, autoclaved, cooled at 45-50 °C and with the addition of 15-20% of defibrinated ram's blood.
3. Mix with care.
4. Distribute into Petri dishes.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical documentation for Bordet Gengou Agar Base code 610006-620006.

QUALITY CONTROL

1. Control of the appearance: freeze-dried product, white colour.
2. Microbiological control.

Prepare the plates using Bordet Gengou Agar Base code 610006 or 620006 enriched with BORDETELLA *Supplement* (1 bottle in 500 ml of medium) and with 15% of defibrinated ram's blood. Plates are inoculated with the strains indicated in the microbiological control table.

Incubation conditions: 48 h at 36 ± 1 °C.

Microbiological control

Control strains		**Growth**
Bordetella pertussis	ATCC 8467	Good
Bordetella parapertussis	MDH 32472	Good
Staphylococcus aureus	ATCC 25923	Inhibited
Escherichia coli	ATCC 25922	Inhibited

PRECAUTIONS

The product BORDETELLA *Supplement* is classifiable as hazardous under current legislation; it is recommended that the Safety Data Sheet be consulted on its use.
BORDETELLA *Supplement* is a selective supplement to be used only for *in vitro* diagnostic use. It is intended for use in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store BORDETELLA *Supplement* at 2-8 °C in its original packaging. In such conditions BORDETELLA *Supplement* will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

REFERENCES

- Bordet, J., and D. Gengou. 1906. Le microbe de la coqueluche. Ann. Inst. Pasteur 20: 731.
- Isenberg, H.D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society of Microbiology, Washington, D.C.
- Regan J., and Lowe F. (1977) J. Clin. Microbiol. **6**: 303-309.

PRESENTATION

Product	REF	Σ
BORDETELLA *Supplement*	**81013**	**10 bottles**

One bottle is sufficient to prepare 500 ml of medium

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	LOT Batch code

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CE IVD

Rev.0 / 06.04.2005

Instructions For Use
ENGLISH

Baird Parker Agar Base

Selective medium for detection and enumeration of coagulase-positive staphylococci in food and animal feed, according to ISO 6888.

DESCRIPTION

Baird Parker Agar Base is a selective medium used with supplements for the isolation and enumeration of *Staphylococcus aureus* from food, foodstuffs and water.

This medium complies with the specification given by ISO 6888 (all parts), FDA-BAM and APHA.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Casein	10.0
Meat Extract	5.0
Yeast Extract	1.0
Sodium Pyruvate	10.0
L-Glycine	12.0
Lithium Chloride	5.0
Agar	17.0
Final pH 7.2 ± 0.2 at 25°C	

METHOD PRINCIPLE

Pancreatic digest of casein and meat extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Sodium pyruvate and glycine are incorporated to stimulate the growth of even damaged *S. aureus* cells without destroying the selectivity. Lithium chloride and the high concentration of glycine inhibit accompanying microflora. Agar is the solidifying agent.

Supplementation with Egg yolk Tellurite Emulsion (ref. 80122, 80123) in addition to being an enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). Presence of potassium tellurite confers further selectivity and determines grey or black colouration of colonies.

If *Proteus* spp are suspected in the test sample, Sulfamethazine Supplement (ref. 81095) may be added to suppress growth and swarming.

For foodstuffs likely to be contaminated by staphylococci forming atypical colonies on Baird Parker Medium or by background flora which can obscure the colonies being sought, the RPF Supplement (ref. 81057) should be used: rabbit plasma, fibrinogen and trypsin inhibitor allow the confirmation of staphylococci on the basis of coagulase reaction.

PREPARATION

Dehydrated medium	Suspend 60 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down.
Supplements	Cool the medium to 45-50°C before adding supplements aseptically.
	Baird Parker Egg Yolk Tellurite Agar: add 5 ml of Egg yolk Tellurite Emulsion to 100 ml of base. If necessary, add 1 ml of reconstituted Sulfamethazine Supplement when *Proteus* spp are suspected.
	Baird Parker RPF Agar: add 10 ml of reconstituted RPF Supplement to 90 ml of base (if the 100 ml bottle is being used, first remove 10 ml of medium from the bottle).
	Mix well avoiding foam formation and under sterile conditions distribute into Petri dishes.

TEST PROCEDURE

Baird Parker Egg Yolk Tellurite Agar (ISO 6888-1/-3)

- For direct enumeration, spread 0.1 ml of sample, initial suspension or decimal dilutions, over the medium surface (use a suitable diluent such as Buffered Peptone Water, ref. 24099).
- For detection and enumeration by the MPN technique, inoculate by subculturing the selective enrichment in Giolitti Cantoni Broth (ref. 620100).

Baird Parker RPF Agar (ISO 6888-2)

Transfer 1 ml of test sample or its initial suspension to two sterile Petri dishes. Repeat the operation with 1 ml of the first decimal dilution and successive dilution. Into each Petri dish, immediately pour freshly prepared complete medium. Carefully mix the inoculum with the culture medium and leave to solidify.

Incubate at 37 ± 1°C for 24-48 hours.

INTERPRETING RESULTS

Baird Parker Egg Yolk Tellurite Agar

Take for enumeration only those plates containing a maximum of 300 typical and/or atypical colonies, from two successive dilutions (one of the plates shall contain at least 15 colonies):

- Typical colonies of *S. aureus* appear black or gray, shining and convex, surrounded by a zone of clearing of the medium. After incubation for at least 24 h, an opalescent ring immediately in contact with the colonies, may appear in this clear zone.

- Atypical colonies are identical in appearance but not surrounded by a clear zone. They can mainly be observed in dairy products.

Confirm typical and atypical colonies by the Coagulase Test (ref. 88030). The majority of other organisms are inhibited but some may grow sparsely, producing white to brown colonies with no clearing of the egg yolk.

Baird Parker RPF Agar
The medium allows the simultaneous enumeration and confirmation to be performed in a single operation. Coagulase-positive staphylococci colonies appear black or grey with a halo of precipitation, indicating coagulase activity.

APPEARANCE
Dehydrated medium: free-flowing, homogeneous, beige.
Prepared medium: opaque, yellow.

STORAGE
The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE
Dehydrated medium: 4 years.
Medium in bottles: 2 years.

QUALITY CONTROL
Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU.
Inoculum for selectivity: 10^4-10^6 CFU.
Inoculum for specificity: 10^3-10^4 CFU.
Incubation conditions: 37 ± 1°C for 24-48 hours.

QC Table.

Microorganism		Specification
Staphylococcus aureus	WDCM 00034	Good growth, black or grey colonies with halo
Escherichia coli	WDCM 00012	Total inhibition
Staphylococcus saprophyticus	WDCM 00159	Black or gray colonies without halo
Staphylococcus epidermidis	WDCM 00009	Black or gray colonies without halo

WARNING AND PRECAUTIONS
The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE
Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 6888-3:2003. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 3 : detection and MPN technique for low numbers.
3. ISO 6888-1:1999/Amd 1:2003. Inclusion of precision data.
4. ISO 6888-1:1999. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium.
5. ISO 6888-2:1999. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) -- Part 2: Technique using rabbit plasma fibrinogen agar medium.
6. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) -- Part 2: Technique using rabbit plasma fibrinogen agar medium
7. D.De Medici, L.Fenicia, L.Orefice and A.Stacchin. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti.
8. Baird Parker , A.C. (1969) The use of Baird Parker's medium for the isolation and enumeration of *Staphylococcus aureus* in "Isolation methods for microbiologists" Shapthon, D.A. & Gould ed. London: Academic Press.
9. Smith, B.A. & Baird Parker, A.C. (1964) - The use of sulphamezathine for inhibiting Proteus spp. on Baird- Parker's isolation medium for Staphylococcus aureus. J. Appl. Bact. 27:78-82.
10. Baird Parker , A.C. (1962) An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bacteriol. 25:12-19.

PRESENTATION		Contents	Ref.
Baird Parker Agar Base	Bottles	6 x 100 ml bottles	420110
Baird Parker Agar Base	Dehydrated medium	500 g of powder	610004
Baird Parker Agar Base	Dehydrated medium	100 g of powder	620004

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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Egg Yolk Tellurite Emulsion

Supplement for detection of coagulase-positive staphylococci, according to ISO 6888.

DESCRIPTION

A sterile, stabilized emulsion of egg yolk containing potassium tellurite for use in Baird Barker Agar Base (ref. 610004, 620004). The complete medium is used for the isolation and enumeration of *Staphylococcus aureus* in food, in accordance with ISO 6888 (part 1 and 3).

COMPOSITION

Chicken egg yolks in an equal volume of saline solution (0.9% NaCl) with potassium tellurite (2 g/l).

METHOD PRINCIPLE

Egg yolk is broken down by staphylococci containing lecithinase to yield clear zones around the colonies. Potassium tellurite is reduced by coagulase-positive staphylococci causing blackening of colonies.

PROCEDURE FOR USE

Aseptically, add 50 ml to 1 litre of Baird Parker Agar Base sterilized in autoclave and cooled to 45-50°C.

Final concentration of tellurite in medium is 0.01% w/v.

TECHNIQUE AND INTERPRETATION OF RESULTS

Refer to the technical sheet of the medium being prepared.

APPEARANCE

Yellowish opaque emulsion. May contain a precipitate that can be resuspended.

STORAGE

Store at 2-8°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration. Mix well before use.

SHELF LIFE

1 year.

QUALITY CONTROL

Cultural response in Baird Parker Agar after 48 hours incubation at 37 ± 1°C:

Microorganism		Specification
Staphylococcus aureus	WDCM 00034	Good growth, black or grey colonies with clear halo
Escherichia coli	WDCM 00012	Total inhibition
Staphylococcus saprophyticus	WDCM 00159	Black or grey colonies without halo
Staphylococcus epidermidis	WDCM 00036	Black or grey colonies without halo

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 6888-3:2003. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 3 : detection and MPN technique for low numbers.
3. ISO 6888-1:1999. Microbiology of food an animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird Parker Agar Medium.
4. Baird-Parker A.C. (1962) An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bacteriol. 25:12-19.

PRESENTATION	Contents	Ref.
Egg Yolk Tellurite Emulsion	4 x 50 ml bottles	80122
Egg Yolk Tellurite Emulsion	6 x 100 ml bottles	80125

TABLE OF SYMBOLS

LOT Batch code	Do not reuse	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	

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Neutralizing Fluid E.P.

Liquid medium for neutralizing antimicrobials, according to the European Pharmacopoeia.

TYPICAL FORMULA	(g/l)
Peptone	1.0
Histidine Hydrochloride	1.0
Lecithin	3.0
Potassium Dihydrogen Phosphate	3.6
Disodium Hydrogen Phosphate Dihydrate	7.2
Sodium Chloride	4.3
Final pH 7.0 ± 0.2 at 25°C	

DESCRIPTION

Neutralizing Fluid E.P. is a liquid medium used for neutralizing the activity of antimicrobial agents.

The medium is formulated according to the European Pharmacopoeia specification for the microbiological examination of non-sterile products.

PRINCIPLE

Peptone supplies amino acids, nitrogen, carbon, minerals, vitamins and other nutrients which support the growth of microorganism. Histidine inactivates aldehydes. Lecithin neutralizes quaternary ammonium compounds. Phosphates serve as buffering agents. Sodium chloride maintains the osmotic balance of the medium.

Supplementation with polysorbate (Tween) 80 is effective against phenolic compounds and mercurial derivates.

PREPARATION

Suspend 20.1 g of powder in 1 liter of deionized or distilled water containing 30 g of polysorbate 80 (ref. 80031). Bring to boil and shake until completely dissolved. Pour into suitable containers. Sterilize at 121°C for 15 minutes.

TECHNIQUE

Neutralizing Fluid E.P. may be incorporated into diluents or media, such as Buffered Peptone Water EP, USP (ref. 400040), preferably before sterilization.

INTERPRETATION OF RESULTS

Refer to technical sheet of the medium being used.

STORAGE AND TRANSPORT CONDITIONS

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store the prepared medium at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

- European Pharmacopeia (2007) Directorate for the Quality of Medicine of the Council of Europe. 2.6.13. Microbiological examination of non-sterile products: test for specific micro-organisms. Council of Europe Strasbourg.

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PRODUCT SPECIFICATIONS

NAME
Neutralizing Fluid E.P.

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610330	500 g	500 g of powder in plastic bottle
620330	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.0 ± 0.2

USE
Neutralizing Fluid E.P. is a liquid medium formulated according to the European Pharmacopoeia specification for the microbiological examination of non-sterile products

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: beige
Ready-to-use medium
Appearance: opalescent
Colour: light amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Cultural response observed when subcultured on Tryptic Soy Agar after an incubation at 30°C for 3 hours in Neutralizing Fluid E.P.
   Inoculum for productivity: 50-100 CFU
   Incubation Conditions: 18-24 h at 32.5 ± 2.5°C, in aerobiosis

Microorganism		Growth
Staphylococcus aureus	ATCC® 6538	Good
Escherichia coli	ATCC® 8739	Good
Pseudomonas aeruginosa	ATCC® 9027	Good
Bacillus subtilis	ATCC® 6633	Good
Salmonella typhimurium	ATCC® 14028	Good
Candida albicans	ATCC® 10231	Good
Aspergillus niger	ATCC® 16404	Good

TABLE OF SYMBOLS

LOT Batch code	Do not reuse	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	

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Pseudomonas Agar Base

Selective medium for detection and enumeration of *Pseudomonas* spp, according to ISO 13720, ISO/TS 11059 and ISO 16266.

TYPICAL FORMULA	(g/l)
Gelatin Peptone	16.0
Casein Hydrolysate	10.0
Potassium Sulfate, Anhydrous	10.0
Magnesium Chloride, Anhydrous	1.4
Agar	15.0
Final pH 7.1 ± 0.2 at 25°C	

DESCRIPTION

Pseudomonas Agar Base is a medium used with supplements for the selective isolation of *Pseudomonas* spp from meat and dairy products, water, environmental samples and clinical specimens.

When supplemented with CFC (Pseudomonas) Supplement (ref. 81049), the medium complies with the recommendations of ISO 13270 for the detection and enumeration of *Pseudomonas* spp in meat and meat products.

