Instructions For Use
ENGLISH

MacConkey Broth

Differential medium for detection of coliform bacteria.

DESCRIPTION

MacConkey Broth is a liquid medium used for the detection and enumeration of coliforms from water, and milk.

TYPICAL FORMULA*	(g/l)
Peptone	20.0
Lactose	10.0
Sodium Chloride	5.0
Bile Salts No.3	5.0
Bromocresol Purple	0.01
Final pH 7.4 ± 0.2 at 25°C	

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

METHOD PRINCIPLE

Peptone provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Lactose is the fermentable carbohydrate. Sodium Chloride maintain the osmotic balance of the medium. Bile salts inhibit the growth of Gram-positive bacteria. Bromocresol purple is the pH indicator.

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. Dispense into tubes containing Durham tubes. Autoclave at 121°C for 15 minutes.

Ensure there are no air bubbles in the Durham tube prior to use as it may lead to a misinterpretation (false-positive reaction in gas production) of results. If needed, invert the tube to allow the air being released from the Durham tube.

TEST PROCEDURE

For the presumptive coliform examination, inoculate a series of tubes with the test sample and its dilutions and incubate at 35-37°C for 18-24 hours.

The number of total coliforms is estimated by counting the number of tubes giving a positive reaction for acid (yellow colouration of the broth) and gas production and comparing the pattern of positive results with standard charts to find MPN (Most Probabile Number) of total 100 ml sample.

For the differential test, subculture from each tube showing positive reaction into a fresh tube of MacConkey Broth. Incubate at 42-44°C for up to 48 h to detect *Escherichia coli*.

INTERPRETING RESULTS

Turbidity of MacConkey Broth indicates microbial growth.

Acid production due to lactose fermentation causes a color change of the medium to yellow. Gas is also produced, collecting in Durham tubes.

The ability to form gas in MacConkey Broth at 44°C is specific for *E. coli,* which is indicative of faecal pollution of the original sample.

Non-fermenting Gram-negative organisms produce good growth but without significant changes in color nor gas formation.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. 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.

QUALITY CONTROL

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

Appearance of Prepared Medium: Clear, purple.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Growth	Acid	Gas
Salmonella Typhimurium	WDCM 00031 (ATCC® 14028; NCTC 12023)	≤100 CFU	18-24 h / 35-37°C	Good	Neg. (purple)	Neg.
Escherichia coli	WDCM 00012 (ATCC® 8739; NCTC 12923)	≤100 CFU	18-24 h / 42-44°C	Good	Pos. (yellow)	Pos.
Staphylococcus aureus	WDCM 00032 (ATCC® 6538; NCTC 10788)	1000 CFU	48 h / 35-37°C	Inhibition	—	—

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 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.
MacConkey Broth	Dehydrated medium	100 g	620171
		500 g	610171

Significant changes from previous version:

Document	Release Date	Change Summary
610171_IFU-0	2022-11-04	Document creation

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

liofilchem.com/ifu-sds

Istruzioni per l'uso
ITALIANO

MacConkey Broth

Terreno differenziale per la ricerca di batteri coliformi.

DESCRIZIONE

MacConkey Broth è un terreno liquido utilizzato per la ricerca ed il conteggio dei coliformi da acqua e latte.

FORMULA TIPICA*	(g/l)
Peptone	20.0
Lattosio	10.0
Sodio Cloruro	5.0
Sali di Bile No.3	5.0
Porpora di Bromocresolo	0.01
pH Finale 7.4 ± 0.2 a 25°C	

*Adattata e/o integrata per soddisfare le specifiche di performance richieste.

PRINCIPIO DEL METODO

Il peptone fornisce 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 inibiscono i batteri Gram positivi. Il porpora di bromocresolo è l'indicatore di pH.

PREPARAZIONE

Terreno disidratato

Sospendere 40.0 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire fino a completa dissoluzione. Distribuire in provette con campanella di Durham. Autoclavare a 121°C per 15 minuti.

Assicurarsi che non vi siano bolle d'aria nella campanella di Durham prima dell'uso in quanto ciò potrebbe portare a un'interpretazione errata (reazione falsa positiva nella produzione di gas) dei risultati. Se necessario, capovolgere la provetta per consentire la fuoriuscita dell'aria dalla campanella di Durham.

PROCEDURA DEL TEST

Per l'esame presuntivo dei coliformi, inoculare una serie di provette con il campione in esame e le sue diluizioni ed incubare a 35-37°C per 18-24 ore.

Il numero di coliformi totali viene stimato contando il numero di provette che danno una reazione positiva per la produzione di acido (colorazione gialla del brodo) e di gas e confrontando l'andamento dei risultati positivi con i grafici standard per trovare l'MPN (numero più probabile) riferito al campione totale (100 ml).

Per il test differenziale, da ciascuna delle provette positive trasferire un volume del brodo di coltura in una nuova provetta di MacConkey Broth. Incubare a 44°C fino a 48 h per rilevare *Escherichia coli*.

INTERPRETAZIONE DEI RISULTATI

La torbidità in MacConeky Broth indica la crescita microbica.

La produzione di acidi causata dalla fermentazione del lattosio provoca il cambiamento di colore del terreno a giallo. Ciò comporta anche la produzione di gas, raccolto nelle campanelle di Durham.

La capacità di formare gas in MacConkey Broth a 44°C è specifica per *E. coli*, che è un indicatore di inquinamento fecale del campione originale.

I microrganismi Gram negativi non fermentanti producono una buona crescita che non è però accompagnata da significativi cambiamenti di colore né da formazione di gas.

CONSERVAZIONE

La polvere è fortemente igroscopica, conservare a 10-30°C, in ambiente asciutto, nel suo contenitore originale chiuso ermeticamente. 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.

CONTROLLO DI QUALITÀ

Aspetto del Terreno Disidratato: Omogeneo, granulometria fine, beige chiaro.

Aspetto del Terreno Preparato: Viola, limpido.

Risultati Attesi dei Test Colturali:

Ceppi di controllo		Inoculo	Incubazione	Crescita	Acido	Gas
Salmonella Typhimurium	WDCM 00031 (ATCC® 14028; NCTC 12023)	≤100 CFU	18-24 h / 35-37°C	Buona	Neg. (viola)	Neg.
Escherichia coli	WDCM 00012 (ATCC® 8739; NCTC 12923)	≤100 CFU	18-24 h / 42-44°C	Buona	Pos. (giallo)	Pos.
Staphylococcus aureus	WDCM 00032 (ATCC® 6538; NCTC 10788)	1000 CFU	48 h / 35-37°C	Inibizione	—	—

Fare riferimento al certificato di analisi (CoA) relativo al lotto effettivo.

AVVERTENZE E PRECAUZIONI

Esclusivamente per uso professionale. Gli operatori devono essere formati e avere una certa esperienza nei metodi di laboratorio. Si prega di legger attentamente le istruzioni prima di utilizzare questo prodotto. L'affidabilità dei risultati del test non può essere garantita se ci sono deviazioni dalle istruzioni riportate in questo documento.

Consultare la scheda di sicurezza (SDS) per informazioni sui pericoli e sulle modalità di manipolazione sicure.

SMALTIMENTO DEI RIFIUTI

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

BIBLIOGRAFIA

Vedere i riferimenti alla fine di questo documento.

TABELLA DEI SIMBOLI

Vedere la tabella dei simboli alla fine di questo documento.

Per le configurazioni disponibili di questo prodotto vedere l'elenco nella lingua inglese.

Questo documento IFU e la SDS sono disponibili dal Support Center online:

liofilchem.com/ifu-sds

References / Riferimenti

1. European Pharmacopoeia (EP) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia (USP) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia (JP) 4.05 Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Departments of the Environment, Health, Social Security and Public Health Laboratory Service (1982) The Bacteriological Examination of Drinking Water Supplies, Report No. 71, HMSO London.
5. World Health Organization (1963) International Standards for Drinking Water, 2nd ed., WHO, Geneva.
6. Murray, Baron, Jorgensen, Landry and Pfaller ed. (2007) Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. MacConkey, A. (1908) Bile salt media and their advantages in some bacteriological examinations. J. Hyg. 8: 322-334.
8. MacConkey, A. (1905) Lactose-fermenting bacteria in faeces. J. Hyg. 8: 333-379.

Table of Symbols / Tabella dei Simboli

LOT	Batch code / Codice del lotto
REF	Catalogue number / Numero di catalogo
	Manufacturer / Fabbricante
	Use by / Utilizzare entro
	Fragile, handle with care / Fragile, maneggiare con cura
	Temperature limitation / Limiti di temperatura
	Contains sufficient for <n> tests / Contenuto sufficiente per <n> saggi
	Consult instructions for use / Consultare le istruzioni per l'uso
	Do not reuse / Non riutilizzare
	Keep away from sunlight / Tenere al riparo dalla luce solare
	Warninng / Avvertimento

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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

Istruzioni per l'uso
ITALIANO

TCBS Agar

Terreno selettivo per la ricerca di *Vibrio* spp enteropatogeno, da campioni clinici e non clinici, secondo ISO 21872.

DESCRIZIONE

Thiosulfate Citrate Bile Sucrose (TCBS) Agar Water è un terreno selettivo utilizzato per la coltivazione di vibrioni.

Questo terreno è conforme con ISO 21872 per l'identificazione di *Vibrio* spp, incluso *Vibrio cholerae* da alimenti, mangimi e campioni ambientali nell'area di produzione e manipolazione degli alimenti stessi.

TCBS Agar è anche raccomandato per l'isolamento di *V. cholerae* e *V. parahaemolyticus* così come altri vibrioni provenienti da campioni fecali.

FORMULA TIPICA	(g/l)
Peptone	10.0
Estratto di Lievito	5.0
Sodio Citrato	10.0
Sodio Tiosolfato	10.0
Ferro(III) Citrato	1.0
Sodio Cloruro	10.0
Bile Bovina Disidratata	8.0
Sucrosio	20.0
Blu di Bromotimolo	0.04
Timol Blu	0.04
Agar	15.0
pH Finale 8.6 ± 0.2 a 25°C	

PRINCIPIO DEL METODO

Peptone ed estratto di lievito forniscono aminoacidi, azoto, carbonio, vitamine e minerali per la crescita dei microrganismi. Il sodio citrato serve per mantenere un pH alcalino ed insieme al sodio tiosolfato ed alla bile di bue costituiscono gli agenti selettivi che inibiscono i microrganismi Gram-positivi e sopprimono i coliformi. Inoltre, le condizioni alcaline del terreno aumentano il recupero di *V. cholerae*. Il sodio tisolfato è utile anche come fonte di zolfo ed, in combinazione con il citrato ferrico, permette di determinare la produzione di solfuro di idrogeno. Il sodio cloruro mantiene il bilancio osmotico del terreno e stimola la crescita dei vibrioni. Il sucrosio è il carboidrato fermentabile. Blu di bromotimolo e timol blu sono gli indicatori di pH. L'agar è l'agente solidificante.

PREPARAZIONE

Terreno disidratato	Sospendere 89.1 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.
Terreno in flaconi	Sciogliere il contenuto di una flacone in bagnomaria a 100°C (con i tappi leggermente svitati) 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

ISO 21872 raccomanda di seguire due fasi di arricchimento consecutive in Alkaline Saline Peptone Water (ASPW) prima di inoculare TCBS Agar.

TCBS Agar può anche essere inoculato direttamente con campioni clinici come ad esempio, tamponi rettali, feci, vomito o con campioni alimentari (*).

Incubare (al riparo dalla luce) a 37 ± 1°C per 18-24 ore in atmosfera aerobica.

(*) NB. Si raccomanda un inoculo massivo. I tamponi con il materiale da testare dovrebbero essere trasportati in Cary Blair Transport Medium (ref. 470290) se si prevede un ritardo nel raggiungere il laboratorio. I campioni clinici da utilizzare per la coltivazione dei vibrioni non dovrebbero essere congelati.

INTERPRETAZIONE DEI RISULTATI

I ceppi di *Vibrio cholerae* producono colonie gialle su TCBS Agar a causa della fermentazione del sucrosio. Anche *Vibrio alginolyticus* produce colonie gialle. Alcuni ceppi di *Proteus* sucrosio positivi possono crescere e formare colonie gialle simili a quelle sviluppate dai vibrioni. *Vibrio parahaemolytics* è un microrganismo che non fermenta il sucrosio e produce colonie blu-verdi, così come *Vibrio fulnificus*. Occasionalmente alcuni isolati di specie di *Pseudomonas* e *Aeromonas* producono anche colonie blu-verdi, ma nel complesso TCBS Agar è altamente selettivo ed ogni colonia H_2S negativa potrebbe appartenere alla specie *Vibrio*.

ASPETTO

Terreno disidratato: omogeneo, fine granulometria, da beige chiaro a verde beige.
Terreno preparato: verde, da chiaro a 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, le provette 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: 3 anni.
Terreno in provette: 2 anni.
Piastre pronte all'uso: 6 mesi.

CONTROLLO DI QUALITÀ

Le provette vengono inoculate con i ceppi microbici indicati nella tabella CQ.
Inoculo per produttività: ≤100 UFC.
Inoculo per selettività: $>10^3$ UFC.
Condizioni di incubazione: ambiente aerobico a 37 ± 1°C per 18-24 ore.

Tabella CQ.

Microrganismo		Crescita	Colore Colonie
Vibrio parahaemolyticus	WDCM 00185	Buona	Verde
Vibrio furnissii	WDCM 00186	Buona	Giallo
Escherichia coli	WDCM 00012	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. 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.