When supplemented with PP (Pseudomonas) Supplement (ref. 81093) the medium complies with the recommendations of ISO/TS 11059 for the isolation and enumeration of *Pseudomonas* spp in milk and milk products.

When supplemented with CN (Pseudomonas) Supplement (ref. 81006) the medium complies with the recommendations of ISO 16266 for the detection and enumeration of *Pseudomonas aeruginosa* in water samples by using the membrane filtration technique.

PRINCIPLE

Gelatin peptone and casein hydrolysate provide amino acids, nitrogen, carbon, minerals, vitamins and other nutrients for organisms growth. Potassium sulfate and magnesium chloride promote pyocyanin production. Agar is the solidifying agent.

Supplementation with Glycerol Supplement (ref. 80021) supplies a carbon and energy source enhancing pyocyanin production.

CFC Supplement contains Cetrimide, Fusidic Acid and Cefaloridin.

PP Supplement contains Primaricin (Natamycin) and Penicillin G.

CN Supplement contains Cetrimide and Nalidixic Acid.

By use of the appropriate selective supplement and incubation conditions the medium becomes selective for *Pseudomonas* spp, including *Burkholderia cepacia* (CFC Agar and PP Agar), or *Pseudomonas aeruginosa* (CN Agar).

PREPARATION

Suspend 52.4 g of powder in 1 liter of deionized or distilled water. Add 10 ml of Glycerol Supplement. Bring to boil and shake until completely dissolved. Sterilize at 121°C for 15 minutes. Cool up to 45-50°C.

To prepare Pseudomonas CFC Agar, aseptically, add rehydrated content of 2 vials (4 ml) of CFC Supplement.

To prepare Pseudomonas PP Agar, aseptically, add rehydrated content of 2 vials (10 ml) of PP Supplement.

To prepare Pseudomonas CN Agar, aseptically, add rehydrated content of 2 vials (4 ml) of CN Supplement.

Mix well and pour in Petri dishes.

TECHNIQUE

Pseudomonas CFC Agar and Pseudomonas PP Agar
Inoculate the medium by using the spread plate technique. Incubate aerobically at 25 ± 1°C for up to 5 hours.

Pseudomonas CN Agar
Inoculate the medium by using the membrane filtration method. Incubate aerobically at 36 ± 2°C for 40-48 hours.

INTERPRETATION OF RESULTS

All colonies grown on either CFC Agar or PP Agar are suspect *Pseudomonas* spp. Colonies which result non-glucose fermenters (ref. 88202) and oxidase positive (ref. 88029, 88003 or 88004) are confirmed as *Pseudomonas* spp.

Examine membranes on CN Agar for growth and fluorescence under UV light after 20-24 h and 40-48 h.

- Count all colonies that produce the green-blue pigment as confirmed *Pseudomonas aeruginosa*.
- Count all non-pyocyanin producing colonies that fluoresce as presumptive *Pseudomonas aeruginosa.* Confirm by using Acetamide Broth (ref. 24144).
- Count all other reddish-brown non-pigmented colonies that do not fluoresce as presumptive *Pseudomonas aeruginosa.* Confirm by using the oxidase test, Acetamide Broth and King's B Medium (ref. 11072).

STORAGE AND TRANSPORT CONDITIONS

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

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CE IVD

REFERENCES

- EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
- ISO/TS 11059:2009 (IDF/RM 225: 2009) Milk and milk products – Method for the enumeration of *Pseudomonas* spp.
- UNI EN ISO 16266:2008. Water quality – Detection and enumeration of *Pseudomonas aeruginosa* by membrane filtration.
- ISO 13720:1995. Meat and meat products – Enumeration of *Pseudomonas* spp.
- Mead, G.C. and B.W. Adams (1977) A selective medium for the rapid isolation of Pseudomonas associated with poultry meat spoilage. Br. Poult. Sci. 18:661-670
- Goto S. and S. Enomoto (1970) Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of P. aeruginosa. Jpn. J. Microbiol. 14:65.

PRODUCT SPECIFICATIONS

NAME
Pseudomonas Agar Base

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610071	500 g	500 g of powder in plastic bottle
620071	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.1 ± 0.2

USE
Pseudomonas Agar Base is a medium used with supplements for the selective isolation of *Pseudomonas* spp from meat and dairy products, water, environmental samples, according to ISO 13720, ISO/TS 11059 and ISO 16266. This medium can be used also for the examination of clinical specimens

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: light beige
Ready-to-use medium
Appearance: slightly opalescent
Colour: amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Inoculum for selectivity: 10^4-10^6 CFU

Pseudomonas CFC Agar Incubation Conditions: 40-48 h at 25 ± 1°C, in aerobiosis

Microorganism		Growth
Pseudomonas fluorescens	WDCM 00115	Good
Pseudomonas fragi	WDCM 00116	Good
Escherichia coli	WDCM 00012	Inhibited

Pseudomonas PP Agar Incubation Conditions: 48 ± 2 h at 25 ± 1°C, in aerobiosis

Microorganism		Growth
Pseudomonas fluorescens	WDCM 00115	Good
Pseudomonas aeruginosa	WDCM 00025	Good
Escherichia coli	WDCM 00012	Inhibited

Pseudomonas CN Agar Incubation Conditions: 40-48 h at 36 ± 2°C, in aerobiosis

Microorganism		Growth
Pseudomonas aeruginosa	WDCM 00024	Good
Enterococcus faecalis	WDCM 00087	Inhibited
Escherichia coli	WDCM 00013	Inhibited

TABLE OF SYMBOLS

LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	Do not reuse

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CE IVD

ENGLISH

CN (Pseudomonas) *Supplement*

Selective supplement for the isolation of *Pseudomonas aeruginosa*

DESCRIPTION

CN (*Pseudomonas*) *Supplement* is a selective supplement for the isolation of *Pseudomonas aeruginosa,* comprising a freeze-dried mixture of Cetrimide and Nalidixic Acid. CN (*Pseudomonas*) *Supplement* is used for selective enrichment of PSEUDOMONAS AGAR BASE medium code 610071 or 620071.

KIT CONTENTS

Each kit contains:

- 10 bottles of freeze-dried CN (*Pseudomonas*) *Supplement*
- 1 Instruction sheet

PRINCIPLE OF THE METHOD

CN (*Pseudomonas*) *Supplement* is recommended for the selective isolation of *Pseudomonas aeruginosa.* The formula for the supplement was described by Goto and Enomoto who demonstrated that the addition of nalidixic acid at a concentration of 15 µg/ml with the anionic surfactant cetrimide at only 200 µg/ml, increased the efficiency of the medium.

COMPOSITION

CN (*Pseudomonas*) *Supplement*		
Contents / bottle		*Contents / l of medium*
Cetrimide Nalidixic acid	100.0 mg 7.5 mg	200.0 mg 15.0 mg

PROCEDURE FOR USE

1. Aseptically reconstitute the contents of a bottle of CN (*Pseudomonas*) *Supplement* with 2 ml of a solution of sterile distilled water and ethanol in the ratio 1: 1. Shake until completely dissolved, avoiding foam formation.
2. Aseptically add the entire contents of a bottle (2 ml) to 500 ml of Pseudomonas Agar Base medium code 610071-620071, supplemented with 5 ml of Glycerol *Supplement* (code 80021), autoclaved and cooled to 45-50 °C.
3. Mix with care.
4. Distribute into Petri dishes.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical documentation for PSEUDOMONAS AGAR BASE code 610071 or 620071.

QUALITY CONTROL

1. Control of the appearance: freeze-dried product, colour white.
2. Microbiological control.

Prepare plates using as base PSEUDOMONAS AGAR BASE code 610071 or 620071 supplemented with Glycerol *Supplement* (code 80021, 5 ml in 500 ml of medium) and with CN (*Pseudomonas*) *Supplement* (1 bottle in 500 ml of medium). The plates are seeded with the strains indicated in the microbiological control table.
Incubation conditions: 24 h at 36+/-1 °C.

Microbiological control

Control strains		**Growth**
Burkholderia cepacia	ATCC 25609	Partially inhibited
Pseudomonas aeruginosa	ATCC 9027	Good
Pseudomonas aeruginosa	ATCC 27852	Good
Pseudomonas putida	ATCC 12633	Good
Staphylococcus aureus	ATCC 25923	Inhibited
Proteus vulgaris	ATCC 13315	Partially inhibited

PRECAUTIONS

The product CN (*Pseudomonas*) *Supplement* is classifiable as hazardous under current legislation; it is recommended that the Safety Data Sheet be consulted on its use.
CN (*Pseudomonas*) *Supplement* is a selective supplement to be used only for *in vitro* diagnostic use. It is intended for use in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store CN (*Pseudomonas*) *Supplement* at 2-8 °C in its original packaging. In such conditions CN (*Pseudomonas*) *Supplement* will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

REFERENCES

- King, E.O., M.K. Ward, and D.E. Raney (1954). Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. 44, 301.
- Goto S. and Enomoto S. (1970) Jap. J. Microbiol. **14:** 65-72.
- Lowbury E.J. And Collins A.G. (1955) J. Clin. Path. **8**: 47-48.

PRESENTATION

product	REF	Σ
CN (*Pseudomonas*) *Supplement*	81006	10 bottles

One bottle is sufficient to prepare 500 ml of medium

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	LOT Batch code

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CE IVD

Rev.0 / 06.04.2005

PSEUDOMONAS (CETRIMIDE) AGAR

Selective medium for *Pseudomonas aeruginosa* isolation, according to European Pharmacopoeia.

TYPICAL FORMULA	(g/l)
Pancreatic digest of gelatin	20.0
Magnesium Chloride	1.4
Dipotassium Sulphate	10.0
Cetrimide	0.3
Agar	15.0

Final pH = 7.2 ± 0.2 at 25 °C.

DIRECTIONS

Suspend 46.7 g of powder in 990 ml of distilled or deionized water.
Add 10 ml of Glycerol supplement (code 80021).
Heat until completely dissolved.
Sterilize in autoclave at 121 °C for 15 minutes.
Dispense in petri dishes.

DESCRIPTION

PSEUDOMONAS (CETRIMIDE) AGAR is recommended by the European Pharmacopoeia for the isolation and identification of *Pseudomonas* strains.
The medium promotes the production of fluorescein (pyoverdin), a green-yellow fluorescent pigment that oxidizes to yellow. It is water-soluble and, unlike pyocyanin (blue-green pigment), is not soluble in chloroform. The pigment diffuses throughout the medium and the fluorescent yellow-green color is observed.
Gelatin pancreatic digest provide the nutrient growth factors: nitrogen, vitamins, minerals and amino acids. Glycerol is the carbon source. Magnesium chloride and dipotassium sulfate enhance the production of pyocyanin, pyoverdin and fluorescein.
Cetrimide is the selective agent as it inhibits the growth of the accompanying microbial flora.

TECHNIQUE

Inoculate the medium using the streak plate method to obtain isolated colonies.
Incubate for 18-48 hours at 36 ± 1 °C.
Examine for the presence of a good growth and pigment production.
Pseudomonas aeruginosa colonies will be green to blue-green with pigment that diffuses into the medium.
The identification of *Pseudomonas aeruginosa* is completed by performing oxidase test and the differential tests for the production of fluorescein and pyocyanin on, respectively, Pseudomonas Agar F (code 610309) and Pseudomonas Agar P (code 610310).

QUALITY CONTROL

Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: very light beige.
Prepared medium
Appearance: slightly opalescent, firm.
Color: light amber.
Incubation conditions: 36 ± 1 °C for 18-48 hours.

Microorganism	ATCC	Growth	Appearance
Pseudomonas aeruginosa	9027	good	green to blue-green
Pseudomonas aeruginosa	27853	good	green to blue-green
Escherichia coli	25922	poor	

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PERFORMANCE AND LIMITATIONS

The quaternary ammonium compound “Cetrimide” inhibits the growth of Gram positive and Gram negative bacteria, except *Psudomonas spp.*
The particular formulation stimulates production of fluorescein and pyocyanin, though some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.
Non-*Pseudomonas aeruginosa* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Pseudomonas aeruginosa*.
Consult appropriate references.

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.
Store prepared plates at 2-8 °C.

REFERENCES

1. King, E.O., and D.E. Raney (1954). Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. **44**, 301.
2. Gilligan, P.H. (1995). Pseudomonas and Burkholderia, p.509-519. In Manual of Clinical Microbiology, 6th ed. American Society for microbiology, Washington, D.C.
3. European Pharmacopoeia, 3rd ed.2001. Supplement.

PRESENTATION

Product	REF	Σ
PSEUDOMONAS (CETRIMIDE) AGAR (10.7 l)	610041	500 g
PSEUDOMONAS (CETRIMIDE) AGAR (2.1 l)	620041	100 g
PSEUDOMONAS (CETRIMIDE) AGAR (107.0 l)	6100415	5 Kg

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	LOT Batch code	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Keep away from heat source	Use by	Caution, consult accompanying documents	

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CE IVD

Instructions For Use
ENGLISH

Phenol Red Broth Base

Liquid medium for carbohydrate fermentation studies.

DESCRIPTION

Phenol Red Broth Base is a liquid medium used with an appropriate carbohydrate for the differentiation of microorganisms on the basis of fermentation reactions.

TYPICAL FORMULA	(g/l)
Casein Peptone	10.0
Meat Extract	3.0
Sodium Chloride	5.0
Phenol Red	0.018

Final pH 7.4 ± 0.2 at 25°C

METHOD PRINCIPLE

Casein peptone and meat extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride maintains the osmotic balance of the medium. Phenol red is the pH indicator. Various fermentable substances may be added in any desired concentration. The concentration of carbohydrate generally employed for testing fermentation reactions of bacteria is 0.5 to 1%.

PREPARATION

Dehydrated medium	Suspend 18 g of the powder in 1 liter of distilled or deionized water. Heat until completely dissolved. If desired, add 5 to 10 g of the specified carbohydrate(*). Mix well. Dispense into test tubes. If necessary, insert Durham tubes. Sterilize in autoclave at 121°C for 15 minutes. *Alternatively, filtered sterilized carbohydrate solutions may be added to the cooled sterilized broth.
Medium in tubes	Under aseptic conditions, add a specific carbohydrate (final concentration 5-10 g/l) as filter-sterilized solution. If necessary, insert Durham tubes. NOTE: Without the addition of carbohydrates, the medium can be used as negative control for fermentation studies.