PRESENTAZIONE		Contenuto	Ref.
TCBS Agar	Piastre da 90 mm pronte all'uso	20 piastre	11195
TCBS Agar	Piastre da 90 mm pronte all'uso	100 piastre	11195*
TCBS Agar	Provette a becco di clarino	Provette 10 x 9 ml	30022
TCBS Agar	Provette a becco di clarino	Provette 20 x 9 ml	31022
TCBS Agar	Flaconi	Flaconi 6 x 100 ml	403140
TCBS Agar	Terreno disidratato	500 g di polvere	611010
TCBS Agar	Terreno disidratato	100 g di polvere	621010

TABELLA DEI SIMBOLI

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

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CE IVD

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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Istruzioni per l'uso
ITALIANO

EE Broth-Mossel

Terreno liquido per la coltivazione e l'arricchimento selettivo delle Enterobacteriaceae da differenti tipologie di campioni, secondo USP/EP/JP.

DESCRIZIONE

Enterobacteriaceae Enrichment Broth-Mossel è un terreno selettivo utilizzato per la ricerca dei batteri Gram negativi bile tolleranti negli alimenti, acqua ed altri materiali di importanza.

Questo terreno è conforme alle raccomandazioni del metodo armonizzato delle Farmacopee Statunitense (USP) Europea (EP) e Giapponese (JP) per l'esame microbiologico dei prodotti non sterili.

FORMULA TIPICA	(g/l)
Digerito Pancreatico di Gelatina	10.0
Glucosio Monoidrato	5.0
Bile di Bue Disidratata	20.0
Potassio Diidrogeno Fosfato	2.0
Sodio Idrogeno Fosfato Bibasico, Anidro	6.4*
Verde Brillante	0.015

pH Finale 7.2 ± 0.2 a 25°C

* Equivalente a 8.0 g di Sodio Idrogeno Fosfato Bibasico Biidrato

PRINCIPIO DEL METODO

Il digerito pancreatico di gelatina fornisce aminoacidi, azoto, carbonio, vitamine e minerali per la crescita dei microrganismi. Il glucosio è il carboidrato fermentabile. Il sodio cloruro mantiene il bilancio osmotico del terreno. Bile di bue e verde brillante sono agenti selettivi efficaci contro i cocchi Gram positivi. Potassio fosfato e sodio fosfato agiscono da tampone.

PREPARAZIONE

Terreno disidratato: Sospendere 43.4 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.

PROCEDURA DEL TEST

Come da Farmacopea, preparare il campione utilizzando una diluizione 1 a 10 con almeno 1 g del prodotto da esaminare scegliendo come diluente Tryptic Soy Broth (ref. 24513 o 452080) ed incubare a 20-25°C per 2-5 ore per recuperare i batteri.

Per test qualitativi (test per assenza), trasferire in EE Broth-Mossel il volume del brodo di pre-arricchimento corrispondente ad 1 g del prodotto da esaminare.

Per test quantitativi, contare le Enterobacteriaceae trovate per millilitro o per grammo di campione utilizzando la tecnica MPN (Most Probable Number).

Per entrambe le tipologie di test, incubare EE Broth-Mossel a 30-35°C per 24-48 ore e procedere con l'analisi seminando parte del brodo su Violet Red Bile Glucose Agar (ref. 11184). Incubare le piastre in atmosfera aerobica a 30-35°C per 18-24 ore.

INTERPRETAZIONE DEI RISULTATI

La torbidità in EE Broth-Mossel indica la crescita microbica; la produzione di acidi provoca il cambiamento di colore del terreno a giallo.

Il mancato sviluppo di colonie su Violet Red Bile Glucose Agar è riportata come assenza di batteri Gram begativi bile tolleranti. La crescita di colonie costituisce un risultato positivo ed il numero probabile di batteri viene determinato consultando la tabella sottostante.

Tabella MPN.

Risultati in base alle quantità di prodotto			Numero probabile di batteri per grammo o per millilitro di prodotto
0.1 g o 0.1 ml	0.01 g o 0.01 ml	0.001 g o 0.001 ml	
+	+	+	$>10^3$
+	+	–	10^3 - 10^2
+	–	–	10^2 - 10
–	–	–	<10

ASPETTO

Terreno disidratato: omogeneo, fine granulometria, da beige a verde chiaro.
Terreno preparato: verde, limpido.

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 provette 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 provette/flaconi: 1 anno.

CONTROLLO DI QUALITÀ

Il terreno viene inoculato con i ceppi microbici indicati nella tabella CQ.
Inoculo per produttività: ≤100 UFC.
Inoculo per selettività: >100 UFC.
Condizioni di incubazione: 18-24 ore a 30-35°C (Pharmacopoeia growth promotion);

Tabella CQ.

Microrganismo		Specifiche
Escherichia coli	ATCC® 8739	Crescita buona
Pseudomonas aeruginosa	ATCC® 9027	Crescita buona
Staphylococcus aureus	ATCC® 6538	Inibizione

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 in ambito professionale 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. 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.

PRESENTAZIONE		Contenuto	Ref.
EE Broth-Mossel	Provette	Provette 20 x 10 ml	24096
EE Broth-Mossel	Flaconi (tappo a vite)	Flaconi 6 x100 ml	402480
EE Broth-Mossel	Flaconi (tappo flip-off)	Flaconi 25 x100 ml	453080
EE Broth-Mossel	Flaconi (tappo perforabile)	Flaconi 6 x 100 ml	495000
EE Broth-Mossel	Terreno disidratato	500 g di polvere	610017
EE Broth-Mossel	Terreno disidratato	100 g di polvere	620017

TABELLA DEI SIMBOLI

LOT Codice del lotto	Tenere al riparo dalla luce	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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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.
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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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MRS Agar

Terreno per l'isolamento ed il conteggio dei batteri lattici mesofili, secondo ISO 15214.

FORMULA TIPICA	(g/l)
Digerito Enzimatico di Caseina	10.0
Estratto di Carne	10.0
Estratto di Lievito	4.0
Triammonio Citrato	2.0
Sodio Acetato	5.0
Magnesio Solfato Eptaidrato	0.2
Manganese Solfato Tetraidrato	0.05
Dipotassio Idrogeno Fosfato	2.0
Glucosio	20.0
Agar	15.0
pH Finale 5.7 ± 0.1	

DESCRIZIONE

MRS Agar è un terreno utilizzato con supplementi per la coltivazione di *Lactobacillus* spp da differenti tipologie di materiale. Il terreno può supportare anche la crescita delle specie di *Pediococcus* e *Leuconostoc* così come di altri batteri secondari.

Il terreno completo soddisfa le raccomandazioni fornite da ISO 15124 ed APHA.

PRINCIPIO

Il digerito enzimatico di caseina e l'estratto di carne forniscono aminoacidi, azoto, carbonio, vitamine e minerali per la crescita dei microrganismi. L'estratto di lievito è una fonte di vitamine, sopratutto del gruppo-B. L'ammonio citrato ed il sodio acetato sono gli agenti selettivi efficaci contro streptococchi e muffe. Il pH basso inibisce inoltre la maggior parte dei microrganismi ad eccezione dei lattobacilli. I solfati di magnesio e manganese sono fonte di ioni e solfato e stimolano la crescita batterica. Il dipotassio fosfato è il tampone. Il glucosio è il carboidrato fermentabile. L'agar è l'agente solidificante.

Il supplemento Tween 80 Supplement (ref. 80031) aggiunto al terreno fornisce una miscela di esteri oleici ed acidi grassi essenziali per la crescita dei batteri lattici mesofili.

PREPARAZIONE

Sospendere 68.3 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire fino a completa dissoluzione. Aggiungere 1 ml di Tween 80 Supplement. Sterilizzare in autoclave a 121°C per 15 minuti.

Nota. Secondo ISO 15214, se si sospetta una contaminazione estesa di lieviti è possibile aggiungere 1.4 g di Acido Sorbico (sciolto il circa 10 ml di una soluzione 1 mol/l di sodio idrossido) in 1 litro di terreno sterilizzato.

TECNICA

1. Utilizzare un diluente adatto come ad esempio Buffered Peptone Water (ref. 24099) per preparare diluizioni seriali del campione al fine di ottenere piastre con un numero di colonie dalle 15 alle 300 unità.
2. Inoculare ciascuna piastra per inclusione utilizzando 1 ml della sospensione del campione. Possono essere applicati più strati se necessario.
3. Incubare a 30°C per 72 ore.

INTERPRETAZIONE DEI RISULTATI

Contare le colonie su tutte le piastre contenenti 15-300 colonie. Riportare la conta come UFC/ml del campione tenendo in considerazione il fattore di diluizione.

Può essere necessario in certi casi e per alcuni prodotti confermare le colonie avvalendosi di tecniche semplici come ad esempio la colorazione di Gram, o il test della catalasi.

CONSERVAZIONE

La polvere è fortemente igroscopica, conservare a 10-30°C, in ambiente asciutto, nel suo contenitore originale chiuso ermeticamente.. Non usare il prodotto dopo la sua data di scadenza indicata sull'etichetta o se il prodotto mostra segni di contaminazione o deterioramento. Conservare le piastre preparate a 2-8°C al riparo dalla luce.

AVVERTENZE E PRECAUZIONI

Solo per uso professionale. Gli operatori devono essere formati e avere una certa esperienza nei metodi di laboratorio. Si prega di leggere il attentamente le istruzioni prima di utilizzare questo prodotto. L'affidabilità dei risultati del test non può essere garantita in caso di deviazioni dalle istruzioni riportate in questo documento.

Consultare la scheda di sicurezza (SDS) per informazioni sui pericoli e sulle modalità di manipolazione sicure.

SMALTIMENTO DEI RIFIUTI

Lo smaltimento del prodotto deve essere effettuato secondo le vigenti regolamentazioni nazionali e locali.

RIFERIMENTI BIBLIOGRAFICI

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2. EN ISO 11133:2014. Microbiology of food, animal feed and water − Preparation, production, storage and performance testing of culture media.
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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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SPECIFICHE DI PRODOTTO

DENOMINAZIONE
MRS Agar

PRESENTAZIONE
Terreno disidratato

CONSERVAZIONE
10-30°C

CONFEZIONAMENTO

Ref.	Contenuto	Confezionamento
610024	500 g	500 g in flacone di plastica
620024	100 g	100 g in flacone di plastica

pH DEL TERRENO
5.7 ± 0.1

IMPIEGO
MRS Agar è un terreno utilizzato con supplementi per la coltivazione di batteri lattici mesofili, secondo ISO 15214

TECNICA
Fare riferimento alla scheda tecnica del prodotto

ASPETTO DEL TERRENO
Terreno in polvere
Aspetto: omogeneo, fine granulometria
Colore: beige
Terreno pronto all'uso
Aspetto: leggermente opalescente
Colore: ambra

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: 50-100 UFC
   Dimensione dell'inoculo per selettività: 10^4-10^6 UFC
   Condizioni di incubazione: 72 ± 3 h a 30 ± 1°C, in microaerofilia

Microrganismo		Crescita
Lactobacillus sakei	WDCM 00015	Buona
Lactobacillus lactis	WDCM 00016	Buona
Escherichia coli	WDCM 00012	Inibita
Bacillus cereus	WDCM 00001	Inibita

TABELLA DEI SIMBOLI

LOT Numero di lotto	Consultare le istruzioni per l'uso	Fabbricante	Data di scadenza
REF Numero di catalogo	Limiti di temperatura	Contenuto sufficiente per <n> test	Tenere al riparo dalla luce del sole

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MRS Agar

Medio para el aislamiento y contaje de bacterias mesofílicas ácido lácticas según la ISO 15214.

FÓRMULA	**(g/l)**
Digerido Enzimático de Caseína	10.0
Extracto de Carne	10.0
Extracto de Levadura	4.0
Citrato de Triamonio	2.0
Acetato Sódico	5.0
Heptahidrato de Sulfato de Magnesio	0.2
Pentahidrato de Sulfato de Manganeso	0.05
Fosfato de Hidrógeno Dipotasio	2.0
Glucosa	20.0
Agar	15.0
pH final 5.7 ± 0.1 a 25°C	

DESCRIPCIÓN

MRS Agar es un medio utilizado con suplementos para el cultivo de *Lactobacillus* spp a partir de diferentes tipos de material. Puede potenciar el crecimiento de especies de *Pediococcus* y *Leuconostoc* y otras bacterias secundarias.

El medio sigue las recomendaciones de la ISO 15214 y APHA.

PRINCIPIO DEL MÉTODO

El digerido enzimático de caseína y los extractos de carne y levadura proporcionan aminoácidos, nitrógeno, carbono, vitaminas y minerales necesarios para el crecimiento de los microorganismos. El extracto de Levadura es una fuente de vitaminas, especialmente del grupo B. El citrato de amonio y el acetato de sodio son los agentes selectivos contra los Estreptococos y los hongos. El bajo pH inhibe a la mayoría de los microorganismos que no sean lactobacilos. Los sulfatos de magnesio y manganeso suministran los iones necesarios para la estimulación del crecimiento. El fosfato de dipotasio es el agente tampón. La glucosa es el carbohidrato fermentable. El agar es el agente solidificante.

El suplemento Tween 80 (ref. 80031) suministra una mezcla de ésteres oléicos y ácidos grasos esenciales para el crecimiento de las bacterias ácido lácticas.

PREPARACIÓN

Suspender 68.3 g del polvo deshidratado en 1 litro de agua destilada o desionizada. Mezclar bien. Calentar hasta la ebullición removiendo frecuentemente hasta la completa disolución. Añadir 1 ml de suplemento Tween 80. Esterilizar en autoclave a 121°C durante 15 minutos.

Nota. Según la ISO 15214, 1.4 g de ácido ascórbico (disuelto en 10 ml de una solución de hidróxido de sodio 1 mol/L) pueden añadirse a 1 litro de medio esterilizado en caso de sospecha de una amplia contaminación de levaduras.

TÉCNICA

1. Utilizar un diluyente apropiado como el Buffered Peptone Water (ref. 24099) para realizar diluciones en serie de la muestra para obtener un contaje de colonias de entre 15 y 300 colonias por placa.
2. Inocular cada placa con 1 ml de muestra en suspensión vertiendola sobre dicha placa. Si es necesario se pueden añadir más estratos.
3. Incubar a 30°C durante 72 horas.

INTERPRETACIÓN DE LOS RESULTADOS

Contar las colonias en todas las placas que contengan entre 15-300 colonias. Informar del contaje como CFU/ml por muestra, permitiendo el uso de factores de dilución.

En algunos casos puede ser necesario una confirmación de las colonias a través de técnicas sencillas como la coloración Gram, o el test de la catalasa.

ALMACENAMIENTO

El polvo deshidratado es muy higroscópico, almacenar a 10-30°C, en un entorno seco, en su frasco original correctamente cerrado. Almacenar las placas preparadas a 2-8°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.

ADVERTENCIAS Y PRECAUCIONES

Solo para uso profesional. Los operadores deben estar capacitados y tener cierta experiencia en los métodos de laboratorio. Lea las instrucciones cuidadosamente antes de usar este producto. No se puede garantizar la confiabilidad de los resultados del ensayo si existen desviaciones de los instrucciones de este documento.

Consulte la Hoja de datos de seguridad (SDS) para obtener información sobre los peligros y las prácticas de manipulación segura.

DESECHO DE RESÍDUOS

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

REFERENCIAS

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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ESPECIFICACIONES DEL PRODUCTO

NOMBRE
MRS Agar

APARIENCIA
Medio deshidratado

ALMACENAMIENTO
10-30°C

PRESENTACIÓN

Ref.	Contenido	Empaquetado
610024	500 g	500 g de polvo deshidratado en frasco de plástico
620024	100 g	100 g de polvo deshidratado en frasco de plástico

pH DEL MEDIO
5.7 ± 0.1

USO
MRS Agar es un medio para el aislamiento y contaje de bacterias mesofílicas ácido lácticas, de acuerdo a la ISO15214

TÉCNICA
Observar la hoja técnica del producto

ASPECTO DEL MEDIO
Medio deshidratado
Aspecto: suelto, homogéneo
Color: beige
Placas listas para su uso
Aspecto: ligeramente opalescente
Colour: ámbar

VIDA ÚTIL
4 años

CONTROL DE CALIDAD
1. Control de características generales, etiqueta e impresión
2. Control microbiológico
   Inóculo de productividad: 50-100 CFU
   Inóculo de selectividad: 10^4-10^6 CFU
   Condiciones de incubación: 72 ± 3 h a 30 ± 1°C, in microaerobiósis

Microorganismo		Crecimiento
Lactobacillus sakei	WDCM 00015	Bueno
Lactobacillus lactis	WDCM 00016	Bueno
Escherichia coli	WDCM 00012	Inhibición
Bacillus cereus	WDCM 00001	Inhibición

TABLA DE SÍMBOLOS

LOT Código de lote	Consultar instrucciones de uso	Fabricante	Utilizar antes de
REF Número de catálogo	Límites de temperatura	Contenido suficiente para <n> análisis	Mantener alejado de la luz solar

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VitalScientific
Diagnostics for Life

ELICAL 2

CALI
CALI-0550 : 4 x 3 mL
CALI-1550 : 3 mL

FTCE-CALI2-5-v9 (01/2025)

CE 0459

Français - FR

USAGE PRÉVU
Ce dispositif de diagnostic *in vitro* est destiné à la calibration des tests quantitatifs listés dans la fiche de valeurs.
Ce dispositif de diagnostic *in vitro* est uniquement destiné aux professionnels.

COMPOSITION
- Produit sous forme lyophilisée préparé à partir de sérum humain contenant des additifs chimiques et biologiques.
- Azide de sodium < 0.1 % (p/p)
- Les concentrations pour chaque analyte à doser sont spécifiques à chaque lot.

☛MATÉRIELS REQUIS MAIS NON FOURNIS
- Réactifs VitalScientific listés dans la fiche de valeurs.
- Equipement général de laboratoire.

TRACABILITE
La traçabilité est indiquée dans la fiche de valeurs incluse dans le coffret.

☛PRÉCAUTIONS D'EMPLOI ET MISES EN GARDE
- Consulter la fiche de données de sécurité (FDS) pour une manipulation appropriée.
- Chaque unité de sang humain utilisée pour la fabrication de ces produits a été testée et trouvée négative/non-réactive pour la présence d'HBsAg, HCV et HIV1/2. Les méthodes utilisées étaient approuvées par la FDA ou en conformité avec la réglementation CE. Cependant le risque d'infection ne pouvant être exclu avec certitude par aucune méthode, ces produits doivent être manipulés comme étant potentiellement infectieux. En cas d'exposition, suivre les directives des autorités sanitaires compétentes.
- Prendre des précautions lors de la manipulation de flacons de verre brisés, car les bords tranchants peuvent blesser l'utilisateur.
- Ces produits contiennent de l'azide de sodium qui peut réagir avec le plomb ou le cuivre et former des azides métalliques potentiellement explosifs. Lors de l'élimination de ces réactifs toujours rincer abondamment avec de l'eau pour éviter l'accumulation d'azides.
- Respecter les précautions d'usage et les bonnes pratiques de laboratoire.
- Utiliser du matériel de laboratoire propre ou à usage unique afin d'éviter toute contamination.

TRAITEMENT DES DÉCHETS
L'élimination de tous les déchets doit être effectuée conformément aux exigences réglementaires locales, d'état et fédérales (veuillez-vous référer à la fiche de données de sécurité (FDS)).

PRÉPARATION
- Ouvrir avec précaution le flacon en évitant la perte de poudre lyophilisée.
- Ajouter **précisément 3 mL d'eau distillée ou désionisée**.
- Reboucher le flacon avec soin et dissoudre le contenu complètement dans les 30 minutes en remuant délicatement le flacon et en évitant la formation de mousse.

DÉTÉRIORATION DU PRODUIT
- Le produit peut présenter un aspect légèrement trouble après reconstitution. Cela n'a aucun effet sur les performances du produit. Toute présence de particules serait le signe d'une détérioration.
- Ne pas utiliser le produit s'il y a des signes évidents de détérioration biologique ou chimique (ex : particules après reconstitution).
- Un flacon endommagé peut avoir un impact sur les performances du produit. Ne pas utiliser le produit si les flacons présentent des signes physiques de détérioration (par exemple, fuite).

STABILITÉ
Avant reconstitution :
- Stocker à 2-8 °C et à l'abri de la lumière.
- Ne pas utiliser après la date d'expiration indiquée sur l'étiquette du flacon.

Après reconstitution :
- Ces produits doivent être immédiatement et correctement refermés afin d'éviter toute contamination ou évaporation.

- Stabilité des constituants :
Entre 15 et 25 °C : 8 heures
Entre 2 et 8 °C : 2 jours
Entre -25 et -15 °C : 4 semaines (ne congeler qu'une seule fois)

Exceptions :
- Stabilité de la bilirubine totale (à conserver à l'abri de la lumière) :
Entre 15 et 25 °C : 6 heures
Entre 2 et 8 °C : 1 jour
Entre -25 et -15 °C : 2 semaines (ne congeler qu'une seule fois)
- Stabilité de la bilirubine directe (à conserver à l'abri de la lumière) :
Entre 15 et 25 °C : 3 heures
Entre 2 et 8 °C : 8 heures
Entre -25 et -15 °C : 2 semaines (ne congeler qu'une seule fois)

***Note** : Conserver les flacons bien fermés et à l'abri de la lumière.*

PROCÉDURE
Pour utiliser ELICAL 2, suivre la procédure décrite dans la fiche technique du réactif utilisé.

☛LIMITATIONS
L'utilisation de ELICAL 2 a été validée avec les systèmes VitalScientific (Automates et réactifs listés dans la fiche de valeurs).
L'utilisation sur un autre système doit être validée par le laboratoire.

VALEURS
Les concentrations sont indiquées dans la fiche de valeurs incluse dans le coffret.
Pour les utilisateurs d'automates Selectra permettant l'import de tests, de calibrants et de contrôles, utiliser le code barre correspondant, disponible sur la fiche de valeurs.

DECLARATION DES INCIDENTS GRAVES
Veuillez notifier au fabricant (par l'intermédiaire de votre distributeur) et à l'autorité compétente de l'Etat membre de l'union européenne dans lequel l'utilisateur et/ou le patient est établi, les cas d'incident grave survenu en lien avec le dispositif.
Pour les autres juridictions, la déclaration d'incident grave doit être effectuée conformément aux exigences réglementaires locales, d'état et fédérales.
En signalant les incidents graves, vous contribuez à fournir davantage d'informations sur la sécurité des dispositifs médicaux de diagnostic *in vitro*.

☛ASSISTANCE TECHNIQUE
Contacter votre distributeur local ou VitalScientific (support@vitalscientific.com).

CH REP
Axon Lab AG
Täfernstrasse 15
CH-5405 Baden-Dättwil
SWITZERLAND

English - EN

INTENDED USE
This *in vitro* diagnostic device is intended for the calibration of quantitative tests listed in the value sheet.
This *in vitro* diagnostic device is for professional use only.

COMPOSITION
- Lyophilized product prepared from human serum spiked with chemical and biological additives.
- Sodium azide < 0.1 % (w/w)
- Concentrations for each analyte to test are lot-specific.

☛MATERIALS REQUIRED BUT NOT PROVIDED
- VitalScientific reagents listed in the value sheet.
- General Laboratory equipment.

TRACEABILITY
The traceability is indicated in the value sheet enclosed in the kit.

☛PRECAUTIONS FOR USE AND WARNINGS
- Consult Safety Data Sheet (SDS) for a proper handling.
- Each unit of human blood used in the manufacture of these products was tested and found to be negative/non-reactive for the presence of HbsAg, HCV and HIV1/2. The methods used were FDA approved or CE compliant. Nevertheless, since the risk of infection cannot be fully excluded these products must be handled as potentially infectious. In case of exposure, follow the guidelines of the competent health authorities.
- Take precautions when handling broken glass vials as sharp edges can injure the user.
- These products contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these reagents always flush with copious amounts of water to prevent azide buildup.
- Take normal precautions and adhere to good laboratory practice.
- Use clean or single use laboratory equipment only to avoid contaminations.

WASTE MANAGEMENT
Disposal of all waste material should be in accordance with local, state and federal regulatory requirements (please refer to the Safety Data Sheet (SDS)).

PREPARATION
- Carefully open the vial, avoiding the loss of lyophilizate.
- Add **in exactly 3 mL of distilled or deionized water**.
- Carefully close the vial and dissolve the contents completely within 30 minutes by occasional gentle stirring avoiding the formation of foam.

PRODUCT DETERIORATION
- The product may present a slightly hazy appearance after reconstitution. This has no effect on the performances of the product. All presence of particules would indicate deterioration.
- Do not use the product if there is visible evidence of contamination or damage (e.g. particle matter after reconstitution).
- Damage to the container may impact on product performance. Do not use the product if there is physical evidence of deterioration (e.g. leakages).

STABILITY
Prior to reconstitution :
- Stored at 2-8 °C and protected from light.
- Do not use after expiration date indicated on the vial label.

After reconstitution :
- These products should be immediately and tightly capped to prevent contamination and evaporation.

- Stability of the components:
Between 15 and 25 °C : 8 hours
Between 2 and 8 °C : 2 days
Between -25 and - 15 °C : 4 weeks (when frozen once)

Exceptions:
- Stability of total bilirubin (when stored protected from light):
Between 15 and 25 °C : 6 hours
Between 2 and 8 °C : 1 day
Between -25 and -15 °C : 2 weeks (When frozen once)
- Stability of direct bilirubin (when stored protected from light):
Between 15 and 25 °C : 3 hours
Between 2 and 8 °C : 8 hours
Between -25 and -15 °C : 2 weeks (when frozen once)

***Note**: Store vials tightly capped and protected from light.*

PROCEDURE
To use ELICAL 2, follow the procedure described in the instructions for use of the reagent used.

☛LIMITATIONS
Using ELICAL 2 has been validated with the VitalScientific systems (Analyzers and reagents listed in the value sheet).
Using any other system should be validated by the laboratory.

VALUES
The concentrations are indicated in the value sheet enclosed in the kit.
For users of Selectra instruments allowing data import for tests, calibrators and controls, use corresponding barcode, available on the value sheet.

DECLARATION OF SERIOUS INCIDENT
Please notify the manufacturer (through your distributor) and competent authority of the Member State of the european union in which the user and/or the patient is established, of any serious incident that has occurred in relation to the device. For other jurisdictions, the declaration of serious incident should be in accordance with local, state and federal regulatory requirements. By reporting a serious incident, you provide information that can contribute to the safety of *in vitro* medical devices.

☛TECHNICAL ASSISTANCE
Contact your local distributor or VitalScientific (support@vitalscientific.com).

Español - ES

USO PREVISTO
Este dispositivo de diagnóstico *in vitro* está diseñado para la calibración de las pruebas cuantitativas listadas en la hoja de valores.
Este dispositivo de diagnóstico *in vitro* esta destinado unicamente para los profesionales.