TEST PROCEDURE

Inoculate tubes with isolated colonies. Tubes without carbohydrates added should also be inoculated to serve as growth controls. Incubate at 35 ± 2°C for 18-48 h with loose caps.

INTERPRETING RESULTS

Examine tubes for growth, acid production, and gas production (if Durham tube is used).

A yellow color in the medium indicates a positive reaction for carbohydrate fermentation. If a Durham tube is used, bubbles in the inverted tube is an indication of gas production. The presence of a single bubble is recorded as positive for the production of gas.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, pinkish-beige.
Prepared medium: clear, bright red to red-orange.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes: 2 year.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Incubation conditions: 18-48 h / 35 ± 2°C.

QC Table.

Microorganism		Specification with Glucose		
		Growth	Acid reaction	Gas formation
Escherichia coli	ATCC® 25922	Good	+ (color change to yellow)	+
Shigella flexneri	ATCC® 12022	Good	+ (color change to yellow)	–
Pseudomonas aeruginosa	ATCC® 27853	Good	– (red medium)	–

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *In Vitro* Diagnostic use and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.
2. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
4. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore.
5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's ManualTM of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
6. Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*. 4th ed. Elsevier Science Publishing Co., New York.
7. Vera, H.D. 1950. Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am.J.PublicHealth, *40*:1267-1272.

PRESENTATION	Category	Packaging	Ref.
Phenol Red Broth	Tubes	20 x 10 ml	24446
Phenol Red Broth Base	Dehydrated media	500 g	610174
Phenol Red Broth Base	Dehydrated media	100 g	620174

TABLE OF SYMBOLS

LOT Batch code	**IVD** In Vitro Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care	Do not reuse
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Keep away from sunlight	

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Rappaport Vassiliadis Soy (RVS) Broth

Selective liquid medium for detection of *Salmonella* according to ISO 6579-1.

Instructions For Use
ENGLISH

DESCRIPTION

Rappaport Vassiliadis Soy (RVS) Broth is a culture medium used for the selective enrichment of *Salmonella* spp. from food, animal feed, environmental and clinical samples.

This medium conforms to the requirements of ISO 6579-1.

TYPICAL FORMULA*	(g/litre)
Enzymatic Digest of Soya	4.5
Sodium Chloride	7.2
Potassium Dihydrogen Phosphate (KH_2PO_4)	1.26
Dipotassium Hydrogen Phosphate (K_2HPO_4)	0.18
Magnesium Chloride Anhydrous	13.4
Malachite Green	0.036

Final pH 5.2 ± 0.2 at 25°C

*Formula may be adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Enzymatic digest of soya provides amino acids, nitrogen, carbon, minerals and vitamins for organisms growth. Sodium chloride maintains the osmotic balance of the medium. Potassium phosphates act as a buffer. Magnesium chloride and malachite green are the selective agents. The low pH helps inhibit non-target organisms.

PREPARATION

Dehydrated medium	Suspend 26.6 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Dispense into final containers. Sterilize in autoclave at 115°C for 15 minutes.

TEST PROCEDURE

Following the method described in the ISO 6579-1, transfer 0.1 ml of the culture obtained in the pre-enrichment (Buffered Peptone Water, ref. 24099) to a tube containing 10 ml of the RVS Broth.

Incubate at 41.5 ± 1°C for 24 ± 3 hours.

Use the culture obtained to inoculate the selective solid media, i.e. XLD agar (ref. 10056) and a second isolation agar.

For more information, see the ISO document.

Notes:

Before use, allow RVS broth to equilibrate at room temperature if it was stored at a lower temperature.

For some products, like dried milk products and cheese, it may be necessary to incubate the selective enrichment medium for an additional 24 h.

After incubation, the selective enrichment can be stored refrigerated at 5 ± 3°C for up to 72 h.

INTERPRETING RESULTS

Turbidity in the RVS broth indicates microbial growth.

Refer to the technical sheets of the solid media.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 3 years.

Medium in tubes: 2 years.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, green.

Appearance of Prepared Medium: Clear, blue.

Expected Cultural Response:

Control strains	WDCM	Inoculum	Incubation	Criteria
Salmonella Typhimurium + *Escherichia coli* + *Pseudomonas aeruginosa*	00031 00012 or 00013 00025	≤100 CFU	24 ± 3 h / 41.5 ± 1°C	>10 characteristic colonies on XLD agar or other medium of choice
Salmonella Enteritidis + *Escherichia coli* + *Pseudomonas aeruginosa*	00030 00012 or 00013 00025			
Escherichia coli	00012 or 00013	>10^3 CFU		Partial inhibition ≤100 colonies on TSA
Enterococcus faecalis	00009 or 00087			<10 colonies on TSA

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For *in vitro* diagnostic use. **For professional use only**. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Rappaport Vassiliadis Soy (RVS) Broth	Tube	20 x 10 ml	24400
Rappaport Vassiliadis Soy (RVS) Broth	Tube	100 x 10 ml	26400
Rappaport Vassiliadis Soy (RVS) Broth	Bottle	6 x 100 ml	402550 •
Rappaport Vassiliadis Soy (RVS) Broth	Dehydrated medium	500 g	610175
Rappaport Vassiliadis Soy (RVS) Broth	Dehydrated medium	100 g	620175

• Not CE-marked

This document is available from the online Support Center:

liofilchem.com/ifu-sds

Instructions For Use
ENGLISH

SIM Medium

Semisolid medium for the identification of Enterobacteriaceae
by sulphide, indole and motility tests.

DESCRIPTION

SIM Medium is used for the identification of microorganisms from clinical specimens and other samples on the basis of hydrogen sulphide production, indole formation, and motility.

This medium is an aid to identify and discriminate between species of the family Enterobacteriaceae - for example, *Enterobacter* has the general characteristics of *Klebsiella* species but can be differentiated because they are motile as well as *Citrobacter*, *Proteus*, *Providencia* and *Serratia; Klebsiella, Enterobacter, Hafnia* and *Serratia* species are usually indole negative whereas *Escherichia* species are positive for indole (except *E. vulneris*); unlike *Shigella*, *Salmonella* species possess flagella and hence are motile and most produce hydrogen sulphide (except *S.* Paratyphi A and *S.* Typhi, which is a weak producer).

TYPICAL FORMULA*	(g/litre)
Casein Peptone	20.0
Meat Peptone	6.1
Ferric Ammonium Sulphate	0.2
Sodium Thiosulphate	0.2
Agar	3.5
Final pH 7.3 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance criteria.

METHOD PRINCIPLE

Peptones provide carbon, nitrogen and amino acids for bacterial growth. Casein peptone in particular contains tryptophan which is converted to indole. Ferric ammonium sulphate and sodium thiosulphate are used to detect hydrogen sulphide (H_2S) production through formation of a black precipitate. Agar is the solidifying agent. The low concentration of agar makes the medium semisolid allowing for visual determination of motility.

PREPARATION

Dehydrated medium	Suspend 30.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil and shake until completely dissolved. Dispense into tubes. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into tubes.

Note: Sufficient volume of medium must be dispensed into tubes to give a depth of about 4 cm. Allow tubes to solidify in a vertical position.

TEST PROCEDURE

1. Inoculate test organism by stabbing two-thirds into the medium.
2. Incubate with loose caps at 35 ± 2°C for 18-24 hours.
3. After incubation, examine tubes for motility and H_2S production.
4. Once H_2S and motility have been recorded, add 3-4 drops of Kovac's Reagent (ref. 87001) to each tube.

Note: Test organisms must be in pure culture. The inoculum should be taken from a solid medium as inoculum from liquid suspensions may delay results. Erroneous results may occur if caps are not loose during incubation.

INTERPRETING RESULTS

Motility	A positive motility test is indicated by a diffuse growth/turbidity extending from inoculating stab line, whereas non-motile organisms grow only along the line of inoculation.
H_2S	Hydrogen sulphide production is shown by a blackening of the medium in those areas where microbial growth has occurred.
Indole	Indole formation is seen as appearance of a pink or red color, whereas test is negative if there is no color change after addition of Kovac's Reagent.

Consult appropriate references for activities of specific microorganisms and for complete identification of Enterobacteriaceae[1-4].

Examples of characteristic reactions.

Microorganisms	Sulphide	Indole	Motility
Citrobacter	+	+	+
Enterobacter	–	–	+
Escherichia	–	+	±
Hafnia	–	–	+
Klebsiella	–	+	–
Morganella	–	+	+
Proteus mirabilis	+	–	+
Proteus vulgaris	+	+	+
Providencia	–	+	+
Salmonella	+	–	+
Serratia	–	–	+
Shigella	–	±	–
Yersinia enterocolitica	–	±	±*

**Y. enterocolitica* are motile at room temperature (25°C) but non-motile at 37°C.

APPEARANCE OF THE MEDIUM

Dehydrated medium: free-flowing, homogeneous, beige.
Prepared medium: semisolid, clear to slightly opalescent, medium amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes and bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Bottles: 2 years.
Tubes: 1 year.

QUALITY CONTROL

To check the performance of the medium the following microbial strains can be used.

Strain		Inoculum	Incubation	Growth	H_2S	Indole	Motility
Escherichia coli	ATCC® 25922	2-3 colonies; direct inoculum	35 ± 2°C / 18-24 h	Good	–	+	+
Salmonella Typhimurium	ATCC® 14028			Good	+	–	+
Shigella flexneri	ATCC® 12022			Good	–	–	–

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. UK Standards for Microbiology Investigations ID 16: Identification of Enterobacteriaceae (2015). Issued by the Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/423601/ID_16i4.pdf
2. UK Standards for Microbiology Investigations ID 24: Identification of *Salmonella* species (2015). Issued by Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/443443/ID_24i3.pdf
3. UK Standards for Microbiology Investigations ID 20: Identification of *Shigella* species (2015). Issued by Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/423180/ID_20i3.pdf
4. UK Standards for Microbiology Investigations ID 21: Identification of *Yersinia* species (2015). Issued by Standards Unit, Microbiology Services, PHE. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/443392/ID_21i3.pdf
5. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (1995) Manual of clinical microbiology. 6th ed. American Society for Microbiology, Washington, D.C.
6. American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed. (1992).
7. Tittsler, R. P. and L. A. Sandholzer (1936) The use of semi-solid agar for the detection of bacteria motility. J. Bact. 31:575.

TABLE OF SYMBOLS

IVD *In vitro* Medical Diagnostic Device	Manufacturer	Temperature limitation	Do not reuse
LOT Batch code	Use by	Contains sufficient for <n> tests	Keep away from sunlight
REF Catalogue number	Fragile, handle with care	Consult Instruction For Use	

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
SIM Medium	Tube	100 x 10 ml	26095
SIM Medium	Bottle	6 x 100 ml	403050
SIM Medium	Dehydrated medium	500 g of powder	610181
SIM Medium	Dehydrated medium	100 g of powder	620181

This document is available from the online Support Center:

liofilchem.com/ifu-sds

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CE IVD

SIMMONS CITRATE AGAR

Differential medium for enterobacteria identification.

TYPICAL FORMULA	(g/l)
Magnesium Sulfate	0.2
Ammonium Dihydrogen Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Brom Thymol Blue	0.08
Agar	15.0

Final pH = 6.8 ± 0.2 at 25 °C.

DIRECTIONS

Suspend 24.3 g of powder in 1 liter of distilled or deionized water. Heat to boiling until completely dissolved. Dispense into final tubes and sterilize in the autoclave at 121°C for 15 minutes. Allow the medium to solidify in a slant position.

DESCRIPTION

SIMMONS CITRATE AGAR is recommended for the differentiation and identification of *Enterobacteriaceae* on the basis of citrate utilization.

TECHNIQUE

Inoculate the medium with the specimen by stabbing the butt and streaking the slope. Incubate at 36 ± 1 °C for 24-48 hours. Organisms able to utilize ammonium dihydrogen phosphate and sodium citrate as the sole sources of nitrogen and carbon respectively will grow on this medium and produce an alkaline reaction as evidenced by a change in the color of the bromthymol blue indicator from green (neutral) to blue (alkaline).

QUALITY CONTROL

Dehydrated medium
Appearance: free-flowing, homogeneous.
Color: yellow, may have green tinge.
Prepared medium
Appearance: slightly opalescent, may have a slight precipitate.
Color: forest green.
Incubation conditions: 36 ± 1°C for 24-48 hours.

Microorganism	ATCC	Growth	Characteristics
Escherichia coli	25922	inhibited	
Enterobacter aerogenes	13048	good	blue
Salmonella typhimuriums	14028	good	blue
Salmonella typhi	19430	good	green

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared tubes at 2-8 °C.

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CE IVD

REFERENCES

1. Ewig W.H. and Edwards P.R. (1960). Bull. Bact. Nomen. And Taxon. **10**:1-12.
2. American Public Health Association (1981). Standard Methods for the Examination of Water and Wastewater, 15th ed. APHA Inc, Washington DC.
3. Matsen J.M., and Sherris J.C. (1969) Appl. Microbiol. **18**: 452-454.

PRESENTATION

Product	REF	Σ
SIMMONS CITRATE AGAR (20.5 l)	610046	500 g
SIMMONS CITRATE AGAR (4.1 l)	620046	100 g

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests	IVD *In Vitro* Diagnostic Medical Device
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	Keep away from heat source

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CE IVD

Instructions For Use
ENGLISH

Sabouraud Dextrose Agar

Medium for the cultivation and enumeration of yeasts and moulds
from different materials, according to EN ISO 11133 and USP/EP/JP.

DESCRIPTION

Sabouraud Dextrose Agar (SDA) is a non selective isolation medium used for the growth and maintenance of pathogenic and non-pathogenic fungi from clinical and nonclinical specimens. It is also used for recovery and total counting of yeasts and moulds in environmental monitoring.

This medium complies with EN ISO 11133 for microbiological examination of food, animal feed and water, where it is described as the main reference medium to carry out quantitative testing on culture media intended for fungi.