COMPOSICIÓN
- Producto liofilizado preparado a partir de suero humano enriquecido con aditivos químicos y biológicos.
- Azida sódica < 0.1 % (p/p)
- Las concentraciones de cada analito a analizar son específicas a cada lote.

☛MATERIALES REQUERIDOS PERO NO INCLUIDOS
- Reactivos VitalScientific listados en la hoja de valores.
- Equipo de laboratorio de uso general.

TRAZABILIDAD
La trazabilidad se indica en la ficha de valores incluida en el kit.

☛PRECAUCIONES DE USO Y ADVERTENCIAS
- Consulte la Hoja de Datos de Seguridad (SDS) para un manejo adecuado.
- Cada unidad de sangre humana utilizada en la fabricación de estos productos fue analizada y resultó ser negativa / no reactiva ante la presencia de HbsAg, VHC y VIH1/2. Los métodos utilizados fueron aprobados de conformidad por la FDA o con la reglamentación CE. Sin embargo, dado que el riesgo de infección no puede excluirse por completo, estos productos deben manejarse como potencialmente infecciosos. En caso de exposición, siga las indicaciones de las autoridades sanitarias competentes.
- Tenga precauciones al manipular viales de vidrio roto, ya que los bordes afilados pueden dañar al usuario.
- Los productos contienen azida sódica que puede reaccionar con el plomo o el cobre de la tubería y potencialmente formar azidas metálicas explosivas. Cuando se eliminen los reactivos, enjuague con agua abundantemente para prevenir la acumulación de azidas.
- Respetar las precauciones de uso y las buenas prácticas de laboratorio.
- Para evitar contaminación utilizar equipo nuevo o completamente limpio.

TRATAMIENTO DE LOS RESIDUOS
Todos los materiales de desecho deben eliminarse de acuerdo con los requisitos regulatorios locales, estatales y federales (diríjase a la hoja de seguridad (SDS)).

PREPARACIÓN
- Destapar cuidadosamente el frasco con el fin de evitar la pérdida de material liofilizado.
- Agregar **exactamente 3 mL de agua destilada o de agua desionizada.**
- Cierre cuidadosamente el vial y disuelva el contenido por completo durante 30 minutos mezclando suavemente para evitar la formación de espuma.

DETERIORACIÓN DEL PRODUCTO
- El producto puede presentar un aspecto ligeramente turbio después de reconstitución, esto no afecta el rendimiento del producto. La presencia de partículas es un signo de deterioro.
- No utilice el producto si este presenta signos evidentes de contaminación o deterioro (p. ej, partículas después de reconstitución).
- Un frasco dañado puede tener un impacto en el rendimiento del producto. No utilice el producto si este tiene signos físicos de deterioro (p. ej, fugas).

ESTABILIDAD
Antes de la reconstitución :
- Conservar a 2-8 °C y protegidos de la luz.
- No utilice después de la fecha de caducidad indicada en la etiqueta del frasco.

Después de reconstitución :
- Estos productos deben ser bien cerrados de inmediato para evitar contaminación y evaporación.

- Estabilidad de los componentes:
Entre 15 y 25 °C : 8 horas
Entre 2 y 8 °C : 2 días
Entre -25 y -15 °C : 4 semanas (no congelar más de una vez)

Excepciones:
- Estabilidad de la bilirrubina total (proteger de la luz) :
Entre 15 y 25 °C : 6 horas
Entre 2 y 8 °C : 1 día
Entre -25 y -15 °C : 2 semanas (no congelar más de una vez)

- Estabilidad de la bilirrubina directa (proteger de la luz) :
Entre 15 y 25 °C : 3 horas
Entre 2 y 8 °C : 8 horas
Entre -25 y -15 °C : 2 semanas (no congelar más de una vez)

Nota : Conservar los frascos bien cerrados y protegidos de la luz.

PROCEDIMIENTO
Para utilizar ELICAL 2, siga el procedimiento descrito en el inserto del reactivo utilizado.

☛LIMITACIONES
El uso de ELICAL 2 ha sido validado con los sistemas VitalScientific (equipos y reactivos enumerados en la hoja de valores).
El uso de cualquier otro sistema debe ser validado por el laboratorio.

VALORES
Las concentraciones son indicadas en la ficha de valores incluida en el kit.
Para usuarios de los equipos Selectra que permiten la importación de pruebas, calibradores y controles, use el código de barras correspondiente, disponible en la hoja de valores.

DECLARACIÓN DE INCIDENTES GRAVES
Por favor notifique al fabricante (por medio de su distribuidor) y autoridad competente del Estado miembro de la Unión Europea en donde el usuario o paciente radique, de cualquier incidente grave que se produzca con relación al dispositivo.
Para otras jurisdicciones, la declaración de incidentes graves debe realizarse
de acuerdo con los requisitos reglamentarios locales, estatales y federales.
Reportando incidentes graves usted contribuye a proporcionar más información sobre la seguridad del dispositivo médico de diagnóstico *in vitro*.

☛ASISTENCIA TÉCNICA
Contacte a su distribuidor local o con VitalScientific (support@vitalscientific.com).

Português - PT

UTILIZAÇÃO PREVISTA
Este dispositivo de diagnóstico *in vitro* é destinado a calibração dos testes quantitativos listados na folha de valores.
Este dispositivo de diagnóstico *in vitro* é apenas para uso profissional.

COMPOSIÇÃO
- Produto liofilizado preparado a partir de soro humano enriquecido com aditivos químicos e biológicos.
- Azida de sódio < 0.1 % (p/p)
- As concentrações de cada analito a ser testado são específicas de cada lote.

☛MATERIAIS NECESSÁRIOS MAS NÃO FORNECIDOS
- Reagentes VitalScientific listados na folha de valores.
- Equipamento geral de laboratório.

RASTREABILIDADE
A rastreabilidade é indicada na folha de valores incluída no kit.

☛PRECAUÇÕES DE USO E AVISOS
- Consulte a ficha de dados de segurança (SDS) para obter um manuseio adequado.
- Cada unidade de sangue humano utilizada na fabricação desses produtos foi testada e se mostrou negativa / não reativa para a presença de HbsAg, HCV e HIV1 / 2. Os métodos utilizados foram aprovados e liberados pela FDA em conformidade com a regulamentação EC. No entanto, como o risco de infecção não pode ser totalmente excluído, esses produtos devem ser manuseados como potencialmente infecciosos. Em caso de exposição, siga as orientações das autoridades sanitárias competentes.
- Tome precauções ao manusear frascos de vidro quebrados, pois bordas afiadas podem ferir o usuário.
- Os produtos contém azida de sódio que pode reagir com o chumbo ou cobre das canalizações formando azidas metálicas explosivas. Ao manusear estes reagentes lave as mãos sempre com grandes quantidades de água para evitar a produção de azida.
- Respeitar as precauções de utilização e as boas práticas de laboratório.
- Utilizar material de laboratório limpo ou destinado a uma única utilização de modo a evitar qualquer contaminação.

TRATAMENTO DOS RESÍDUOS
O descarte de todo material residual deve estar de acordo com os requisitos regulamentares locais, estaduais e federais (consulte a Ficha de dados de segurança (SDS)).

PREPARAÇÃO
- Abrir cuidadosamente o frasco, evitando a perda de pó liofilizado.
- Acrescentar **exatamente 3 mL de água destilada ou deionizada**.
- Feche cuidadosamente o frasco para injetáveis e dissolva completamente o conteúdo dentro de 30 minutos, mexendo suave e ocasionalmente, evitando a formação de espuma.

DETERIORAÇÃO DO PRODUTO
- O produto pode apresentar uma aparência um pouco turva após a reconstituição. Isto não tem efeito sobre o desempenho do produto. A presença de partículas indica deterioração.
- Não use o produto se houver evidência visível de contaminação ou dano (por exemplo, partículas após a reconstituição).
- Não utilizar o calibrador caso haja danos na embalagem que possam causar algum efeito sobre o desempenho do produto (ex. vazamentos).

ESTABILIDADE
Antes da reconstituição:
- Conservado a 2-8 °C e ao abrigo da luz.
- Não utilizar após as datas de validade indicadas nos rótulos
dos frascos.

Após a reconstituição:
- Esses produtos devem ser tampados imediatamente e firmemente para evitar contaminação e evaporação.

- Estabilidade dos constituintes:
Entre 15 e 25 °C : 8 horas
Entre 2 e 8 °C : 2 dias
Entre -25 e -15 °C : 4 semanas (congelar apenas uma única vez)

Excepções:
- Estabilidade da bilirrubina total (a conservar ao abrigo da luz) :
Entre 15 e 25 °C : 6 horas
Entre 2 e 8 °C : 1 dia
Entre -25 e -15 °C : 2 semanas (congelar apenas uma única vez)
- Estabilidade da bilirrubina direta (a conservar ao abrigo da luz) :
Entre 15 e 25 °C : 3 horas
Entre 2 e 8 °C : 8 horas
Entre -25 e -15 °C : 2 semanas (congelar apenas uma única vez)

***Observação**: Conservar os frascos bem fechados e ao abrigo da luz.*

PROCEDIMENTO
Para usar ELICAL 2, siga o procedimento descrito nas instruções de uso do reagente utilizado.

☛LIMITAÇÕES
O uso de ELICAL 2 foi validado com o sistema VitalScientific (analisador e reagentes listados na folha de valores).
O uso de qualquer outro sistema deve ser validado pelo laboratório.

VALORES
As concentrações são indicadas na folha de valores colocada no kit.
Para usuários de instrumentos Selectra que permitem a importação de dados para testes, calibradores e controles, se o código de barras correspondente, disponível na folha de valores.

DECLARAÇÃO DE INCIDENTE GRAVE
Notifique o fabricante (através do seu distribuidor) e a autoridade competente da União Européia em que o usuário e/ou o paciente está estabelecido, de qualquer incidente grave que tenha ocorrido em relação ao dispositivo.
Para outras jurisdições, a declaração de incidente grave deve estar de acordo com as normas locais, requisitos regulatórios estaduais e federais.
Ao relatar um incidente grave, você fornece informações que podem contribuir para o segurança de dispositivos médicos *in vitro*.

☛ASSISTÊNCIA TÉCNICA
Entre em contato com o seu distribuidor local ou com a VitalScientific (support@vitalscientific.com).

Ελληνικά - EL

ΠΡΟΒΛΕΠΟΜΕΝΗ ΧΡΗΣΗ
Τα εν λόγω *in vitro* διαγνωστικά προορίζονται για τον ποιοτικό προσδιορισμό της απόδοσης των ποσοτικών τεστ της καταγεγραμμένα στο φύλλο τιμών.
Τα εν λόγω *in vitro* διαγνωστικά προορίζονται μόνο για επαγγελματική χρήση.

ΣΥΣΤΑΣΗ
- Τα λυοφιλοποιημένα προϊόντα που ετοιμάζονται για ανθρώπινο ορό είναι δεσμευμένα με χημικά και βιολογικά πρόσθετα.
- Αζίδια του νατρίου < 0.1 % (w/w)
- Οι ελεγχόμενες συγκεντρώσεις για κάθε αναλυτή είναι συγκεκριμένες σύμφωνα με την παρτίδα.

☛ΑΠΑΙΤΟΥΜΕΝΑ ΥΛΙΚΑ ΠΟΥ ΔΕΝ ΣΥΜΠΕΡΙΛΑΜΒΑΝΟΝΤΑΙ
- Αντιδραστήρια VitalScientific της καταγεγραμμένα στο φύλλο τιμών.
- Γενικός Εργαστηριακός Εξοπλισμός.

ΙΧΝΗΛΑΣΙΜΟΤΗΤΑ
Η ιχνηλασιμότητα ορίζεται στο φύλλο τιμών που συμπεριλαμβάνεται στο κιτ.

☛ΠΡΟΦΥΛΑΞΕΙΣ ΧΡΗΣΗΣ ΚΑΙ ΠΡΟΕΙΔΟΠΟΙΗΣΕΙΣ
- Συμβουλευτείτε το Δελτίο Δεδομένων Ασφαλείας (SDS) για σωστό χειρισμό.
- Κάθε μονάδα ανρθρώπινου αίματος που χρησιμοποιείται για την Παρασκευή αυτών των προϊόντων έχει ελεγχθεί και βρεθεί αρνητική/ μη-αντιδρούσα στην παρουσία HbsAg, HCV και HIV1/2. Οι μέθοδοι που χρησιμοποιούνται είναι εγκεκριμένες από τον FDA ή σύμφωνες με την ρυθμίσεως CE. Μολονότι και εφόσον ο κίνδυνος μόλυνσης δεν μπορεί να αποκλειστεί εντελώς, τα εν λόγω προϊόντα πρέπει να χειρίζονται ως πιθανώς μολυσματικά. Σε περίπτωση έκθεσης, ακολουθείστε τις οδηγίες των αρμόδιων υγειονομικών αρχών.
- Πάρτε προφυλάξεις όταν χειρίζεστε σπασμένα γυάλινα φιαλίδια καθώς οι αιχμηρές γωνίες μπορεί να τραυματίσουν τον χρήστη.
- Τα συγκεκριμένα προϊόντα περιέχουν αζίδια του νατρίου που μπορεί να αντιδράσει με το χαλκό ή το μόλυβδο των σωληνώσεων δημιουργώντας πιθανά εκρηκτικά αζωτούχα μέταλλα. Όταν απορρίπτονται στην αποχέτευση τα αντιδραστήρια αυτά, να ξεπλένετε πάντοτε με άφθονο νερό για την αποφυγή συσσώρευσης αζιδίων.
- Λάβετε συνήθεις προφυλάξεις και εφαρμόστε καλή εργαστηριακή πρακτική.
- Χρησιμοποιήστε μόνο καθαρά ή μίας χρήσης εργαστηριακά σκεύη για την αποφυγή επιμολύνσεων.