Its formula conforms to the recommendations of the harmonized method in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) for the microbiological examination of non sterile products. The medium is also available as gamma-irradiated triple bagged plates, particularly suitable for use in restricted areas like isolators and clean rooms.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Dextrose	40.0
Agar	15.0

Final pH 5.6 ± 0.2 at 25°C

METHOD PRINCIPLE

Pancreatic digest of casein and peptic digest of animal tissue provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Dextrose is an energy source. Agar is the solidifying agent. The high concentration of dextrose and the acidic pH of the medium permit selectivity of fungi.

The medium can be supplemented with chloramphenicol to increase bacterial inhibition and recovery of dermatophytes.

PREPARATION

Dehydrated medium	Suspend 65 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

For use in medical microbiology

Streak the specimen as soon as possible after it is received in the laboratory to obtain isolated colonies.
Prepared tubed slants primarily are intended for use with pure cultures for maintenance or other purposes.
Incubation conditions may vary according to the type of specimen and the microorganisms being tested for.

For use in food, animal feed and water testing

Refer to EN ISO 11133 for specific instructions.

For use in industrial microbiology

Control of non-sterile products

Refer to the procedure described in the harmonized chapters of the Pharmacopoeia.

Passive Air Monitoring

Take the lid off the settle plate and leave the medium exposed to the air for a period of time no longer than 4 hours (settling plates filled with 30 ml of medium may compensate for water loss during extended incubation periods). Plates can be placed according to the 1/1/1 scheme (for 1 h, about 1 above the floor, at least 1 m from the walls or any obstacle).

Surfaces and Personnel Hygiene Monitoring

Take a swab sample for irregular surfaces or use the sampling template 10x10 (ref. 96762) to sample a well defined area of the test surface. Inoculate a 90 mm plate by streaking the swab over the agar surface. Furthermore, the medium is suitable for personnel hygiene monitoring to detect microbial contamination of gloves or hands e.g. in a 5-finger-print.

Incubate the plates at 20-25°C for 5-7 days or at 30-35°C for 24-48 hours.

INTERPRETING RESULTS

Transfer of growth from slants to plated media may be required in order to obtain pure cultures of fungi.
Examine for fungal colonies exhibiting typical microscopic and colonial morphology. Biochemical tests may be required for final identification.

The total combined yeasts/moulds count (TYMC) is considered to be equal to the number of CFU found per each plate.
When an acceptable criterion for microbiological quality is prescribed it is interpreted as follows:

- 10^1 CFU: maximum acceptable count = 20;
- 10^2 CFU: maximum acceptable count = 200;
- 10^3 CFU: maximum acceptable count = 2000, and so forth.

In procedures intended for environmental and personnel hygiene monitoring, observe daily for the formation of colonies.

APPEARANCE
Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: slightly opalescent, light amber.

STORAGE
The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE
Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Medium in tubes: 1 year.
Ready-to-use plates (90 and 60 mm): 6 months.
Contact plates (55 mm): 9 months

QUALITY CONTROL
The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU.
Incubation conditions: 32.5 ± 2.5°C for 24-48 h (*C. albicans*) and at 22.5 ± 2.5°C for up to 5 days (all listed organisms), under aerobic atmosphere.

QC Table.

Microorganism		Growth
Candida albicans	WDCM 00054	Good
Aspergillus brasiliensis	WDCM 00053	Good
Saccharomyces cerevisiae	WDCM 00058	Good

WARNING AND PRECAUTIONS
The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE
Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY
1. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. European Pharmacopoeia 6.5 (2009) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
3. United States Pharmacopoeia 32 NF 27 (2009) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Japanese Pharmacopoeia 4.05 (2008) Microbiological examination of non-sterile products: Test for specified microorganisms.
5. Sabouraud, R. (1892) Ann. Dermatol. Syphilol. 3:1061.

PRESENTATION	Category	Packaging	Ref.	
Sabouraud Dextrose Agar	90 mm plates	20 plates	10035	
Sabouraud Dextrose Agar	90 mm plates	100 plates	10035*	
Sabouraud Dextrose Agar	90 mm plates (triple-wrapped and gamma-irradiated)	20 plates	10035S	ƒ
Sabouraud Dextrose Agar	90 mm plates (triple-wrapped and gamma-irradiated, 30 ml filling volume)	20 plates	10114S	ƒ
Sabouraud Dextrose Agar	60 mm plates	20 plates	163402	ƒ
Sabouraud Dextrose Agar	60 mm plates	450 plates	173402	ƒ
Sabouraud Dextrose Agar	55 mm contact plates	20 plates	15327	ƒ
Sabouraud Dextrose Agar	55 mm contact plates irradiated	20 plates	15327S	ƒ
Sabouraud Dextrose Agar	Tubes - Bottles	10 x 9 ml slant tubes	30093	
Sabouraud Dextrose Agar	Tubes - Bottles	20 x 9 ml slant tubes	31093	
Sabouraud Dextrose Agar	Tubes - Bottles	6 x 500 ml bottles	470040	
Sabouraud Dextrose Agar	Tubes - Bottles	6 x 200 ml bottles	412280	
Sabouraud Dextrose Agar	Tubes - Bottles	25 x 200 ml bottles	452280	
Sabouraud Dextrose Agar	Tubes - Bottles	6 x 100 ml bottles	402280	
Sabouraud Dextrose Agar	Dehydrated culture medium	500 g of powder	610103	
Sabouraud Dextrose Agar	Dehydrated culture medium	100 g of powder	620103	
Sabouraud Dextrose Agar	Dehydrated culture medium	5 kg of powder	6101035	

ƒ: Not CE Marked

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

SLANETZ BARTLEY AGAR + TTC

Selective medium for fecal streptococci enumeration and isolation, according to the formulation reported by ISO 7899-2:2000.

TYPICAL FORMULA (g/l)

Tryptose	20.0
Glucose	2.0
Yeast Extract	5.0
Dipoatssium Hydrogen Phosphate	4.0
Sodium Azide	0.4
Triphenyl Tetrazolium Chloride	0.1
Agar	13.0
Final pH 7.2 ± 0.2	

DESCRIPTION

SLANETZ BARTLEY AGAR + TTC is a selective medium for fecal streptococci enumeration and isolation in water and foods by membrane filtration or pour plate technique prepared according to the formulation reported by ISO 7899-2:2000.

PRINCIPLE

Tryptose is a peptone obtained by the enzymatic hydrolysis of a mix containing meat, yeast and casein. It is utilized for the growth and isolation of fastidious microorganisms. Glucose is a source of energy. Yeast extract is a source of aminoacids and vitamins of group B. Dipotassium hydrogen phosphate allows to maintain the osmotic balance of the medium. Sodium azide inhibits the growth of Gram-negative and staphylococci. TTC is a redox indicator and it is colorless in the oxidized form and is reduced in the insoluble red triphenyl formazan. Agar is the solidifying agent.

PREPARATION

Suspend 44.5 g in 1 litre of distilled or deionized water. Heat until completely dissolved. Autoclave at 98°C for 2 minutes. Cool to 45-50°C. Mix well, dispense into Petri dishes and allow to solidify.

TECHNIQUE

Inoculate the plates by streaking the sample onto the agar surface. Incubate at 36+/-1 for 18-24 hours.

INTERPRETATION of RESULTS

Observe the colonies on the agar surface: all the colonies which cultivate with the typical red or reddish-brown color can be considered enterococci.

STORAGE

10-30°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

WARNING and PRECAUTIONS

The product is not classified as hazardous by current legislation and does not contain harmful substances in concentrations of ≥1%. The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL of WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. Burkwal, M.K., and P.A. Hartman. (1964). App. Microbiol. 12,18.
2. Slanetz, L.W., and C.H. Bartley (1957). J. Bact., 74,591.
3. ISO 7899-2: 2000 Recherche et denombrement des streptocoques fecaux. Partie 2: methode par filtration sur membrane.

PRODUCT SPECIFICATIONS

NAME
SLANETZ BARTLEY AGAR + TTC

PRESENTATION
Dehydrated culture medium.

STORAGE
10-30°C

PACKAGING

Code	Content	Packaging
610147	500 g	500 g of powder in plastic bottle
620147	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.2 ± 0.2

USE
SLANETZ BARTLEY AGAR + TTC is a selective medium for fecal streptococci enumeration and isolation in water and foods by membrane filtration or pour plate technique prepared according to the formulation reported by ISO 7899-2:2000.

TECHNIQUE
Refer to technical sheet of the product.

APPEARANCE of the MEDIUM
Dehydrated medium
Appearance: free-flowing, homogeneous.
Colour: light beige
Prepared medium
Appearance: slightly opalescent
Colour: light to medium amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print

2. Microbiological control
   Inoculum for productivity: 10-100 UFC/ml
   Inoculum for selectivity: 10^4-10^5 UFC/ml.
   Inoculum for specificity: ≤ 10^4 UFC/ml
   Incubation conditions:18-24 h at 36 ± 1°C,in aerobiosis

Microorganism		Growth	Characteristics
Enterococcus faecalis	ATCC 19433	Good	Red colonies
Enterococcus faecalis	ATCC 29212	Good	Red colonies
Streptococcus pyogenes	ATCC 19615	Inhibited	
Escherichia coli	ATCC 25922	Inhibited	

TABLE OF SYMBOLS

IVD	*In Vitro* Diagnostic Medical Device		Do not reuse		Manufacturer		Contains sufficient for <n> tests		Temperature limitation
REF	Catalogue number		Fragile, handle with care		Use by		Caution, consult accompanying documents	LOT	Batch code

CE IVD

S.S. AGAR (MODIFIED)

Terreno selettivo per l'isolamento di *Salmonella* spp. e *Shigella* spp.

FORMULA TIPICA	(g/l)
Peptone	5.5
Estratto di Carne	5.0
Lattosio	10.0
SodioTiosolfato	8.5
Estratto di Lievito	5.0
Sodio Citrato	1.0
Sali di Bile N.3	1.5
Ammonio Ferrico Citrato	1.5
Verde Brillante	0.33 mg
Rosso Neutro	0.025
Agar	14.0
pH Finale 7.0 ± 0.2	

DESCRIZIONE

S.S. AGAR (MODIFIED) è un terreno altamente selettivo per l'isolamento di *Salmonella* spp ed alcune specie di *Shigella* da materiale clinico, alimenti ed altri campioni.

PRINCIPIO

I microrganismi Gram-positivi ed i coliformi sono inibiti dai componenti selettivi: verde brillante, sali di bile, tiosolfato e citrato. La differenziazione dei microrganismi è ottenuta attraverso l'introduzione del lattosio nel terreno: gli organismi che fermentano il lattosio producono acidificazione che, in presenza del rosso neutro, determina la formazione di colonie rosse. I lattosio non-fermentanti producono colonie incolori. Il tiosolfato, in combinazione con il ferro, agisce come un indicatore per la produzione di solfuro che è indicata da un annerimento del centro delle colonie.

PREPARAZIONE

Sospendere 52.0 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire fino a completa dissoluzione. NON AUTOCLAVARE. Raffreddare il terreno a 45-50°C. In condizioni di asepsi dispensare in piastre Petri e lasciar solidificare il terreno mantenendo i coperchi parzialmente rimossi.

TECNICA

Inoculare strisciando il campione da analizzare sulla superficie del terreno al fine di isolare colonie pure da campioni contenenti una flora mista. Incubare a 36+/-1°C per 18-24 ore.

INTERPRETAZIONE DEI RISULTATI

Salmonella spp ed altri microorganismi non fermentanti il lattosio possono produrre colonie opache, tralucenti o trasparenti, con o senza il centro nero. Le colonie di Shigella sono incolori. I pochi organismi che fermentano il lattosio, che riescono a crescere sul terreno, si differenziano per le colonie rossastre di aspetto mucoide.

CONSERVAZIONE

Il prodotto può essere conservato a 10-30°C al riparo dalla luce, fino alla data di scadenza indicata in etichetta. Eliminare se vi sono segni evidenti di deterioramento o contaminazione.

AVVERTENZE E PRECAUZIONII

Il prodotto non contiene sostanze nocive in concentrazioni superiori ai limiti fissati dalla normativa vigente, perciò non è classificato come pericoloso; per il suo impiego si consiglia comunque di consultare la scheda di sicurezza. Il prodotto è destinato esclusivamente per Uso Diagnostico *in vitro* e deve essere utilizzato da parte di personale qualificato.

SMALTIMENTO DEI RIFIUTI

Lo smaltimento del prodotto deve essere effettuato secondo le vigenti regolamentazioni nazionali e locali.

RIFERIMENTI BIBLIOGRAFICI

1. Gray L.D. (1995). Escherichia, Salmonella, Shigella and Yersinia, p. 450-456. In Manual of clinical microbiology, 6th ed. American society of microbiology.
2. Leifson E. (1935). J. Pathol. Bacteriol. 40: 581.
3. Rose, H.M., and M.H. Kolodny (1942). J. Lab. Clin. Med. 27: 1081-1083.

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CE IVD

SPECIFICHE DI PRODOTTO

DENOMINAZIONE
S.S. AGAR (MODIFIED)

PRESENTAZIONE
Terreno disidratato

CONSERVAZIONE
10-30°C

CONFEZIONAMENTO

Ref.	Contenuto	Modalità di confezionamento
610042	500 g	500 g di polvere in flacone in plastica
620042	100 g	100 g di polvere in flacone in plastica
6100425	5 kg	5 kg di polvere in contenitore in plastica

pH DEL TERRENO
7.0 ± 0.2

IMPIEGO
S.S. AGAR è un terreno altamente selettivo per l'isolamento di *Salmonella* spp ed alcune specie di *Shigella* da materiale clinico, alimenti ed altri campioni

TECNICA
Fare riferimento alla scheda tecnica del prodotto

ASPETTO DEL TERRENO
Terreno disidratato
Aspetto: omogeneo
Colore: rosa chiaro
Terreno preparato
Aspetto: opaco
Colore: viola

VALIDITÀ DALLA DATA DI PRODUZIONE
4 anni

CONTROLLO DI QUALITÀ
1. Controllo caratteristiche generali, etichettatura e stampa
2. Controllo microbiologico
   Dimensione dell'inoculo per produttività: 10-100 UFC/ml
   Dimensione dell'inoculo per selettività : 10^4-10^5 UFC/ml
   Dimensione dell'inoculo per specificità: ≤10^4 UFC/ml
   Condizioni di incubazione: 18-24 h a 35 ± 2°C in aerobiosi

Microrganismo		Crescita	Caratteristiche
Shigella flexneri	ATCC® 12022	Buona	Colonie incolori
Salmonella typhimurium	ATCC® 14028	Buona	Colonie incolori con o senza centro nero
Enterococcus faecalis	ATCC® 29212	Inibita	---
Staphylococcus aureus	ATCC® 25923	Inibita	---
Escherichia coli	ATCC® 25922	Parzialmente inibita	Colonie rosa o rosse

TABELLA DEI SIMBOLI

LOT Numero di lotto	IVD Per uso diagnostico *in vitro*	Fabbricante	Data di scadenza
REF Numero di catalogo	Limiti di temperatura	Contenuto sufficiente per <n> test	Attenzione, consultare le istruzioni per l'uso

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CE IVD

ENGLISH

Lactophenol Cotton Blue Droppers

Blue stain for microscopic examination of fungi,
in dropper bottles.