ΔΙΑΧΕΙΡΙΣΗ ΑΠΟΒΛΗΤΩΝ
Απόρριψη όλων των αποβλήτων πρέπει να γίνεται σύμφωνα με τις τοπικές, εθνικές και ομοσπονδιακές ρυθμιστικές απαιτήσεις (παρακαλώ ανατρέξτε στο Δελτίο Δεδομένων Ασφαλείας (SDS)).

ΠΡΟΕΤΟΙΜΑΣΙΑ
- Χτυπήστε απαλά την φιάλη ώστε το υλικό ελέγχου να βρίσκεται στο κάτω μέρος της φιάλης.
- Ανοίξτε προσεκτικά το φιαλίδιο, αποφεύγοντας την απώλεια της λυοφιλιωμένης ουσίας.
- Προσθέστε **ακριβώς 3 mL απεσταγμένου/ απιονισμένου νερού**.
- Κλείστε το φιαλίδιο προσεκτικά και διαλύστε εντελώς το περιεχόμενο εντός 30 λεπτών με ελαφρύ ανακάτεμα αποφεύγοντας την δημιουργία αφρού.

ΑΛΛΟΙΩΣΗ ΤΟΥ ΠΡΟΪΟΝΤΟΣ
- Μετά την ανασύσταση : Ο βαθμονομητής μπορεί να παρουσιάζει μία ελαφρά θολή εμφάνιση. Αυτή δεν έχει καμία επίδραση στην απόδοση του προϊόντος. Η παρουσία μεριδίων μπορεί να υποδεικνύει καταστροφή.
- Μην χρησιμοποιείτε το προϊόν αν υπάρχει εμφανής ένδειξη μόλυνσης ή αλλοίωσης (πχ σωματίδια μετά την ανασύσταση).
- Αλλοίωση στο φιαλίδιο μπορεί να έχει επίδραση στην απόδοση του προϊόντος. Να μην χρησιμοποιείται το προϊόν αν υπάρχει εμφανής απόδειξη αλλοίωσης (πχ διαρροή)

ΣΤΑΘΕΡΟΤΗΤΑ
Πριν την ανασύσταση:
- Αποθήκευση στους 2-8ο C μακριά από το φως.
- Να μην χρησιμοποιείται πέραν των ημερομηνιών λήξης που
αναγράφονται στις ετικέτες των φιαλιδίων.

Μετά την ανασύσταση:
- Μη χρησιμοποιείτε μετά τις ημερομηνίες λήξης που αναφέρονται στις ετικέτες των φιαλιδίων.

- Σταθερότητα των συστατικών:
Στους 15 έως 25ο C : 8 ώρες
Στους 2 έως 8ο C : 2 ημέρες
Στους -25 έως -15ο C : 4 εβδομάδες (μία φορά κατάψυξη)

Εξαιρέσεις:
- Σταθερότητα ολικής χολερυθρίνης (όταν αποθηκεύεται μακριά από φως)
Στους 15 έως 25ο C : 6 ώρες
Στους 2 έως 8ο C : 1 ημέρα
Στους -25 έως -15ο C : 2 εβδομάδες (μία φορά κατάψυξη)
-Σταθερότητα άμεσης χολερυθρίνης (όταν αποθηκεύεται μακριά από φως)
Στους 15 έως 25ο C : 3 ώρες
Στους 2 έως 8ο C : 8 ώρες
Στους -25 έως -15ο C : 2 εβδομάδες (μία φορά κατάψυξη)

***Σημείωση**: Αποθηκεύστε τα φιαλίδια ερμητικά πωματισμένα και προστατευμένα από το φως όταν δε χρησιμοποιούνται.*

ΔΙΑΔΙΚΑΣΙΑ
Για την χρήση ELICAL 2, ακολουθείστε την περιγραφόμενη διαδικασία στις οδηγίες χρήσης του αντιδραστηρίου της που χρησιμοποιείται.

☛ΠΕΡΙΟΡΙΣΜΟΙ
Την χρήση ELICAL 2 έχει επικυρωθεί με τα συστήματα της VitalScientific (Αναλυτές και αντιδραστήρια είναι καταγεγραμμένα στο φύλλο τιμών).
Η χρήση οποιουδήποτε άλλου συστήματος πρέπει να επικυρωθεί από το εργαστήριο.

ΤΙΜΕΣ
Οι συγκεντρώσεις υποδεικνύονται στο φύλλο τιμών μου συμπεριλαμβάνεται στο κιτ.
Για να επιτρέπεται η εισαγωγή δεδομένων τέστ, βαθμονομητών και ορών ελέγχου στους χρήστες μηχανημάτων Selectra, χρησιμοποιείστε τον ανάλογο γραμμικό κώδικα διαθέσιμο στο φύλλο τιμών.

ΔΗΛΩΣΗ ΣΟΒΑΡΟΥ ΑΤΥΧΗΜΑΤΟΣ
Παρακαλώ ενημερώστε τον κατασκευαστή (μέσω του διανομέα σας) και τις αρμόδιες αρχές του Κράτους Μέλους της ευρωπαϊκής ένωσης στον οποίο ο χρήστης ή/και ο ασθενής είναι εγκατεστημένος, για κάθε σοβαρό ατύχημα που μπορεί να συμβεί σε σχέση με το μηχάνημα. Για λοιπές δικαιοδοσίες, η δήλωση σοβαρού ατυχήματος πρέπει να συμφωνεί με τις τοπικές, εθνικές και ομοσπονδιακές ρυθμιστικές απαιτήσεις. Αναφέροντας ένα σοβαρό ατύχημα, παρέχετε πληροφορίες που μπορεί να συμβάλλουν στην ασφάλεια των *in vitro* ιατρικών μηχανημάτων.

☛ΤΕΧΝΙΚΗ ΒΟΗΘΕΙΑ
Επικοινωνήστε με τον τοπικό σας διανομέα ή την VitalScientific (support@vitalscientific.com).

☛SYMBOLES/ SYMBOLS/ SÍMBOLOS/ ΣΥΜΒΟΛΑ
- Les symboles utilisés sur notre documentation sont décrits dans la norme ISO-15223-1 hormis certains présentés dans le glossaire de symboles disponible sur le site Web VitalScientific (Symbols glossary).

- Symbols used on our documentation are defined on ISO-15223-1 standard, except for some presented in the symbols glossary available on the VitalScientific Website. (Symbols glossary).

- Los símbolos utilizados en nuestra documentación están definidos en la norma ISO-15223-1, excepto algunos presentados en el glosario de símbolos disponible en el sitio web VitalScientific (Symbols glossary).

- Os símbolos utilizados em nossa documentação são definidos na norma ISO-15223-1, exceto alguns apresentados no glossário de símbolos disponível no site Web da VitalScientific. (Symbols glossary).

- Τα σύμβολα που χρησιμοποιούνται στα έγγραφα ορίζονται στο πρότυπο ISO-15223-1, εκτός κάποιων που παρουσιάζονται στο γλωσσάρι συμβόλων στην ιστοσελίδα της VitalScientific (Symbols glossary).

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

Istruzioni per l'uso
ITALIANO

Nutrient Agar ISO 16266

Terreno per la coltivazione di microrganismi non esigenti e
la conferma di *Pseudomonas aeruginosa*, secondo ISO 16266.

DESCRIZIONE

Nutrient Agar ISO 16266 è un terreno utilizzato per la coltivazione di microrganismi non esigenti da campioni di acqua.

Questo terreno è formulato secondo ISO 16266 per la ricerca ed il conteggio di *Pseudomonas aeruginosa* nell'acqua con la tecnica delle membrane filtranti.

FORMULA TIPICA*	(g/litro)
Peptone	5.0
Estratto di Carne	1.0
Estratto di Lievito	2.0
Sodio Cloruro	5.0
Agar	15.0
pH Finale 7.4 ± 0.2 a 25°C	

*Adattata e/o integrata con supplementi per soddisfare le specifiche di performance richieste

PRINCIPIO DEL METODO

Il peptone e l'estratto di carne forniscono aminoacidi, azoto, carbonio, vitamine e minerali per la crescita degli organismi. L'estratto di lievito è una fonte di vitamine, sopratutto del gruppo-B. Il sodio cloruro mantiene il bilancio osmotico del terreno. L'agar è l'agente solidificante.

PREPARAZIONE

Terreno disidratato	Sospendere 28 g di polvere in 1 litro di acqua distillata o deionizzata sterile Mescolare bene. Riscaldare agitando di frequente e bollire fino a 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 i tappi leggermente svitati) 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

Secondo ISO 16266, trasferire la membrana e le colonie presuntive di *Pseudomonas aeruginosa* sul terreno in piastra.

Incubare a 36 ± 2°C per 20-24 ore in atmosfera aerobica.

In alternativa il terreno può essere inoculato per spatolamento o strisciando direttamente il campione sulla superficie dell'agar.

INTERPRETAZIONE DEI RISULTATI

Osservare le colonie sviluppate sul terreno. Confermare *P. aeruginosa* con il test dell'ossidasi (ref. 88029).

CONSERVAZIONE

La polvere è fortemente igroscopica, conservare a 10-30°C, in ambiente asciutto, nel suo contenitore originale chiuso ermeticamente. Conservare i flaconi, le provette 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.

Evitare rapidi cambiamenti di temperatura del terreno in piastre per prevenire la formazione di condensa.

VALIDITÀ

Terreno disidratato: 4 anni.
Terreno in flaconi: 2 anni.
Terreno in provette a becco di clarino: 1 anno.
Piastre pronte all'uso: 6 mesi.

CONTROLLO DI QUALITÀ

Aspetto del Terreno Disidratato: Omogeneo, fine granulometria, beige.

Aspetto del Terreno Preparato: Ambra, leggermente opalescente.

Risultati Attesi dei Test Microbiologici:

Ceppo di controllo		Inoculo	Incubazione	Specifiche
Pseudomonas aeruginosa	WDCM 00025 (ATCC 27853, NCTC 12903)	50-100 UFC	20-24 h 36 ± 1°C	Crescita buona
Escherichia coli	WDCM 00013 (ATCC 25922, NCTC 12241)			

Fare riferimento al certificato di analisi (CoA) relativo al lotto effettivo.

AVVERTENZE E PRECAUZIONI

Esclusivamente per uso professionale. Gli operatori devono essere formati e avere una certa esperienza nei metodi di laboratorio. Si prega di legger attentamente le istruzioni prima di utilizzare questo prodotto. L'affidabilità dei risultati del test non può essere garantita se ci sono deviazioni dalle istruzioni riportate in questo documento.

Consultare la scheda di sicurezza (SDS) per informazioni sui pericoli e sulle modalità di manipolazione sicure.

SMALTIMENTO DEI RIFIUTI

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

BIBLIOGRAFIA

Vedere i riferimenti alla fine di questo documento.

TABELLA DEI SIMBOLI

Vedere la tabella dei simboli alla fine di questo documento.

Il prodotto è disponibile in diverse configurazioni. Vedere l'elenco nella lingua inglese.

Questo documento IFU e la SDS sono disponibile dal Support Center online:
liofilchem.com/ifu-sds

Instrucciones de Uso
ESPAÑOL

Nutrient Agar ISO 16266

Medio para el cultivo de organismos no exigentes y para la confirmación de *Pseudomonas aeruginosa*, según la ISO 16266.

ESCRIPCIÓN

Nutrient Agar ISO 16266 es un medio para el cultivo de organismos no exigentes a partir de muestras de agua.

Su formulación sigue la ISO 16266 para la detección y contaje de *Pseudomonas aeruginosa* en aguas mediante la técnica de filtración de membrana.

FÓRMULA*	(g/litro)
Peptona	5.0
Extracto de Carne	1.0
Extracto de Levadura	2.0
Cloruro Sódico	5.0
Agar	15.0
pH final 7.4 ± 0.2 a 25°C	

Ajustada y/o complementada según sea necesario para cumplir con las especificaciones de rendimiento.

PRINCIPIO DEL MÉTODO

La peptona y el extracto de carne suministran los aminoácidos, nitrógeno, carbono, vitaminas y minerales necesarios para el crecimiento de los microorganismos. El extracto de levadura es una fuente de vitaminas, especialmente del grupo B. El cloruro sódico mantiene el equilibrio osmótico del medio. El agar es el agente solidificante

PREPARACIÓN

Medio deshidratado	Suspender 28 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

Según la ISO 16266, se debe transferir la membrana filtrante y las colonias presuntas de *Pseudomonas aeruginosa* al medio en placa.

Incubar aerobicamente a 36 ± 2°C durante 20-24 horas.

Como alternativa, se puede inocular el medio por estriación o vertiendo la muestra sobre el agar.

INTERPRETACIÓN DE LOS RESULTADOS

Observar el crecimiento de colonias. Confirmar *P. aeruginosa* a través del test de la oxidasa (ref. 88029).

ASPECTO

Medio deshidratado: suelto, homogéneo, beige claro.
Medio preparado: ligeramente opalescente, ámbar claro

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 los tubos 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.

Evite los cambios rápidos en la temperatura del medio en placas para evitar la condensación.

VIDA ÚTIL

Medio deshidratado: 4 años.
Medio en botellas: 2 años.
Tubos semitendidos: 1 año
Placas preparadas: 6 meses.

CONTROL DE CALIDAD

Aspecto del Medio Deshidratado: Suelto, homogéneo, beige claro.

Apariencia del Medio Preparado: Ligeramente opalescente, ámbar claro.

Respuesta Cultural Esperada Cultural:

Cepa de control		Inóculo	Incubación	Especificación
Pseudomonas aeruginosa	WDCM 00025 (ATCC 27853, NCTC 12903)	50-100 UFC	20-24 h 36 ± 1°C	Buen crecimiento
Escherichia coli	WDCM 00013 (ATCC 25922, NCTC 12241)			

Consulte el Certificado de análisis (CoA) para el lote real.

ADVERTENCIAS Y PRECAUCIONES

Solo para uso profesional. Los operadores deben estar capacitados y tener cierta experiencia en los métodos de laboratorio. Lea atentamente las instrucciones antes de utilizar este producto. No se puede garantizar la confiabilidad de los resultados del ensayo si existen desviaciones de las instrucciones de este documento.

Consulte la Hoja de datos de seguridad (SDS) para obtener información sobre los peligros y las prácticas de manipulación segura.

DESECHO DE RESÍDUOS

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

BIBLIOGRAFÍA

Consulte las referencias al final de este documento.

TABLA DE SÍMBOLOS

Consulte la tabla de símbolos al final de este documento.

El producto está disponible en diferentes configuraciones. Ver la lista en inglés.

Este documento IFU y la SDS están disponibles en el Centro de soporte en línea:
liofilchem.com/ifu-sds

References / Riferimenti / Referencias

1. ISO 16266:2008. Water Quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration.
2. Marshall, R.T. (1993) Standard methods for the microbiological examination of dairy products, 16th ed.

Table of Symbols / Tabella dei Simboli / Tabla de Símbolos

LOT	Batch code / Codice del lotto / Código de lote
REF	Catalogue number / Numero di catalogo / Número de catálogo
	Manufacturer / Fabbricante / Fabricante
	Use by / Utilizzare entro / Utilizar antes de
	Fragile, handle with care / Fragile, maneggiare con cura / Frágil, manipular con cuidado
	Temperature limitation / Limiti di temperatura / Límites de temperatura
	Contains sufficient for <n> tests / Contenuto sufficiente per <n> saggi / Contenido suficiente para <n> análisis
	Consult instructions for use / / Consultare le istruzioni per l'uso / Consultar las instrucciones de uso /
	Do not reuse / Non riutilizzare / No reutilizar
	Keep away from sunlight / Tenere al riparo dalla luce solare / Mantener alejado de la luz solar /

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Instructions For Use
ENGLISH

Nutrient Broth

Liquid medium for the cultivation of nonfastidious microorganisms.

DESCRIPTION

Nutrient Broth is a liquid medium used for the cultivation of a wide variety of organisms from clinical specimens and other materials.

This medium can be enriched with other ingredients such as blood, serum, sugars, etc., for special purposes.

TYPICAL FORMULA	(g/l)
Beef Extract	1.0
Peptone	5.0
Yeast Extract	2.0
Sodium Chloride	5.0

Final pH 6.8 ± 0.2 at 25°C

METHOD PRINCIPLE

Beef extract and peptone 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.

PREPARATION

Dehydrated medium: Suspend 13 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

Inoculate broth with test sample. Incubate at 35 ± 2°C for 18-24 hours or longer if necessary.

INTERPRETING RESULTS

Turbidity indicates microbial growth.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, white to light beige.
Prepared medium: clear to 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 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 medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU
Incubation conditions: aerobically at 35 ± 2°C for 18-24 hours.

QC Table.

Microorganism		Growth
Escherichia coli	ATCC® 25922	Good
Staphylococcus aureus	ATCC® 25923	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. Association of Official Analytical Chemists (1995) Official methods of analysis of AOAC International, 16th ed.
2. Marshall, R.T. (ed.) (1993) Standard methods for the microbiological examination of dairy products, 16th ed.
3. American Public Health Association (1923) Standard methods of water analysis, 5th ed.

PRESENTATION		Contents	Ref.
Nutrient Broth	Tubes	20 x 10 ml tubes	24103
Nutrient Broth	Tubes	50 x 5 ml tubes	27503
Nutrient Broth	Bottles	6 x 100 ml bottles	402000
Nutrient Broth	Bottles	6 x 500 ml bottles	470050
Nutrient Broth	Dehydrated medium	500 g of powder	610037
Nutrient Broth	Dehydrated medium	100 g of powder	620037
Nutrient Broth	Dehydrated medium	5 kg of powder	6100375

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

Istruzioni per l'uso
ITALIANO

Nutrient Broth

Terreno liquido per la coltivazione di microrganismi non esigenti.

DESCRIZIONE

Nutrient Broth è un terreno liquido utilizzato per la coltivazione di un'ampia varietà di microrganismi da campioni clinici ed altri materiali.

Questo terreno può essere arricchito con altri ingredienti come sangue, siero, zuccheri, ecc., per scopi specifici.

FORMULA TIPICA	(g/l)
Estratto di Manzo	1.0
Peptone	5.0
Estratto di Lievito	2.0
Sodio Cloruro	5.0

pH Finale 6.8 ± 0.2 a 25°C

PRINCIPIO DEL METODO

Estratto di manzo e peptone forniscono aminoacidi, azoto, carbonio, vitamine e minerali per la crescita dei microrganismi. L'estratto di lievito è una fonte di vitamine, soprattutto del gruppo B. Il sodio cloruro mantiene il bilancio osmotico del terreno.

PREPARAZIONE

Terreno disidratato: Sospendere 13 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire fino a completa dissoluzione. Sterilizzare in autoclave a 121°C per 15 minuti.

PROCEDURA DEL TEST

Inoculare il brodo con il campione. Incubare a 35 ± 2°C per 18-24 ore o per un tempo maggiore se necessario.

INTERPRETAZIONE DEI RISULTATI

La torbidità è indice di crescita microbica.

ASPETTO

Terreno disidratato: omogeneo, fine granulometria, da bianco a beige chiaro.
Terreno preparato: ambra chiaro, da limpido a 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 provette 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 provette/flaconi: 2 anni.

CONTROLLO DI QUALITÀ

Il terreno viene inoculato con i ceppi microbici indicati nella tabella CQ.
Inoculo per produttività: ≤100 UFC.
Condizioni di incubazione: ambiente aerobico a 35 ± 2°C per 18-24 ore.

Tabella CQ.

Microrganismo		Crescita
Escherichia coli	ATCC® 25922	Buona
Staphylococcus aureus	ATCC® 25923	Buona

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. Association of Official Analytical Chemists (1995) Official methods of analysis of AOAC International, 16th ed.
2. Marshall, R.T. (ed.) (1993) Standard methods for the microbiological examination of dairy products, 16th ed.
3. American Public Health Association (1923) Standard methods of water analysis, 5th ed.

PRESENTAZIONE		Contenuto	Ref.
Nutrient Broth	Provette	Provette 20 x 10 ml	24103
Nutrient Broth	Provette	Provette 20 x 5 ml	27503
Nutrient Broth	Flaconi	Flaconi 6 x 100 ml	402000
Nutrient Broth	Flaconi	Flaconi 6 x 500 ml	470050
Nutrient Broth	Terreno disidratato	500 g di polvere	610037
Nutrient Broth	Terreno disidratato	100 g di polvere	620037
Nutrient Broth	Terreno disidratato	5 k g di polvere	6100375

TABELLA DEI SIMBOLI

LOT Codice del lotto	**IVD** Dispositivo Medico Diagnostico *in vitro*	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

ENGLISH

KPC&MBL&OXA-48 disc kit (acc. to EUCAST)

Disc tests for confirmation of carbapenemase-producing Enterobacteriaceae.

DESCRIPTION

Carbapenemases are β-lactamases that hydrolyze penicillins, in most cases cephalosporins and to various degree carbapenems. The most important carbapenemases are categorized as three types of enzymes:

1. *Klebsiella pneumoniae* carbapenemases (KPC) belonging to the Ambler class A of β-lactamases;
2. VIM, IMP and NDM belonging to the class B metallo β-lactamases (MBL);
3. Class D β-lactamases of OXA-48-like enzymes.

Since the 1990s, the increased consumption of carbapenems to treat infections caused by extended-spectrum β-lactamases (ESBL) producing bacteria has in turn contributed to the dramatic increase in carbapenem-resistant Enterobacteriaceae (CRE). Bacterial acquisition of carbapenemases is crucial to the emergence of CRE. Indeed, the corresponding genes are mostly plasmid-located and associated with various mobile genetic structures and may confer resistance to virtually all β-lactams. Infections with carbapenemase-producing Enterobacteriaceae are associated with high mortality posing a serious threat especially to hospitalized patients. Carbapenemases are considered to be of high epidemiological importance and one of the most important concerns is the spread in the community, particularly in *E. coli*. Adequate treatment and control of CRE infections is predicted upon their accurate and prompt diagnosis from patient samples in the clinical laboratory.
Carbapenem resistance can also arise in both ESBL and AmpC-producing organisms (Ambler class A and C, respectively) by mutation that regulate the synthesis of porins contained in the outer membrane.

Carbapenem MICs for carbapenemase-producing enterobacteriaceae may be below the clinical breakpoints, however, the epidemiological cut-off (ECOFF) defined by EUCAST can be used to detect carbapenemase-producers. Meropenem, offering the best compromise between sensitivity and specificity, is recommended for screening.
Following detection of reduced susceptibility to carbapenems, a phenotypic method for confirmation of carbapenemase-production should be applied. Meropenem discs supplemented with different inhibitors of carbapenemase are used to evaluate carbapenem-resistant strains.

CONTENTS OF THE PACKAGES

5 x 50 discs cartridges, each packaged in a “blister” with a dryer.

METHOD PRINCIPLE

Enterobacteriaceae suspected to be producers of carbapenemases may be confirmed by using meropenem and evaluating the synergistic effects when combined with the following inhibitors:

- **Phenylboronic acid** inhibits class A carbapenemase;
- **EDTA** inhibits class B carbapenemase;
- **Cloxacillin**, which inhibits AmpC β-lactamases, permits to differentiate between AmpC hyperproduction plus porin loss and carbapenemase-production.

There is no currently available inhibitor for class D carbapenemases. However, when there is no synergy of meropenem with any of the above mentioned inhibitors, 30 µg **Temocillin** disc (TMO) can be used in order to differentiate between ESBL plus porin loss and OXA-48 like enzymes.

For each combined disc test (CDT), discs containing meropenem alone and in combination with a carbapenemases inhibitor are applied. The inhibition zone around the meropenem disc combined with inhibitor is compared with the zone around the 10 µg meropenem disc (MRP).

GATHERING AND KEEPING SAMPLES

The colonies that are to be subjected to the susceptibility test are taken up by culture media that have been previously swabbed with the sample under examination.

TEST PROCEDURE

1. Using a fresh, pure culture prepare a suspension of the test organism equal to 0.5 McFarland Standard.
2. Using a sterile cotton swab, spread the adjusted suspension over the entire area of a Mueller Hinton agar plate.
3. Apply Meropenem, Meropenem + EDTA, Meropenem + Phenylboronic acid, Meropenem + Cloxacillin, on the inoculated plate, ensuring sufficient space between individual discs to allow for proper measurement of inhibition zones.
4. Incubate at 36±1°C for 18-24 hours.

EVALUATING THE RESULTS

At the end of the incubation period, measure the inhibition halos and interpret as indicated in the table below.

Interpretative Table. Synergy of meropenem with carbapenem inhibitors for confirmation of CRE.

Increase in inhibition zone of meropenem with the following inhibitor			Temocillin (TMO)*	β-lactamases
Phenylboronic acid (MR+BO)	EDTA (MR+ED)	Cloxacillin (MR+CL)		
≥ **4 mm**	< 5 mm	< 5 mm	---	KPC
< 4 mm	≥ **5 mm**	< 5 mm	---	MBL
≥ **4 mm AND**	< 5 mm	≥ **5 mm**	---	AmpC + porin loss or efflux
< 4 mm	< 5 mm	< 5 mm	**< 11 mm**	OXA-48-like

*Temocillin susceptibility test is recommended only in cases where no synergy is detected.

QUALITY CONTROL

Each production batch of discs is subjected to the quality control with the following bacterial strains:

Microorganism		
Klebsiella pneumoniae	ATCC® 700603	Negative control
Klebsiella pneumoniae	ATCC® BAA-2146	MBL positive
Klebsiella pneumoniae	ATCC® BAA-1705	KPC positive
Klebsiella pneumoniae	NCTC 13442	OXA-48 positive

LIMITS

Diffusion susceptibility tests use an *in vitro* technique and cannot therefore reproduce the extremely complex *in vivo* conditions. Nevertheless, it is a useful and important tool that helps the clinician choose the correct therapy. Many variable factors influence the final result of the diffusion susceptibility test. The main ones are: the culture medium used, impregnation of the discs, inoculation of the medium, temperature, time and incubation atmosphere of the plates, pre-incubation and pre-diffusion conditions, depth of the medium, etc.

PRECAUTIONS

The discs cannot be classified as being hazardous according to current legislation but fall within the specific field of application where a safety data sheet must be supplied because they can cause phenomena of sensitization in sensitive subjects if they come into contact with the skin.
The discs are disposable products. They are only for diagnostic *in vitro* use and are intended for professional use. They must be used in the laboratory by properly trained operators using approved aseptic and safety methods for pathogenic agents.