DESCRIPTION

Lactophenol Cotton Blue Droppers is a mounting medium and a staining agent used in the preparation of slides for microscopic examination of fungi from clinical specimens, food and environmental samples.

KIT CONTENTS

- 10 bottles of Lactophenol Cotton Blue Droppers.
- 1 instruction sheet.

METHOD PRINCIPLE

Upon the addition of Lactophenol Cotton Blue, fungi stain blue allowing for easier visualization and examination under microscope.

COMPOSITION

Aqueous solution of the following ingredients (per 100 ml):
Glycerol 50 mg, Phenol 25 mg, Lactic acid 25 mg, Methyl blue (Cotton blue) 50 mg.

TEST PROCEDURE

1. Dispense a drop of Lactophenol Cotton Blue stain on a slide.
2. Using an inoculating needle, carefully spread the fungal culture into a thin preparation.
3. Place a coverslip edge on the drop and slowly lower it. Avoid trapping air bubbles under the coverslip. Wait for about 5 minutes.
4. Observe under microscope.

INTERPRETATION OF RESULTS

Yeast cells, mycelia and fruiting structures are stained a delicate blue colour, while the background appear a faint, pale blue.

QUALITY CONTROL FOR THE USER

Prepare a wet mount with the stain according to laboratory methodology and carry out microscopic analysis.

Control strains		**Appearance**
Aspergillus niger	ATCC® 16404	Delicate blue hyphae and fruiting structures with a pale blue background
Trichophyton mentagrophytes	ATCC® 9533	Delicate blue hyphae and fruiting structures with a pale blue background

PRECAUTIONS

Lactophenol Cotton Blue Droppers is classifiable as hazardous under current legislation; it is recommended that the Safety Data Sheet be consulted on its use. The product is intended for *in vitro* diagnostic use and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE AND TRANSPORT CONDITIONS

2-8°C away from light, until the expiry date on the label. However, our stability studies have shown that the storage or transport at 18-25°C for 4 days, or at 35-39°C for 48 hours, do not alter in any way the performance of the product. Eliminate if signs of deterioration or contamination are evident.

ELIMINATING USED MATERIAL

After use, used Lactophenol Cotton Blue Droppers and the material that has come into contact with the sample must be decontaminated and disposed of in accordance with the laboratory procedures for the decontamination and disposal of potentially infected material.

BIBLIOGRAPHY

1. Larone D.H. (1995) Medically important fungi: a guide to identification. Washington DC: ASM Press.
2. Lennette, E.H., A. Balows, W.J. Hausler, Jr., and H.J. Shadomy (ed.). (1985) Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
3. Finegold, S.M., and W.J. Martin. (1982) Bailey and Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Co., St. Louis.

PRESENTATION

Product	**Ref.**	**Content**
Lactophenol Cotton Blue Droppers	87008	10 x 10 ml bottles

TABLE OF SYMBOLS

LOT	Batch code	IVD	*In vitro* Diagnostic Medical Device		Manufacturer		Use by		Fragile, handle with care
REF	Catalogue number		Temperature limitation	Σ	Contains Sufficient for <n> tests		Caution, consult accompanying documents		Do not reuse

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CE IVD

Rev.0 / 01.10.2015

Enterosystem 24R

System for the identification of Gram-negative, oxidase negative enterobacteria.

Ref. 71619 - 79619

Tabella interpretativa.

Pozzetto	**Test**	**Colore pozzetto**	
		Reazione positiva	**Reazione negativa**
1-ONPG	Idrolisi ONPG	giallo	incolore
2-LDC	Decarbossilazione lisina	rosso	giallo-arancio
3-ODC	Decarbossilazione ornitina	rosso	giallo-arancio
4-ADC	Decarbossilazione arginina	rosso	giallo-arancio
5-PD	Deaminazione fenilalanina	nero-marrone	giallo
6-CIT	Utilizzazione citrato	blu-verde scuro	verde chiaro
7-UR	Idrolisi urea	rosso-fucsia	giallo-arancio
8-H_2S	Produzione idrogeno solforato	nero	giallo
9-MLN	Utilizzazione malonato	blu-verde	giallo
10-VP	Voges-Proskauer (aggiunta reagenti)	rosa-rosso	giallo
11-IND	Indolo (aggiunta reagente di Kovac)	anello rosso	giallo
12-GLU	Glucosio	giallo	blu-verde
13-MAN	Mannitolo	giallo	blu-verde
14-INO	Inositolo	giallo	blu-verde
15-SOR	Sorbitolo	giallo	blu-verde
16-SAC	Saccarosio	giallo	blu-verde
17-ARA	Arabinosio	giallo	blu-verde
18-RAF	Raffinosio	giallo	blu-verde
19-RAM	Ramnosio	giallo	blu-verde
20-MEL	Melibiosio	giallo	blu-verde
21-LAC	Llattosio	giallo	blu-verde
22-TRE	Trealosio	giallo	blu-verde
23-XYL	Xilosio	giallo	blu-verde
24-DUL	Dulcitolo	giallo	blu-verde

FORMAZIONE DEL CODICE NUMERICO

I test biochimici sono distinti in 8 gruppi da 3 ed ognuno può assumere un valore di 1, 2, o 4:

- Valore 1 : primo test positivo in ogni gruppo (**ONPG**, **ADC**, **UR**, **VP**, **MAN**, **SAC, RAM, TRE**);
- Valore 2 : secondo test positivo in ogni gruppo (**LDC**, **PD**, **H_2S**, **IND**, **INO**, **ARA, MEL, XYL**);
- Valore 4 : terzo test positivo in ogni gruppo (**ODC**, **CIT**, **MLN**, **GLU**, **SOR**, **RAF, LAC, DUL**);
- Valore 0 : reazioni negative in ogni gruppo.

Si compone il codice ad 8 cifre sommando i valori ottenuti per ciascun gruppo.

L'esempio sottostante mostra come si ottiene un profilo numerico.

	Gruppo 1			Gruppo 2			Gruppo 3			Gruppo 4			Gruppo 5			Gruppo 6			Gruppo 7			Gruppo 8		
Test	**ONPG**	**LDC**	**ODC**	**ADC**	**PD**	**CIT**	**UR**	**H_2S**	**MLN**	**VP**	**IND**	**GLU**	**MAN**	**INO**	**SOR**	**SAC**	**ARA**	**RAF**	**RAM**	**MEL**	**LAC**	**TRE**	**XYL**	**DUL**
Valore	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
Risultato	+	–	+	+	–	+	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–
Somma dei valori	5			5			4			5			7			7			7			1		
CODICE NUMERICO: 55457771 IDENTIFICAZIONE: *Enterobacter cloacae*																								

CONTROLLO QUALITÀ

Ogni lotto di Enterosystem 24R viene sottoposto al controllo qualità utilizzando i seguenti ceppi di riferimento:

Escherichia coli ATCC® 25922, *Salmonella* Typhimurium ATCC® 14028, *Proteus mirabilis* ATCC® 25933, *Klebsiella pneumoniae* ATCC® 13883, *Enterobacter cloacae* ATCC® 13047.

PERFORMANCE

I risultati ottenuti con il sistema Enterosystem 24R concordano con quelli ottenuti utilizzando altri test microbiologici e biochimici per identificazione microbica.

FATTORI CHE POSSONO INVALIDARE I RISULTATI

Coltura contaminata; imprecisa standardizzazione dell'inoculo; materiale da esaminare inadatto; uso di sistemi e reagenti supplementari scaduti; temperatura e tempi di incubazione non rispettati.

PRECAUZIONI

Il prodotto Enterosystem 24R non contiene sostanze nocive in concentrazioni superiori ai limiti fissati dalla normativa vigente, perciò non è classificato come pericoloso; per il suo corretto impiego si consiglia comunque di consultare la Scheda di Sicurezza. Enterosystem 24R è un dispositivo monouso da usare solo per uso diagnostico *in vitro*, è destinato ad un ambito professionale e deve essere usato in laboratorio da operatori adeguatamente addestrati, con metodi approvati di asepsi e di sicurezza nei confronti degli agenti patogeni.

CONSERVAZIONE

Conservare Enterosystem 24R a 2-8°C nella sua confezione originale. Non conservare vicino a fonti di calore ed evitare eccessive variazioni di temperatura. In queste condizioni il prodotto è valido fino alla data di scadenza indicata in etichetta. Non utilizzare oltre questa data. Eliminare se vi sono segni di deterioramento.

ELIMINAZIONE DEL MATERIALE USATO

Dopo l'utilizzazione Enterosystem 24R ed altri dispositivi venuti a contatto con il materiale clinico devono essere decontaminati e smaltiti in accordo con le tecniche in uso in laboratorio per la decontaminazione e lo smaltimento di materiale potenzialmente infetto.

PRESENTAZIONE

Prodotto	Ref.	Confezione
Enterosystem 24R	71619	20 test
Enterosystem 24R	79619	4 test

TABELLA DEI SIMBOLI

IVD Dispositivo medico diagnostico *in vitro*	Non riutilizzare	Fabbricante	Contenuto sufficiente per <n> saggi	Limiti di temperatura
REF Numero di catalogo	Fragile, maneggiare con cura	Utilizzare entro	Attenzione, vedere le istruzioni per l'uso	**LOT** Codice del lotto

CE **IVD**

ENGLISH

Enterosystem 24R

System for the identification of Gram-negative, oxidase negative enterobacteria.

DESCRIPTION

Enterosystem 24R is a 24-well system containing desiccated biochemical substrates for the identification of Gram-negative bacteria that belong to the family of Enterobacteriaceae. The system is inoculated with the suspension of the organism to be examined and incubated in thermostat. The wells are examined for color changes and the resulting pattern of positive and negative reactions determines the numerical profile used for identification. The complete list of those organisms that is possible to identify with this system is provided in the Identification Table at the end of this document.

CONTENT OF THE PACKAGE

Ref. 71619	**Ref. 79619**
• 20 Enterosystem 24R • 20 Vials of Physiological Solution (7.0 mL) • 1 Cartridge of Xylose Disc (20 discs) • 1 Cartridge of Arabinose Disc (20 discs) • Instructions sheet and Data chart	• 4 Enterosystem 24R • 4 Vials of Physiological Solution (7.0 mL) • 1 Cartridge of Xylose Disc (20 discs) • 1 Cartridge of Arabinose Disc (20 discs) • Instructions sheet and Data chart

ITEMS NECESSARY BUT NOT INCLUDED IN THE PACKAGE

- Enterosystem 18R Reagent (ref. 80252): Vaseline Oil, Indole Test Reagent, VP Test Reagents
- Gram Color Kit (ref. 80293)
- Oxidase Test Stick (ref. 88029)
- McFarland 0.5 Barium Sulphate Standard (ref. 80400)
- Identification Software online (free-access)

PRINCIPLE OF THE METHOD

Enterosystem 24R allows the identification of Gram-negative, oxidase negative enterobacteria of clinical significance. 24 different tests are carried out, each in every single well of the system. These wells are inoculated with a bacterial suspension that reconstitutes the dehydrated media contained in. The reactions occurring in the wells during incubation produce color changes which are read according to the Interpretive Table. The organism numerical profile is determined and the identification is obtained by using the Identification Software on Liofilchem website.

CONFIGURATION

Well	**Test**	Well	**Test**
1-ONPG	Hydrolysis of ONPG (Ortho-nitrophenyl-β-galactoside)	**13-MAN**	Utilization of mannitol
2-LDC ⌴	Decarboxylation of lysine	**14-INO**	Utilization of inositol
3-ODC ⌴	Decarboxylation of ornithine	**15-SOR**	Utilization of sorbitol
4-ADC ⌴	Decarboxylation of arginine	**16-SAC**	Utilization of saccharose
5-PD	Deamination of phenylalanine	**17-ARA**	Utilization of arabinose
6-CIT	Utilization of citrate	**18-RAF**	Utilization of raffinose
7-UR ⌴	Hydrolysis of urea	**19-RAM**	Utilization of rhamnose
8-H_2S ⌴	Production of hydrogen sulphide	**20-MEL**	Utilization of melibiose
9-MLN	Utilization of malonate	**21-LAC**	Utilization of lactose
10-VP *	Production of acetoin (Voges-Proskauer test)	**22-TRE**	Utilization of trehalose
11-IND *	Production of indole (Kovac's test)	**23-XYL**	Utilization of xylose
12-GLU	Utilization of glucose	**24-DUL**	Utilization of dulcitol

⌴ : overlay the well with vaseline oil

* : after incubation, add the indicated reagent to perform the test

COLLECTION OF THE SAMPLE

Enterosystem 24R is not for use directly with clinical or other specimens. The microorganism to be identified must first be isolated on a culture medium suitable for growth of enterobacteria such as MacConkey Agar (ref. 10029), Eosin Methylene Blue Agar (ref. 10048), Salmonella and Shigella Agar (ref. 10036), Hektoen Enteric Agar (ref. 10043) as well as a non selective blood agar (e.g. Tryptic Soy Agar with 5% Sheep Blood, ref. 11037).

TEST PROCEDURE

PREPARATION OF BACTERIAL SUSPENSION

- The microorganism to be identified must be recently isolated (18-24 h); bacterial cultures older than 48 hours can provide not reliable results.
- Before inoculating the microorganism to be examined, Gram staining and oxidase testing are required. Use Enterosystem 24R with Gram-negative, oxidase negative bacteria only.
- Take one or more morphologically similar well isolated colonies from the agar culture medium and suspend in physiological solution. The final turbidity should be equal to 0.5 McFarland. This suspension must be used immediately after preparation.

  Note: A drop from the inculum tube, either before or after inoculating the system, can be spread onto an agar slant or plate (any appropriate media) for purity check.

INOCULATION OF THE SYSTEM

1. Take a system from its wrapper and bring it to room temperature.
2. Write down the name of the patient and the date of the start of the examination.
3. Transfer a disc of Arabinose Disc into the well **17-ARA** and a disc of Xylose Disc into the well **23-XYL**.
4. Dispense 0.2 mL of bacterial suspension into each well of the system and overlay with 1 drop of vaseline oil the wells **2-LDC**, **3-ODC**, **4-ADC**, **7-UR** and **8-H_2S**.
5. Cover the system with the lid provided and incubate at 36±1°C for 18-24 hours.

INTERPRETATION OF THE RESULTS

At the end of the incubation period:

1. Add 2 drops of Alpha-napthol and 1 drop of NaOH 40% to the well **10-VP** (wait 15-20 min for reading after adding the reagents).
2. Add 2 drops of Kovac's reagent to the well **11-IND** (wait 1-2 min for reading after adding the reagent).
3. Watch for the color change in the wells and interpret the results by referring to the Interpretive Table.
4. Note the results on the test results form and determine the 8-digit code following instructions provided as outlined under NUMERICAL CODE FORMATION.
5. Identify the organism by using the Identification Software.

ENGLISH

POTASSIUM TELLURITE 3.5% *Supplement*

Selective supplement for the isolation of Staphylococci

DESCRIPTION

POTASSIUM TELLURITE 3.5% *Supplement* is a supplement consisting of a 3.5% potassium tellurite aqueous solution for microbiological use, used in culture medium Giolitti Cantoni Broth Base code 610100 or 620100, for isolation of staphylococci and in other media the composition of which provides for the inclusion of potassium tellurite.

KIT CONTENTS

Each kit contains:

- Bottles containing POTASSIUM TELLURITE 3.5% *Supplement*
- 1 Instruction sheet

PRINCIPLE OF THE METHOD

POTASSIUM TELLURITE 3.5% *Supplement* is a selective supplement used chiefly in the isolation of staphylococci. These micro-organisms, which reduce the tellurite to tellurium, grow with grey-black colonies. Potassium tellurite is also included in the composition of other culture media.

COMPOSITION

POTASSIUM TELLURITE 3.5% *Supplement*	
Contents / ml	
Potassium tellurite	35.0 mg
Distilled water	1.0 ml

PROCEDURE FOR USE

1. Aseptically add 2.9 ml of POTASSIUM TELLURITE 3.5% *Supplement* to 500 ml of Vogel Johnson Agar medium code 610186 or 620186, or 0.3 ml (0.03 ml for analysis of meat-based products) to 19 ml of Giolitti Cantoni Broth Base code 610100 or 620100, autoclaved and cooled to 45-50 °C. When potassium tellurite is included in the composition of other media, refer to the specific instructions for the medium concerned on the quantity of POTASSIUM TELLURITE 3.5% *Supplement* that should be added to it.
2. Mix with care.
3. Distribute into the final containers.

TECHNIQUE AND INTERPRETATION OF THE RESULTS

Refer to the technical documentation for Vogel Johnson Agar code 610186 or 620186 or Giolitti Cantoni Broth Base code 610100 or 620100, or for the specific medium being prepared.

QUALITY CONTROL

1. Visual inspection: clear, colourless solution.
2. Microbiological control.

Prepare the plates using as base Vogel Johnson Agar medium code 610186 or 620186 supplemented with POTASSIUM TELLURITE 3.5% *Supplement* (2.9 ml in 500 ml of medium). The plates are seeded with the strains indicated in the microbiological control table.

Incubation conditions: 24-48 h at 36+/-1 °C.

Microbiological control

Control strains		**Growth**	**Colonies**
Staphylococcus aureus	ATCC 25923	Good	Black
Escherichia coli	ATCC 25922	Inhibited	-------

PRECAUTIONS

The product POTASSIUM TELLURITE 3.5% *Supplement* is not classified as dangerous under current legislation; it is nevertheless recommended that the Safety Data Sheet be consulted on its correct use.
POTASSIUM TELLURITE 3.5% *Supplement* is a supplement to be used only for *in vitro* diagnostic use. It is intended for use in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store POTASSIUM TELLURITE 3.5% *Supplement* at 2-8 °C in its original packaging. In such conditions POTASSIUM TELLURITE 3.5% *Supplement* will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

REFERENCES

- United States Pharmacopoeia XXI (1985) Microbial Limit Tests. Rockville. Md.
- Vogel, R.A., and Johnson, M.J. (1961). Pub. Hlth. Lab. **18**: 131.
- Giolitti C. and Cantoni C. (1966) J. Appl. Bact. **29**: 395.

PRESENTATION

product	REF	Σ
POTASSIUM TELLURITE 3.5% *Supplement*	80291	5 bottles X 10 ml
POTASSIUM TELLURITE 3.5% *Supplement*	80491	10 bottles X 10 ml
POTASSIUM TELLURITE 3.5% *Supplement*	80492	10 bottles X 1 ml

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	LOT Batch code

Liofilchem®
Made in Italy
www.liofilchem.net

CE IVD

Rev.1/ 27.07.2011

Instructions For Use
ENGLISH

Tryptic Soy Broth
Casein Soya Bean Digest Broth

Liquid medium for the isolation and cultivation of
a wide variety of organisms, according to USP/EP/JP.

DESCRIPTION

Tryptic Soy Broth (TSB) is a nutritious medium used for the detection, isolation and cultivation of fastidious and nonfastidious microorganisms including bacteria and fungi from clinical specimens, environmental sources and other materials.

This medium meets the requirements of the harmonized method in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) for sterility testing and for microbiological examination of non-sterile products. It is recommended by the Clinical and Laboratory Standards Institute (CLSI) for inoculum preparation in antibiotic susceptibility testing.

TSB is also available as triple-wrapped and gamma-irradiated bottles, particularly suitable for use in restricted areas and for aseptic process simulations (media fill trial) in the pharmaceutical industry.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Casein	17.0
Papaic Digest of Soya Bean	3.0
Sodium Chloride	5.0
Dipotassium Hydrogen Phosphate	2.5
Glucose Monohydrate	2.5
Final pH 7.3 ± 0.2 at 25°C	

METHOD PRINCIPLE

Pancreatic digest of casein and papaic digest of soya bean provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Sodium chloride maintains osmotic balance in the medium. Dipotassium phosphate is a buffering agent. Glucose is an energy source.

PREPARATION

Dehydrated medium — Suspend 30 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

TEST PROCEDURE

For use in clinical microbiology, inoculate TSB directly with the clinical specimen or with a small amount of growth from an overnight culture on a solid medium. Usually, incubation at 35 ± 2°C for 18-24 hours is adequate.

For use in industrial microbiology, inoculate the sample or material to be tested into the medium. Incubate under appropriate atmosphere at 30-35°C for 18-72 hours (for the bacteria) and at 20-25°C for a maximum of 5 days (for the fungi).

For sterility testing, the way of inoculation depends on the type and size of test material. If membrane filtration is carried out, a suitable diluent such as Fluid A (ref. 400010) may be used. For direct inoculation, it is recommended a minimum 1:10 dilution of the sample to the culture medium. Incubate at 20-25°C for 14 days.

For media fill test in biopharmaceutical manufacturing, two incubation temperatures are used. The initial incubation at 20-25°C for 7 days, and then at 30-35°C for further 7 days.

For all applications notice that:
- it is important to provide sufficient aeration during incubation by slightly loosening the caps;
- Fluid Thioglycollate Medium (ref. 24124) should be used for the cultivation of strict anaerobes.

INTERPRETING RESULTS

The presence of turbidity compared to an uninoculated control or a pellicle formation indicate microbial growth. Subculture to suitable solid media for complete identification of the isolated colonies.

If the material being tested renders the medium turbid and a visual examination is not possible at the end of the incubation period, subculture to fresh TSB or onto appropriate solid media to ensure that turbidity is caused by the sample only and it is not a result of microorganisms multiplying in the broth.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige.
Prepared medium: clear to very slightly opalescent, light amber to amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and tubes at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles/tubes: 2 years.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Incubation conditions: bacteria at 32.5 ± 2.5°C for 18-24 h and up to 72 h (*Clostridium*)
yeasts and molds at 22.5 ± 2.5°C for up to 5 days.

QC Table.

Microorganism		Growth
Staphylococcus aureus	ATCC® 6538	Good
*Staphylococcus aureus**	ATCC® 25923	Good
Escherichia coli	ATCC® 8739	Good
*Escherichia coli**	ATCC® 25922	Good
Pseudomonas aeruginosa	ATCC® 9027	Good
Bacillus subtilis	ATCC® 6633	Good
Salmonella Typhimurium	ATCC® 14028	Good
Clostridium sporogenes	ATCC® 11437	Good
Candida albicans	ATCC® 10231	Good
Aspergillus brasiliensis	ATCC® 16404	Good

*CLSI recommended organisms

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard - Tenth Edition. CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
2. United States Pharmacopeial Convention (2014) The United States Pharmacopeia 38/National Formulation 33, Supp. 2. Chapter <61> Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter <62> Microbiological examination of non-sterile products: Test for specified products. Chapter <71> Sterility Tests. Rockville, Md., USA.
3. European Directorate for the Quality of Medicines and Healthcare (2014) The European Pharmacopoeia. 8th Ed. Chapter 2.6.12 Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.
4. PDA Technical Report No. 13 (2014 Revised) Fundamentals of an Environmental Monitoring Program.
5. Japanese Ministry of Health, Labour and Welfare (2011) The Japanese Pharmacopoeia. 16th Ed. Chapter 4.05 Microbial Limit Test I. Microbiological examination of non-sterile products: Total viable aerobic count and II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

6. Pharmaceutical Inspection Convention Co-operation Scheme (PIC/S). Recommendation on the Validation of Aseptic Processes (2011) Revision 6.
7. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard - Tenth Edition. CLSI document M02-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
8. FDA (2004) Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice.

PRESENTATION		**Contents**	**Ref.**
Tryptic Soy Broth	Tubes	50 x 5 ml tubes	27500
Tryptic Soy Broth	Tubes	20 x 9 ml tubes	24469
Tryptic Soy Broth	Tubes	20 x 10 ml tubes	24513
Tryptic Soy Broth	Tubes	100 x 10 ml tubes	26513
Tryptic Soy Broth	Tubes	10 x 15 ml tubes	20129
Tryptic Soy Broth	Bottles	6 x 100 ml bottles (flip-off cap)	400030
Tryptic Soy Broth	Bottles	6 x 100 ml bottles (screw cap)	452080
Tryptic Soy Broth	Bottles (triple wrapped and gamma-irradiated)	6 x 100 ml bottles (screw cap)	452080S
Tryptic Soy Broth	Bottles	6 x 100 ml bottles (crimp cap)	495010
Tryptic Soy Broth	Bottles	25 x 100 ml bottles (flip-off cap)	453030
Tryptic Soy Broth	Bottles	25 x 100 ml bottles (screw cap)	455208
Tryptic Soy Broth	Bottles	6 x 200 ml bottles (screw cap)	442080
Tryptic Soy Broth	Bottles	6 x 225 ml bottles (screw cap)	432080
Tryptic Soy Broth	Bottles	6 x 500 ml bottles (screw cap)	470370
Tryptic Soy Broth	Dehydrated medium	500 g of powder	610053
Tryptic Soy Broth	Dehydrated medium	100 g of powder	620053
Tryptic Soy Broth	Dehydrated medium	5 kg of powder	6100535

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

Instructions For Use
ENGLISH

TCBS Agar

Selective medium for detection of enteropathogenic *Vibrio* spp
from clinical and nonclinical samples, according to ISO 21872.

DESCRIPTION

Thiosulfate Citrate Bile Sucrose (TCBS) Agar is a medium used for the selective isolation and cultivation of vibrios.

This medium conforms to ISO 21872 for the identification of *Vibrio* spp, including *Vibrio cholerae* from food, animal feeding stuffs and environmental samples in the area of food production and food handling.

TCBS Agar is also recommended for isolating *V. cholerae* and *V. parahaemolyticus* as well as other vibrios from stool specimens.

TYPICAL FORMULA	(g/l)
Peptone	10.0
Yeast Extract	5.0
Sodium Citrate	10.0
Sodium Thiosulfate	10.0
Iron(III) Citrate	1.0
Sodium Chloride	10.0
Dried Bovine Bile	8.0
Sucrose	20.0
Bromothymol Blue	0.04
Thymol Blue	0.04
Agar	15.0
Final pH 8.6 ± 0.2 at 25°C	

METHOD PRINCIPLE

Peptone and yeast extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Sodium citrate serves to maintain an alkaline pH and along with sodium thiosulfate and oxbile are selective agents, inhibiting Gram-positive organisms and suppressing coliforms. Moreover, the alkaline pH of the medium enhances the recovery of *V. cholerae*. Sodium thiosulfate serves also as a sulfur source and, in combination with ferric citrate, detects hydrogen sulfide production. Sodium chloride maintains the osmotic balance of the medium and stimulates vibrios growth. Sucrose is the fermentable carbohydrate. Bromothymol blue and thymol blue are pH indicators. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 89.1 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into final containers.

TEST PROCEDURE

ISO 21872 recommends to follow two successive enrichment steps in Alkaline Saline Peptone Water (ASPW) before inoculating TCBS Agar.

TCBS Agar can be also inoculated directly with specimens such as rectal swabs, feces, vomitus or with food samples (*).

Incubate (protected from light) at 37 ± 1°C for 18-24 hours in an aerobic atmosphere.

(*) NB. Heavy inoculation is recommended. Swabs containing specimen material should be transported to the laboratory in Cary Blair Transport Medium (ref. 470290) if a delay in reaching the laboratory is anticipated. Specimens for cultivation of vibrios should not be frozen.