STORAGE

Store the unopened blister at -20°C to +8°C till the expiry date. Allow unopened cartridge to come to room temperature before removing it from the blister for minimising condensation on the discs. Leftover discs from an opened cartridge should be stored at 2-8°C for no more than 7 days. Return unused discs to the refrigerator as soon as the application of the discs has been completed. Dispose of expire discs.

ELIMINATING USED MATERIAL

After use, the discs and the material that comes into contact with the sample must be decontaminated and disposed of in accordance with current laboratory techniques for the decontamination and disposal of potentially infected material.

REFERENCES

- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.

PRESENTATION

DESCRIPTION		PACKAGING	REF
Meropenem 10 µg	MRP	50 Discs	99007
Meropenem + EDTA	MR+ED	50 Discs	
Meropenem + Phenylboronic acid	MR+BO	50 Discs	
Meropenem + Cloxacillin	MR+CL	50 Discs	
Temocillin 30 µg	TMO	50 Discs	

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 accompanying documents	Do not reuse

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CE IVD

ITALIANO

KPC&MBL&OXA-48 disc kit (acc. to EUCAST)

Dischi per i test di conferma di Enterobacteriaceae produttrici di carbapenemasi.

DESCRIZIONE

Le carbapenemasi sono β-lattamasi che idrolizzano penicilline, nella maggior parte dei casi cefalosporine e con efficienze variabili carbapenemi. Le carbapenemasi più importanti sono raggruppate in tre categorie di enzimi secondo il sistema di classificazione di Ambler:

1. KPC (*Klebsiella pneumoniae* carbapenemasi) appartengono alla classe A;
2. VIM, IMP ed NDM appartengono alla classe B delle metallo β-lattamasi (MBL);
3. OXA-48, appartengono alla classe D.

A partire dagli anni '90, l'incremento nel consumo di carbapenemi per trattare infezioni causate da batteri produttori di β-lattamasi a spettro esteso (ESBL) ha contribuito a sua volta al notevole aumento di Enterobacteriaceae resistenti ai carbapenemi (CRE). L'acquisizione batterica di carbapenemasi è cruciale per la diffusione dei ceppi CRE. Infatti, i geni corrispondenti sono presenti principalmente su plasmidi ed associati a diverse strutture genetiche mobili capaci virtualmente di conferire resistenza a tutti i β-lattami. Le infezioni ad opera di Enterobacteriaceae produttrici di carbapenemasi sono associate ad un alto tasso di mortalità ponendo una seria minaccia sopratutto per i pazienti ospedalizzati. Le carbapenemasi sono considerate di primaria importanza da un un punto di vista epidemiologico e la diffusione nella comunità, in modo particolare in *E. coli*, è una delle preoccupazioni maggiori. Un adeguato trattamento e controllo delle infezioni da CRE si basa su una diagnosi accurata e tempestiva dal campione pervenuto nel laboratorio clinico. La resistenza ai carbapenemi può insorgere anche in microrganismi produttori di ESBL e AmpC (Classe A e C secondo Ambler, rispettivamente) a causa di una mutazione che regola la sintesi di porine presenti nella membrana esterna.

Nelle Enterobacteriaceae produttrici di carbapenemasi le MIC riferite ai carbapenemi possono risultare al di sotto dei breakpoint clinici, comunque può essere utilizzato il cut-off epidemiologico (ECOFF) definito dall'EUCAST per la ricerca degli organismi produttori di tali enzimi. Per il test di screening è raccomandato il meropenem che offre il miglior compromesso tra sensibilità e specificità. Per la conferma della produzione di carbapenemasi si dovrebbe applicare un metodo fenotipico in grado di determinare la ridotta sensibilità ai carbapenemi. Per valutare i ceppi carbapenemi-resistenti vengono utilizzati dei dischi contenenti meropenem ed inibitori delle carbapenemasi

CONTENUTO DEL KIT

Cartucce 5 x 50 dischi, ciascuna confezionata in un "blister" con essiccante.

PRINCIPIO DEL METODO

Enterobacteriaceae sospettate di essere produttrici di carbapenemasi vengono confermate utilizzando il meropenem e valutando gli effetti sinergici quando è in combinazione con i seguenti inibitori:

- **Acido fenilboronico** inibisce le carbapenemasi di classe A;
- **EDTA** inibisce le carbapenemasi di classe B;
- **Cloxacillina** inibisce le β-lattamasi AmpC e permette di differenziare tra produzione di carbapenemasi ed iperproduzione di AmpC e perdita di porine.

Non sono attualmente disponibili inibitori delle carbapenemasi di classe D. Comunque, quando non si riscontra nessuna sinergia tra tra il meropenem e gli inibitori sopra menzionati, può essere utilizzato il disco da 30 µg di **Temocillin** (TMO) per distinguere tra ESBL più perdita di porine e presenza di enzimi come OXA-48.

Per ciascun test combinato (CDT), vengono applicati sia il disco contenente 10 µg di meropenem (MRP) ed un disco contenente meropenem in combinazione con un inibitore di carbapenemasi. Al termine dell'incubazione vengono quindi confrontate le zone di inibizione.

RACCOLTA E MANTENIMENTO DEI CAMPIONI

Le colonie da sottoporre al test di sensibilità sono prelevate da terreni di coltura precedentemente inoculati con il campione da esaminare.

PROCEDURA DEL TEST

1. Utilizzare una coltura pura recente per preparare una sospensione del microrganismo equivalente allo standard 0.5 McFarland.
2. Utilizzare un tampone di cotone sterile per diffondere la sospensione sull'intera superficie di una piastra di Mueller Hinton II agar.
3. Applicare Meropenem, Meropenem + EDTA, Meropenem + Acido fenilboronico, Meropenem + Cloxacillina, sulla piastra inoculata, assicurandosi di lasciare uno spazio sufficiente tra i dischi che permetta di misurare correttamente le zone di inibizione.
4. Incubare a 36±1°C per 18-24 ore.

VALUTAZIONE DEI RISULTATI

Alla fine del periodo di incubazione, misurare gli aloni di inibizione ed interpretare come indicato nella tabella sottostante.

Tabella Interpretativa. Sinergia del meropenem con inibitori di carbapenemi per la conferma di CRE.

Aumento della zona di inibizione dei meropenem con il seguente inibitore			Temocillina (TMO)*	β-lattamasi
Acido fenilboronico (MR+BO)	EDTA (MR+ED)	Cloxacillina (MR+CL)		
≥ **4 mm**	< 5 mm	< 5 mm	---	KPC
< 4 mm	≥ **5 mm**	< 5 mm	---	MBL
≥ **4 mm E**	< 5 mm	≥ **5 mm**	---	AmpC + perdita porine o efflusso
< 4 mm	< 5 mm	< 5 mm	**< 11 mm**	OXA-48-like

*Il test di sensibilità alla Temocillina è raccomandato solo nei casi dove non si riscontrano sinergie.

CONTROLLO QUALITÀ

Ciascun lotto di dischi prodotto è sottoposto al controllo qualità con i seguenti ceppi batterci:

Microrganismo		
Klebsiella pneumoniae	ATCC® 700603	Controllo negativo
Klebsiella pneumoniae	ATCC® BAA-2146	MBL positivo
Klebsiella pneumoniae	ATCC® BAA-1705	KPC positivo
Klebsiella pneumoniae	NCTC 13442	OXA-48 positivo

LIMITI

I test di sensibilità per diffusione utilizzano tecniche *in vitro* e non possono riprodurre esattamente le condizioni che si trovano *in vivo*. Ciononostante, rappresentano uno strumento utile ed importante che aiuta il clinico nella scelta della terapia corretta. Diverse variabili influenzano il risultato finale di tale test: Le principali sono: il terreno di coltura utilizzato, l'impregnazione dei dischi, l'inoculo del terreno, temperatura, tempo ed atmosfera di incubazione delle piastre, condizioni di pre-incubazione e pre-diffusione, spessore del terreno, ecc.

PRECAUZIONI

I dischi non possono essere classificati come pericoloso ai sensi della legislazione vigente, ma rientrano nello specifico campo di applicazione della normativa relativa all'obbligo di fornitura di scheda di sicurezza, perché può causare fenomeni di sensibilizzazione in soggetti sensibili in caso di contatto con la pelle.
I dischi sono dispositivi monouso. Sono destinati esclusivamente ad uso diagnostico *in vitro*, in ambito professionale. Devono essere utilizzati in laboratorio da operatori adeguatamente addestrati, con metodi approvati di asepsi e di sicurezza nei confronti degli agenti patogeni.

CONSERVAZIONE

Il blister integro deve essere conservato a –20°C/+8°C fino alla data di scadenza indicata sulla confezione. Portare le cartucce a temperatura ambiente prima di rimuoverle dal blister per minimizzare la formazione di condensa su dischi. I dischi rimasti in una cartuccia aperta devono essere conservati a 2-8°C per non più di 7 giorni. Riporre i dischi inutilizzati in frigorifero non appena l'applicazione è stata completata. Non utilizzare oltre la data di scadenza.

ELIMINAZIONE DEL MATERIALE USATO

Dopo l'utilizzazione i dischi ed il materiale venuto a contatto con il campione devono essere decontaminati e smaltiti in accordo con le tecniche in uso in laboratorio per la decontaminazione e lo smaltimento di materiale potenzialmente infetto.

RIFERIMENTI BIBLIOGRAFICI

- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.

PRESENTAZIONE

DESCRIZIONE		CONFEZIONAMENTO	REF
Meropenem 10 µg	MRP	50 Dischi	99007
Meropenem + EDTA	MR+ED	50 Dischi	
Meropenem + Phenylboronic acid	MR+BO	50 Dischi	
Meropenem + Cloxacillin	MR+CL	50 Dischi	
Temocillin 30 µg	TMO	50 Dischi	

TABELLA DEI SIMBOLI

LOT Codice del lotto	**IVD** Dispositivo Medico Diagnostico *in Vitro*	Fabbricante	Utilizzare entro	Fragile, utilizzare con cura
REF Numero di catalogo	Temperatura limitata	Contenuto sufficiente per <n> test	Attenzione,consultare i documenti in accompagnamento	Non riutilizzare

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CE IVD

ESPAÑOL

KPC&MBL&OXA-48 disc kit (acc. to EUCAST)

Pruebas para la confirmación de Enterobacterias productoras de carbapenemasas a través de discos.

DESCRIPCIÓN

Las Carbapenemasas son enzimas β-lactamasas que hidrolizan a la mayoría de las penicilinas, cefalosporinas, y algunos tipos de carbapenémicos. Hay tres tipos principales de carbapenemasas:

1. Carbapenemasas *Klebsiella pneumoniae* (KPC) que pertenecen a la clase A de Ambler de β-lactamasas;
2. VIM, IMP y NDM que pertenecen a la clase B metalo β-lactamasas (MBL);
3. β-lactamasas de Clase D de enzimas tipo OXA-48.

En los años 90 debido al incremento de consumo de fármacos carbapenémicos para el tratamiento de infecciones de bacterias productoras de β-lactamasas de amplio espectro (ESBL) ha significado un aumento de las Enterobacterias resistentes a carbapenémicos (CRE).
La adquisición de las carbapenemasas por parte de las bacterias es esencial para entender a las CRE. De hecho, los genes responsables son normalmente plásmidos localizados y asociados a diferentes estructuras genéticas móviles y pueden conferir resistencia a todos los fármacos β-lactámicos. Las infecciones con Enterobacterias productoras de carbapenemasas suelen caracterizarse por una elevada mortalidad creando un serio problema especialmente en pacientes hospitalizados. Las Carbapenemasas están consideradas de alta importancia epidemiológica y una de las mayores preocupaciones es la transmission a otros patógenos, especialmente a *E. coli*. Un tratamiento y control adecuados de las infecciones de CRE puede garantizarse una vez hayamos diagnosticado con precision las muestras de pacientes en el laboratorio clínico.
La resistencia a Carbapenémicos puede darse también en organismos productores de ESBL y AmpC (clases de Ambler A y C, respectivamente) por una mutación en la regulación de la síntesis de porinas presentes en la membrana externa.

Las CIM de los Carbapenémicos en Enterobacterias productoras de carbapenemasas puede ser inferior a los puntos de corte clínicos, sin embargo, el punto de corte epidemiológico (ECOFF) definido por el EUCAST puede utilizarse para identificar a los productores de carbapenemasas. Se recomienda el uso del Meropenem para el cribado, ya que ofrece la mejor relación entre sensibilidad y especificidad.
Para continuar con la identificación de la susceptibilidad reducida a los carbapenémicos, se debe utilizar un método fenotípico para la confirmación de la producción de carbapenemasa. Para evaluar las cepas resistentes a carbapenémicos se utilizan discos de Meropenem con diferentes inhibidores de carbapenemasas como suplemento

CONTENIDO DEL KIT

5 x 50 discos en cartuchos, cada uno empaquetado en “blíster” con desecante.

PRINCIPIO DEL MÉTODO

Las Enterobacterias aparentemente productoras de carbapenemasas se deben confirmar utilizando meropenem y valorando los efectos de sinergia en combinación con los siguientes inhibidores:

- **Ácido fenilborónico** inhibe las carbapenemasas de clase A;
- **EDTA** inhibe las carbapenemasas de clase B;
- **Cloxacilina**, inhibe las β-lactamasas AmpC, y permite la diferenciación entre la sobreproducción de AmpC con pérdida de porinas y la producción de carbapenemasas.