INTERPRETING RESULTS

Strains of *Vibrio cholerae* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *Vibrio alginolyticus* also produce yellow colonies. It's possible that a few sucrose-positive *Proteus* strains can grow to form yellow, vibrid-like colonies. *Vibrio parahaemolyticus* is a sucrose non-fermenting organism and produces blue-green colonies, as does *Vibrio vulnificus*. Occasional isolates of *Pseudomonas* and *Aeromonas* species also produce blue-green colonies, but overall TCBS Agar is highly selective and any H_2S-negative colony is possibly *Vibrio* species.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige to green beige.
Prepared medium: clear to slightly opalescent, green.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Medium in tubes: 1 year.
Ready-to-use plates: 6 months.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Inoculum for selectivity: >10^3 CFU.
Incubation conditions: aerobically at 37 ± 1°C for 18-24 hours.

QC Table.

Microorganism		Growth	Colony Color
Vibrio parahaemolyticus	WDCM 00185	Good	Green
Vibrio furnissii	WDCM 00186	Good	Yellow
Escherichia coli	WDCM 00012	Inhibited	---

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 21872-1:2007. Microbiology of food and animal feeing stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*.
3. ISO 21872-2:2007. Microbiology of food and animal feeing stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 2: Detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*.
4. American Public Health Association (1992): compendium of methods for the microbiological examination of foods, 3rd edition.
5. Dewitt, W.E., E.J. Gangarosa, I. Huq, and A. Zarifi (1971) Holding media for the transport of Vibrio cholerae from field to laboratory. Am. J. Trop. Med. Hyg. 20:685-688.
6. Kobayashi, T., S. Enomoto, R. Sakazaki, and S. Kuwahara (1963) A new selective medium for pathogenic vibrios: T.C.B.S. Agar (Modified Nakanishiís Agar). Jap. J. Bacteriol. 18:387-391.

PRESENTATION		Contents	Ref.
TCBS Agar	90 mm ready-to-use plates	20 plates	11195
TCBS Agar	90 mm ready-to-use plates	100 plates	11195*
TCBS Agar	Slant tubes	10 x 9 ml tubes	30022
TCBS Agar	Slant tubes	20 x 9 ml tubes	31022
TCBS Agar	Bottles	6 x 100 ml bottles	403140
TCBS Agar	Dehydrated medium	500 g of powder	611010
TCBS Agar	Dehydrated medium	100 g of powder	621010

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

X FACTOR TEST
V FACTOR TEST
V+X FACTOR TEST

ENGLISH

DESCRIPTION
X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST are constituted by paper discs with special features, containing the respective coagulation factors, used for differentiating *Haemophilus* spp.

CONTENT OF THE PACKAGES
Each package contains:
- 2 cartridges with 50 discs each, packaged in a heat-sealed container
- 1 dryer
- 1 instruction sheet

PRINCIPLE OF THE METHOD
Different strains of *Haemophilus* grow on a culture medium only in presence of the coagulation factor or factors (X, V, or both) which they need. These different requirements allow the differentiation and the identification of *Haemophilus* spp.

COMPOSITION
– Each disc of X FACTOR TEST contains Hemin.
– Each disc of V FACTOR TEST contains NAD (Nicotinamide-Adenine-Dinucleotide).
– Each disc of V+X FACTOR TEST contains Hemin and NAD (Nicotinamide-Adenine-Dinucleotide).

TEST PROCEDURE
1. Take the cartriges container from the refrigerator and leave it on the test bench until it reaches room temperature (about 30 minutes). This will prevent humidity being deposited on the discs when the package is opened, which could prejudice their long-term stability.
2. Using a sterile swab, evenly inoculate the surface of a plate of Tryptic Soy Agar (ref. 10037) or Mueller Hinton Agar (ref. 10031) with a pure suspension of the microorganism to test.
3. Using sterile tools, press one disc of X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST on the inoculated surface, at a distance of 120° from one to another and at 1-2 cm from the edge of the plate. Incubate at 36±1°C for 24-48 in 5-10% carbon dioxide atmosphere.

INTERPRETATION OF THE RESULTS
If the organism requires X Factor alone, it will grow only at the edge of the X and the X+V FACTOR TEST discs; if it requires V Factor alone, it will grow only at the edge of the V and the X+V FACTOR TEST discs; if both X and V Factors are required, it will grow only at the vicinity of the X+V FACTOR TEST discs.
Some examples are indicated in the following table:

	Without Factors	Factor X	Factor V	Factors V+X
Haemophilus influenzae	-	-	-	+
Haemophilus aegyptius	-	-	-	+
Haemophilus parainfluenzae	-	-	+	+
Haemophilus ducreyi	-	+	-	+
Bordetella pertussis	+	+	+	+

QUALITY CONTROL
Each batch of X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST is subjected to microbial control, inoculating pure suspensions of *Haemophilus influenzae* ATCC 19418 and *Haemophilus parainfluenzae* ATCC 7901 on plates of Tryptic Soy Agar.

PRECAUTIONS
X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST cannot be classified as being hazardous according to the current legislation.
X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST is a disposable device to be used only for diagnostic use *in vitro.* It must be used in the laboratory by properly trained personnel, using approved aseptic and safety methods for handling pathogenic agents.

STORAGE
Store X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST at 2-8°C in the original packaging. Keep away from sources of heat and avoid excessive changes in temperature. In such conditions, X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

DISPOSAL OF USED MATERIAL
After use, X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST and material that has come into contact with the sample must be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.

BIBLIOGRAPHY

- Kilian M. (1980) *Haemophilus*. in Manual of Clinical Microbiology. Eds. Lennette et al. Amer. Soc. for Microbiol. 3rd edn. Washington.

PRESENTATION

Product	REF	µg	Σ
X FACTOR TEST	**9503**	**5**	**100**
V FACTOR TEST	**9504**	**4**	**100**
V+X FACTOR TEST	**9505**	**4+5**	**100**

TABLE OF SYMBOLS

IVD	*In Vitro* Diagnostic Medical Device		Do not reuse
REF	Catalogue number		Fragile,handle with care
	Manufacturer	Σ	Contains sufficient for <n> tests
	Use by		Caution, consult accompanying documents
	Temperature limitation	LOT	Batch code

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CE IVD

F00005
Rev. 1/ 28.03.2013

Violet Red Bile Lactose Agar

Selective medium for the enumeration of coliforms
in food, water and other materials, according to APHA and ISO 4832.

DESCRIPTION

Violet Red Bile Lactose Agar is a selective medium used for the isolation and enumeration of coliform bacteria in food, water and other materials of sanitary importance, according to APHA and ISO 4832.

TYPICAL FORMULA	(g/l)
Enzymatic Digest of Animal Tissues	7.0
Yeast Extract	3.0
Lactose	10.0
Sodium Chloride	5.0
Bile Salts	1.5
Neutral Red	0.03
Crystal Violet	0.002
Agar	14.0

Final pH 7.4 ± 0.2 at 25°C

METHOD PRINCIPLE

Enzymatic digest of animal tissues provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Lactose is the fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Bile salts and Crystal violet are selective agents effective against Gram-positive cocci. Neutral red is the pH indicator. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 40.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.
Medium in tubes/bottles	Melt the content of the tube/bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the tube/bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

1. Perform serial dilutions of the test sample in order to achieve a colony count of between 10 and 150 colonies per plate. Use a suitable diluent such as Buffered Peptone Water (ref. 24099) or Maximum Recovery Broth (ref. 20071).
2. Inoculate the medium by pour plating or spread plating method.
3. Incubate aerobically at 30°C or 37°C, depending on the organisms under study, for 24 ± 2 hours.

For environmental hygiene monitoring, use a swab and the sampling template 10x10 (ref. 96762) to sample a well defined area of the test surface. Then, inoculate the medium by streaking the swab over the plate. Otherwise, RODAC plates can be directly used for surface sampling by firmly pressing the agar medium against the test area for a few seconds.

INTERPRETING RESULTS

Select plates containing 10-150 colonies. Count the purplish-red colonies with a diameter of at least 0.5 mm.

Atypical colonies (e.g. smaller size) and all colonies derived from milk products should be confirmed by using Brilliant Green Lactose Bile Broth 2% (ref. 20102).

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, beige to reddish-beige.
Prepared medium: slightly opalescent, reddish-purple.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU.
Inoculum for selectivity: 10^4-10^6 CFU.
Inoculum for specificity: 10^3-10^4 CFU.
Incubation conditions: aerobically at 30 ± 1°C for 24 ± 2 hours.

QC Table.

Microorganism		Specification
Escherichia coli	WDCM 00012	Good growth, purplish-red colonies with or without precipitation halo
Enterococcus faecalis	WDCM 00009	Inhibition
Pseudomonas aeruginosa	WDCM 00025	Colorless to beige colonies

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 4832:2006. Microbiology of food and animal feeing stuffs – Horizontal method for the enumeration of coliforms – Colony count technique.
3. Davidson, Roth, and Gambrel-Lenarz (2004) In Wehr and Frank (ed.) Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
4. Kornacki and Johnson (2001) In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington D.C.

PRESENTATION		Contents	Ref.
Violet Red Bile Lactose Agar	90 mm ready-to-use plates	20 plates	11183
Violet Red Bile Lactose Agar	90 mm ready-to-use plates	100 plates	11183*
Violet Red Bile Lactose Agar	55 mm ready-to-use RODAC plates (in blister packs)	20 plates	15326
Violet Red Bile Lactose Agar	55 mm ready-to-use RODAC plates	20 plates	15326L
Violet Red Bile Lactose Agar	Tubes	20 x 22 ml tubes	31076
Violet Red Bile Lactose Agar	Tubes	10 x 22 ml tubes	34076
Violet Red Bile Lactose Agar	Bottles	6 x 100 ml bottles	402460
Violet Red Bile Lactose Agar	Dehydrated medium	500 g of powder	610058
Violet Red Bile Lactose Agar	Dehydrated medium	100 g of powder	620058
Violet Red Bile Lactose Agar	Dehydrated medium	5 kg of powder	6100585

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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Violet Red Bile Glucose Agar

Selective medium for detection and enumeration of Enterobacteriaceae in food, water and other materials, according to USP/EP/JP and ISO 21528.

Instructions For Use
ENGLISH

DESCRIPTION

Violet Red Bile Glucose Agar is a selective medium used for the detection and enumeration of bile-tolerant Gram-negative bacteria in food, water and other materials of sanitary importance.

This medium complies with the recommendations of the harmonized method in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP).

The medium is also formulated in accordance with ISO 21528 (all parts).

TYPICAL FORMULA*	(g/litre)
Enzymatic Digest of Animal Tissues	7.0
Yeast Extract	3.0
Glucose	10.0
Sodium Chloride	5.0
Bile Salts	1.5
Neutral Red	0.03
Crystal Violet	0.002
Agar	14.0

Final pH 7.4 ± 0.2 at 25°C

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Enzymatic digest of animal tissues provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Glucose is the fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Bile salts and Crystal violet are selective agents effective against Gram-positive cocci. Neutral red is the pH indicator. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 40.5 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

1. Use a suitable diluent such as Buffered Peptone Water (ref. 24099) to prepare the sample. The European Pharmacopoeia recommends to perform a pre-incubation step in Tryptic Soy Broth (ref. 24444) for 2-5 h at 20-25°C to resuscitate bacteria followed by 24-48 h enrichment at 30-35°C in EE Broth-Mossel (ref. 24096).
2. Inoculate Violet Red Bile Glucose Agar by pour plating or spread plating method.
3. Incubate aerobically at 30-35°C for 18-24 hours or 37°C for 24 ± 2 hours, depending on the method used.

For environmental hygiene monitoring, use a swab and the sampling template 10x10 (ref. 96762) to sample a well defined area of the test surface. Then, inoculate the medium by streaking the swab over the plate. Otherwise, contact plates can be directly used for surface sampling by firmly pressing the agar medium against the test area.

INTERPRETING RESULTS

Select plates containing less than 150 colonies. Count characteristic pink to red colonies (with or without precipitation halo).

Confirm by subculturing to a non selective agar medium looking for oxidase reaction (ref. 88029) and glucose fermentation (ref. 88202). Colonies that are oxidase-negative and glucose-positive are confirmed as Enterobacteriaceae.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, beige to reddish-beige.
Prepared medium: slightly opalescent, reddish-purple.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
90 mm ready-to-use plates: 6 months.
Contact plates: 9 months.

QUALITY CONTROL

To check the performance of the medium, QC testing should be carried out following specific requirements for the method used.

ISO compliance

Control strain		Inoculum	Incubation	Criteria	Specification
Escherichia coli	WDCM 00012 or WDCM 00013	50-100 CFU	24 ± 2 h / 37 ± 1°C	$P_R \geq 0.5$	Pink to red colonies with or without precipitation halo
Salmonella Typhimurium	WDCM 00031				
Salmonella Enteritidis	WDCM 00030				
Enterococcus faecalis	WDCM 00009 or WDCM 00087	10^4-10^6 CFU		Total inhibition	—

A productivity ratio (P_R) of 0.5 is equivalent to a recovery rate of 50%

Pharmacopoeia growth promotion

Control strain		Inoculum	Incubation	Expected results
Escherichia coli	ATCC® 8739 (WDCM 00012)	≤ 100 CFU	18-24 h / 30-35°C	Recovery ≥ 50%, pink to red colonies with precipitation halo
Pseudomonas aeruginosa	ATCC® 9027 (WDCM 00026)			Recovery ≥ 50%, colourless to slightly red colonies

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Violet Red Bile Glucose Agar	90 mm Plate	20 plates	11184
Violet Red Bile Glucose Agar	Contact Plate	20 plates	15375
Violet Red Bile Glucose Agar	Bottle	6 x 100 ml	402540
Violet Red Bile Glucose Agar	Bottle	25 x 100 ml	450254
Violet Red Bile Glucose Agar	Bottle	6 x 500 ml	470031
Violet Red Bile Glucose Agar	Dehydrated medium	100 g	620059
Violet Red Bile Glucose Agar	Dehydrated medium	500 g	610059
Violet Red Bile Glucose Agar	Dehydrated medium	5 kg	6100595

This document is available from the online Support Center:
liofilchem.com/ifu-sds

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Instructions For Use
ENGLISH

XLD Agar

Selective medium for detection of *Salmonella* and *Shigella* spp in food, environmental samples and other materials, according to ISO 6579 and ISO 21567.

DESCRIPTION

XLD (Xylose Lysine Deoxycholate) Agar is a selective medium used for the isolation and differentiation of pathogen Enterobacteriaceae, especially *salmonellae* and *shigellae* from food, environmental samples and clinical specimens.

XLD Agar is formulated according to ISO 6579 and ISO 21567 for the detection of *Salmonella* and *Shigella* spp, respectively.

TYPICAL FORMULA	(g/l)
Yeast Extract	3.0
Sodium Chloride	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
L-Lysine	5.0
Sodium Thiosulfate	6.8
Iron(III) Ammonium Citrate	0.8
Phenol Red	0.08
Sodium Deoxycholate	1.0
Agar	15.0
Final pH 7.4 ± 0.2 at 25°C	

METHOD PRINCIPLE

Yeast extract is a source of vitamins, particularly of B-group. Sodium chloride maintains the osmotic balance of the medium. Xylose, lactose and sucrose are the fermentable carbohydrates. Lysine is the decarboxylase substrate. Sodium thiosulfate and ferric ammonium serve as indicators of hydrogen sulphide production under alkaline conditions. Phenol red is the pH indicator. Sodium deoxycholate is the selective agent inhibiting most Gram-positive bacteria. Agar is the solidifying agent.

PREPARATION

Dehydrated medium	Suspend 55.4 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.
Medium in bottles	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Immediately cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

TEST PROCEDURE

Inoculate the plates by spread method. Incubate aerobically at 37 ± 1°C for up to 48 hours.

INTERPRETING RESULTS

After incubation observe the color of the colonies and interpret the results as indicated in the ID Table.

ID Table.

Microorganism	Appearance of colonies
Salmonella, Edwarsiella spp	Red with black center
Shigella, *Providencia*, *Pseudomonas* spp, *Salmonella paratyphi* (H_2S-negative strains)	Red
*Salmonella thyphosa (*xilose-positive strains)	Orange
Escherichia coli, *Enterobacter*, *Aeromonas*, *Klebsiella*, *Serratia* spp	Yellow with yellow zone
Citrobacter spp (lactose-positive strains)	Yellow with yellow zone, sometimes with black center
Proteus spp	Yellow with yellow zone and black center

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, pink.
Prepared medium: slightly opalescent, red.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 2 years.
Medium in bottles: 1 year.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU
Inoculum for selectivity: >10^3 CFU
Incubation conditions: aerobically at 37 ± 1°C for 24 ± 3 hours.

QC Table.

Microorganismo		Growth	Specification
Salmonella Typhimurium	WDCM 00031	Good	Red colonies with black center
Salmonella Enteritidis	WDCM 00030	Good	Red colonies with black center
Shigella flexneri	ATCC® 12022	Good	Red colonies
Escherichia coli	WDCM 00013	Poor	Yellow colonies
Enterococcus faecalis	WDCM 00087	Inhibited	---

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use.
The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. EN ISO 21567:2004 Microbiology of food and animal feeing stuffs – Horizontal method for the detection of *Shigella* spp.
3. ISO 6579:2002 Microbiology of food and animal feeing stuffs – Horizontal method for the detection of *Salmonella* spp.
4. Vanderzant C. and D.F. Splittstoesser (1992) Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington D.C.
5. Rollender W. et al. (1969) Comparison of xylose lysine deoxycholate agar and MacConkey Agar for the isolation of Salmonella and Shigella from clinical specimens. Am J Clin Pathol; 51/2:284-386
6. Taylor W.J. (1965) Isolation of Shigellae I. Xylose lysine agars: new media for isolation of enteric pathogens. Am J Clin Pathol; 44:471-475.
7. Taylor W.J. and Harris B (1965) Isolation of Shigellae II. Comparison of plating media and enrichment broths. Am J Clin Pathol; 44X:476-479.

PRESENTATION		Contents	Ref.
XLD Agar	90 mm ready-to-use plates	20 plates	10056
XLD Agar	90 mm ready-to-use plates	100 plates	10056*
XLD Agar	Bottles	6 x 100 ml bottles	402570
XLD Agar	Dehydrated medium	500 g of powder	610060
XLD Agar	Dehydrated medium	100 g of powder	620060
XLD Agar	Dehydrated medium	5 kg of powder	6100605

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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CE IVD

X FACTOR TEST
V FACTOR TEST
V+X FACTOR TEST

ENGLISH

DESCRIPTION
X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST are constituted by paper discs with special features, containing the respective coagulation factors, used for differentiating *Haemophilus* spp.

CONTENT OF THE PACKAGES
Each package contains:
- 2 cartridges with 50 discs each, packaged in a heat-sealed container
- 1 dryer
- 1 instruction sheet

PRINCIPLE OF THE METHOD
Different strains of *Haemophilus* grow on a culture medium only in presence of the coagulation factor or factors (X, V, or both) which they need. These different requirements allow the differentiation and the identification of *Haemophilus* spp.

COMPOSITION
– Each disc of X FACTOR TEST contains Hemin.
– Each disc of V FACTOR TEST contains NAD (Nicotinamide-Adenine-Dinucleotide).
– Each disc of V+X FACTOR TEST contains Hemin and NAD (Nicotinamide-Adenine-Dinucleotide).

TEST PROCEDURE
1. Take the cartriges container from the refrigerator and leave it on the test bench until it reaches room temperature (about 30 minutes). This will prevent humidity being deposited on the discs when the package is opened, which could prejudice their long-term stability.
2. Using a sterile swab, evenly inoculate the surface of a plate of Tryptic Soy Agar (ref. 10037) or Mueller Hinton Agar (ref. 10031) with a pure suspension of the microorganism to test.
3. Using sterile tools, press one disc of X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST on the inoculated surface, at a distance of 120° from one to another and at 1-2 cm from the edge of the plate. Incubate at 36±1°C for 24-48 in 5-10% carbon dioxide atmosphere.

INTERPRETATION OF THE RESULTS
If the organism requires X Factor alone, it will grow only at the edge of the X and the X+V FACTOR TEST discs; if it requires V Factor alone, it will grow only at the edge of the V and the X+V FACTOR TEST discs; if both X and V Factors are required, it will grow only at the vicinity of the X+V FACTOR TEST discs.
Some examples are indicated in the following table:

	Without Factors	Factor X	Factor V	Factors V+X
Haemophilus influenzae	-	-	-	+
Haemophilus aegyptius	-	-	-	+
Haemophilus parainfluenzae	-	-	+	+
Haemophilus ducreyi	-	+	-	+
Bordetella pertussis	+	+	+	+

QUALITY CONTROL
Each batch of X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST is subjected to microbial control, inoculating pure suspensions of *Haemophilus influenzae* ATCC 19418 and *Haemophilus parainfluenzae* ATCC 7901 on plates of Tryptic Soy Agar.

PRECAUTIONS
X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST cannot be classified as being hazardous according to the current legislation.
X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST is a disposable device to be used only for diagnostic use *in vitro.* It must be used in the laboratory by properly trained personnel, using approved aseptic and safety methods for handling pathogenic agents.

STORAGE
Store X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST at 2-8°C in the original packaging. Keep away from sources of heat and avoid excessive changes in temperature. In such conditions, X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

DISPOSAL OF USED MATERIAL
After use, X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST and material that has come into contact with the sample must be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.

BIBLIOGRAPHY

- Kilian M. (1980) *Haemophilus*. in Manual of Clinical Microbiology. Eds. Lennette et al. Amer. Soc. for Microbiol. 3rd edn. Washington.

PRESENTATION

Product	REF	µg	Σ
X FACTOR TEST	**9503**	**5**	**100**
V FACTOR TEST	**9504**	**4**	**100**
V+X FACTOR TEST	**9505**	**4+5**	**100**

TABLE OF SYMBOLS

IVD	*In Vitro* Diagnostic Medical Device		Do not reuse
REF	Catalogue number		Fragile,handle with care
	Manufacturer	Σ	Contains sufficient for <n> tests
	Use by		Caution, consult accompanying documents
	Temperature limitation	LOT	Batch code

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CE IVD

F00005
Rev. 1/ 28.03.2013

X FACTOR TEST
V FACTOR TEST
V+X FACTOR TEST

ENGLISH

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- 1 instruction sheet

PRINCIPLE OF THE METHOD
Different strains of *Haemophilus* grow on a culture medium only in presence of the coagulation factor or factors (X, V, or both) which they need. These different requirements allow the differentiation and the identification of *Haemophilus* spp.

COMPOSITION
– Each disc of X FACTOR TEST contains Hemin.
– Each disc of V FACTOR TEST contains NAD (Nicotinamide-Adenine-Dinucleotide).
– Each disc of V+X FACTOR TEST contains Hemin and NAD (Nicotinamide-Adenine-Dinucleotide).

TEST PROCEDURE
1. Take the cartriges container from the refrigerator and leave it on the test bench until it reaches room temperature (about 30 minutes). This will prevent humidity being deposited on the discs when the package is opened, which could prejudice their long-term stability.
2. Using a sterile swab, evenly inoculate the surface of a plate of Tryptic Soy Agar (ref. 10037) or Mueller Hinton Agar (ref. 10031) with a pure suspension of the microorganism to test.
3. Using sterile tools, press one disc of X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST on the inoculated surface, at a distance of 120° from one to another and at 1-2 cm from the edge of the plate. Incubate at 36±1°C for 24-48 in 5-10% carbon dioxide atmosphere.

INTERPRETATION OF THE RESULTS
If the organism requires X Factor alone, it will grow only at the edge of the X and the X+V FACTOR TEST discs; if it requires V Factor alone, it will grow only at the edge of the V and the X+V FACTOR TEST discs; if both X and V Factors are required, it will grow only at the vicinity of the X+V FACTOR TEST discs.
Some examples are indicated in the following table:

	Without Factors	Factor X	Factor V	Factors V+X
Haemophilus influenzae	-	-	-	+
Haemophilus aegyptius	-	-	-	+
Haemophilus parainfluenzae	-	-	+	+
Haemophilus ducreyi	-	+	-	+
Bordetella pertussis	+	+	+	+

QUALITY CONTROL
Each batch of X FACTOR TEST, V FACTOR TEST and V+X FACTOR TEST is subjected to microbial control, inoculating pure suspensions of *Haemophilus influenzae* ATCC 19418 and *Haemophilus parainfluenzae* ATCC 7901 on plates of Tryptic Soy Agar.

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STORAGE
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X FACTOR TEST	**9503**	**5**	**100**
V FACTOR TEST	**9504**	**4**	**100**
V+X FACTOR TEST	**9505**	**4+5**	**100**

TABLE OF SYMBOLS

IVD	*In Vitro* Diagnostic Medical Device		Do not reuse
REF	Catalogue number		Fragile,handle with care
	Manufacturer	Σ	Contains sufficient for <n> tests
	Use by		Caution, consult accompanying documents
	Temperature limitation	LOT	Batch code

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CE IVD

F00005
Rev. 1/ 28.03.2013

Instructions For Use
ENGLISH

Chromatic™ Clostridium difficile

Chromogenic medium for detection of *C. difficile* in clinical specimens and environmental samples.

DESCRIPTION

Chromatic™ Clostridium difficile is a chromogenic selective medium used for the isolation and identification of *Clostridium difficile* in clinical specimens and other materials of sanitary importance.

TYPICAL FORMULA	(g/l)
Peptones	20.0
Yeast Extract	5.0
Sodium Chloride	5.0
Growth Factors	8.0
Chromogenic and Selective Mix	2.7
Agar	15.0

Final pH 7.8 ± 0.2 at 25°C

METHOD PRINCIPLE

Peptone provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Sodium chloride maintains the osmotic balance of the medium. Growth factors are necessary for the optimal recovery of *C. difficile*. Chromogenic and selective mix allow to identify the microorganism on the basis of the color/fluorescence of the colonies while inhibiting most of bacteria, yeasts and moulds. Agar is the solidifying agent.

TEST PROCEDURE

Inoculate samples by direct streaking on the medium surface or after a first enrichment step.
Incubate anaerobically at 35 ± 2°C for 24 hours.

INTERPRETING RESULTS

After incubation observe the bacterial growth: typical colonies of *C. difficile* appear colorless and fluorescent under UV lamp (365 nm). Further biochemical or molecular test should be performed for confirmation.

APPEARANCE

Slightly opalescent, light amber.

STORAGE

Store at 2-8°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

4 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: 50-100 CFU
Inoculum for selectivity: 10^4-10^6 CFU
Incubation conditions: anaerobically at 35 ± 2°C for 18-24 hours.

QC Table.

Microorganism		Growth	Specification
Clostridium diffcile	ATCC® 43255	Good	Colorless colonies, fluorescent under UV light
Clostridium perfringens	ATCC® 13124	Inhibited	---
Enterococcus faecalis	ATCC® 29212	Inhibited	---
Escherichia coli	ATCC® 25922	Inhibited	---
Candida albicans	ATCC® 10231	Inhibited	---

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

1. Van Broeck J.et al. (2014) Evaluation of a new 24 h culture medium for the isolation of Clostridium difficile from stool samples. National Reference Centre Clostridium difficile, Université Catholique de Louvain, Brussels, Belgium. Poster P0731, ECCMID, Barcelona.
2. Crobach MJT. et al. (2009) European Society of Clinical Microbiology and Infectious Diseases (ESCMID): Data review and recommendations for diagnosing Clostridium difficile- infection (CDI) - Clin Microbiol Infect. 15:1053- 1066.
3. Delmee M. et al. (2005) Laboratory diagnosis of Clostridium difficile-associated diarrhea: a plea for culture. J Med Microbiology. 54:187-191.
4. Bartlett JG., Gerding D. (2008) Clinical Recognition and Diagnosis of Clostridium difficile Infection - Clin Infect Dis. 46:S12-S18.

PRESENTATION		Contents	Ref.
Chromatic™ Clostridium difficile	90 mm ready-to-use plates	20 plates	11632

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

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