Actualmente no existe ningún inhibidor de las carbapenemasas de clase D. Sin embargo, cuando no hay sinergia entre el meropenem y los inhibidores mencionados, se puede utilizar un disco de 30 µg de **Temocilina** (TMO) para diferenciar entre ESBL con pérdida de porinas y enzimas del tipo OXA-48.

Para cada prueba de combinación de discos (CDT), se utilizan discos que contienen meropenem a solas y meropenem con inhibidor de carbapenemasas. La zona de inhibición alrededor del disco de meropenem en presencia de inhibidor se compara con la zona alrededor del disco que contiene el disco de 10 µg meropenem a solas (MRP).

RECOGIDA Y MANTENIMIENTO DE LAS MUESTRAS

Las colonias que van a ser analizadas con las pruebas de susceptibilidad se deben retirar del medio de cultivo que se ha inoculado previamente con la muestra a examinar.

PROCEDIMIENTO DE LA PRUEBA

1. Utilizar colonias puras, frescas para preparar la suspensión equivalente a 0.5 McFarland Standard.
2. Utilizar un hisopo de algodón estéril y extender la suspensión sobre la superficie de una placa de Mueller Hinton.
3. Aplicar los discos de Meropenem, Meropenem + EDTA, Meropenem + Ácido fenilborónico, Meropenem + Cloxacilina, en la placa inoculada, asegurándose de que haya suficiente espacio entre los discos para poder leer correctamente las zonas de inhibición.
4. Incubar a 35±2°C durante 18-24 horas.

EVALUACIÓN DE LOS RESULTADOS

Al finalizar el tiempo de incubación, medir los halos de inhibición e interpretar siguiendo la tabla aquí presente.

Tabla interpretativa. Para la confirmación de CRE es necesario que exista una sinergia entre los inhibidores de meropenem y carbapenem

Aumento de la zona de inhibición del meropenem con el siguiente inhibidor			Temocilina (TMO)*	β-lactamasas
Ácido fenilborónico (MR+BO)	EDTA (MR+ED)	Cloxacilina (MR+CL)		
≥ **4 mm**	< 5 mm	< 5 mm	---	KPC
< 4 mm	≥ **5 mm**	< 5 mm	---	MBL
≥ **4 mm AND**	< 5 mm	≥ **5 mm**	---	AmpC + pérdida de porinas o eflujo
< 4 mm	< 5 mm	< 5 mm	**< 11 mm**	Tipo OXA-48

*El test de susceptibilidad con la Temocilina sólo se recomienda en situaciones donde no se observe sinergia.

CONTROL DE CALIDAD

Cepas adecuadas para el control de calidad de las pruebas de detección de ESBL:

Microorganismo		
Klebsiella pneumoniae	ATCC® 700603	Control negativo
Klebsiella pneumoniae	ATCC® BAA-2146	MBL positivo
Klebsiella pneumoniae	ATCC® BAA-1705	KPC positivo
Klebsiella pneumoniae	NCTC 13442	OXA-48 positivo

LÍMITES

Las pruebas de susceptibilidad por difusión son una técnica in vitro y no pueden reproducir las complejas condiciones in vivo. Sin embargo, son una herramienta útil que ayuda al médico a elegir la terapia más adecuada. Existen muchas variables que pueden afectar al resultado de la prueba de susceptibilidad por difusión. Las principales son: el medio de cultivo empleado, el impregnado de los discos, el inóculo del medio, la temperatura, la atmósfera y tiempo de incubación de las placas, las condiciones de pre-incubación y pre-difusión, la profundidad del medio, etc.

PRECAUCIONES

Los discos no están considerados como peligrosos según la legislación vigente pero deben ir acompañados de una hoja de seguridad porque podrían causar fenómenos de sensibilización en personas sensibles si entran en contacto con su piel.
Los discos son productos desechables. Su uso está restringido al ámbito diagnóstico in vitro para uso profesional. Deben ser utilizados en laboratorio por usuarios debidamente adiestrados en condiciones asépticas aprobadas y métodos de seguridad para agentes patógenos.

ALMACENAMIENTO

Almacenar el blister sin abrir a -20°C a +8°C hasta su fecha de caducidad. Permitir que el cartucho sin abrir se atempere antes de que el blister sea abierto para reducir la posible condensación en los discos. Los discos restantes sin utilizar del cartucho abierto deben almacenarse a 2-8°C durante un máximo de 7 días. Reintroducir los discos en el frigorífico tan pronto como se hayan utilizado. Eliminar los discos caducados.

DESECHADO DEL MATERIAL EMPLEADO

Después de su utilización, los discos y el material que ha entrado en contacto con las muestras deben ser descontaminados y desechados siguiendo las técnicas generales de laboratorio para la descontaminación y desechado de material potencialmente infectado.

REFERENCIAS

- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.

PRESENTACIÓN

DESCRIPCIÓN		PRESENTACIÓN	REF
Meropenem 10 µg	MRP	50 Discos	99007
Meropenem + EDTA	MR+ED	50 Discos	
Meropenem + Phenylboronic acid	MR+BO	50 Discos	
Meropenem + Cloxacillin	MR+CL	50 Discos	
Temocillin 30 µg	TMO	50 Discos	

TABLA DE SÍMBOLOS

LOT	Código de lote	**IVD**	Dispositivo médico diagnóstico in vitro		Fabricante		Utilizar antes de		Frágil, manipular con cuidado
REF	Número de catálogo		Límites de temperatura	Σ	Contenido suficiente para <n> análisis		Atención, consultar el documento adjunto		No reutilizar

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CE IVD

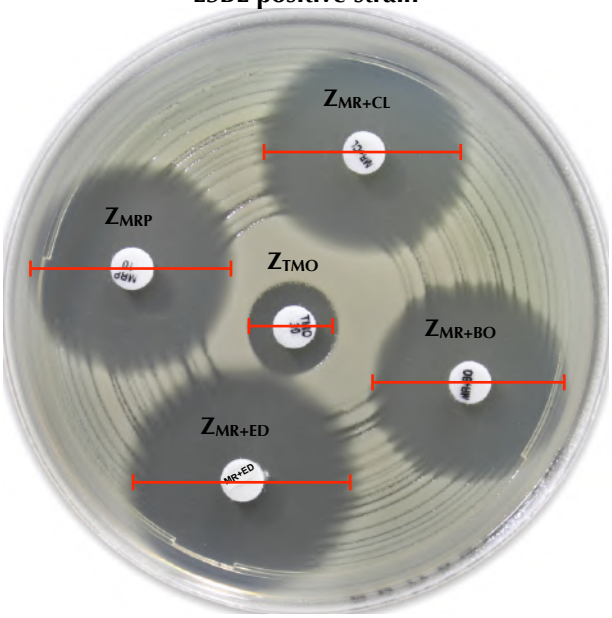
Ref. 99007

Appendix I

KPC&MBL&OXA-48 disc kit (acc. to EUCAST)

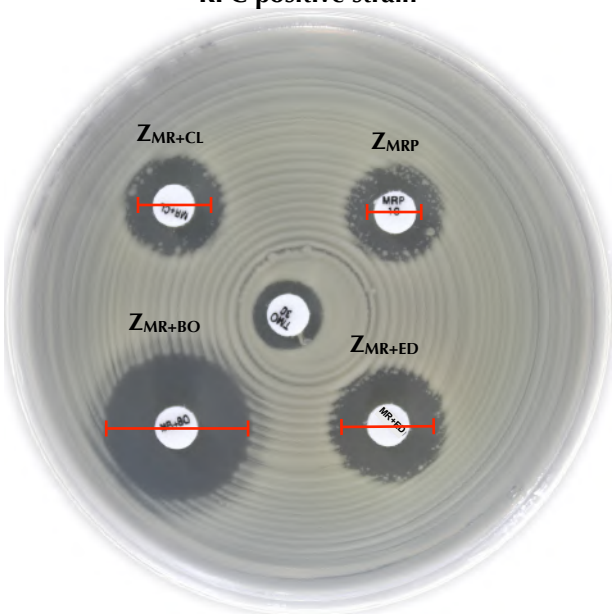
Disc tests for confirmation of carbapenemase-producing Enterobacteriaceae.

ESBL positive strain



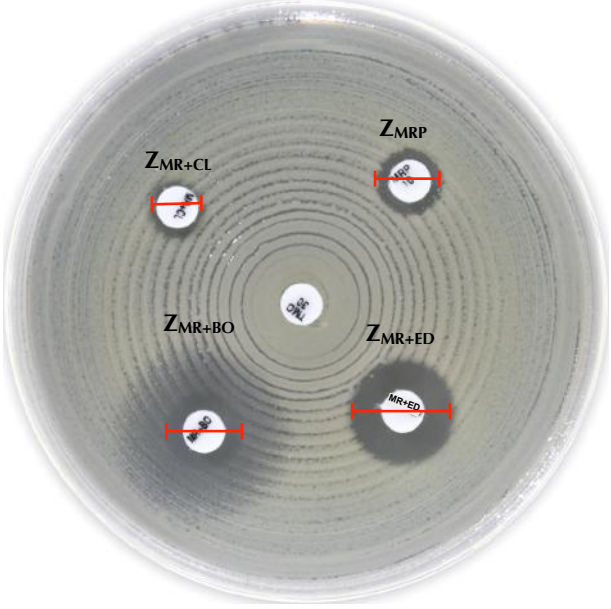
$Z_{MR+BO} - Z_{MRP} < 4$ mm
$Z_{MR+ED} - Z_{MRP} < 5$ mm
$Z_{MR+CL} - Z_{MRP} < 5$ mm
$Z_{TMO} > 11$ mm

KPC positive strain



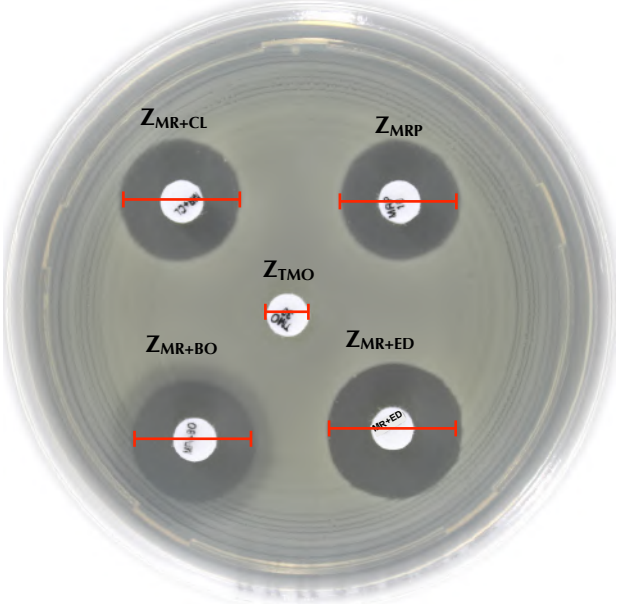
$Z_{MR+BO} - Z_{MRP} \geq 4$ mm
$Z_{MR+ED} - Z_{MRP} < 5$ mm
$Z_{MR+CL} - Z_{MRP} < 5$ mm

MBL positive strain



$Z_{MR+BO} - Z_{MRP} < 4$ mm
$Z_{MR+ED} - Z_{MRP} \geq 5$ mm
$Z_{MR+CL} - Z_{MRP} < 5$ mm

OXA-48 positive strain



$Z_{MR+BO} - Z_{MRP} < 4$ mm
$Z_{MR+ED} - Z_{MRP} < 5$ mm
$Z_{MR+CL} - Z_{MRP} < 5$ mm
$Z_{TMO} < 11$ mm

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CE IVD

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Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Troponin-I (cTnI) Test System
Product Code: 3825-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Circulating Troponin-I concentrations in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

The cardiac-specific isoform of Troponin I (cTnI) has been known as a marker of heart damage and myocardial cell death, due to myocardial infarction, for just over 10 years. Troponin-I (cTnI, 24 kDa) is the inhibitory subunit of the Troponin complex of striated muscle. Most of the Troponin (I & T) proteins are located within the contractile apparatus of the striated muscle. The concentration of these subunits is increased in circulation for many days after AMI, because release from the structural elements requires degradation of myofibril itself. This location and release from the specific myocardial tissue and their sustained appearance in circulation make Troponin subunits reliable bio-markers of AMI. Unlike CK-MB and Myoglobin, Troponin does not suffer from non-specificity issues. The immunological determination of cTnI does have some issues surrounding it but those are mainly due to lack of mass standardization, the presence of post-translationally modified cTnI in the circulation and variations in the antibody cross-reactivities to the various detectable forms of cTnI. However, cTnI, unlike Myoglobin, has not been found in circulation in marathon runners and other cases of skeletal injury or trauma. Careful selection of antibodies and diligent preparation of stable cTnI for calibration permits cTnI to be an excellent biochemical marker of myocardial damage.

Troponin I (cTnI) is the inhibitory subunit of the Troponin complex, which regulates the calcium modulated interaction of actin and myosin in striated muscle. The complex is a heterodimer consisting of troponins C, I and T, which are tightly bound to the contractile apparatus; hence, circulating concentrations are low. Even though Troponin C and T are both considered to be equally good markers for AMI, the NH_2 terminus of cTnI has 31 additional amino acids not present in the skeletal isoforms and has generated an interest in easily creating cTnI-specific MoAb's, by researchers. Troponin I, however, has its own problems that researchers are dealing with. Troponin I is not very stable. A major portion of it is bound with tropinin C (cTnC) and the remaining small part is free in circulation. Again, the post translational modifications, including selective degradation, covalent complex formation and phosphorylation of cTnI in the post-ischemic myocardium complicate the matters further more. Also, the cTnI proteolysis is even more extensive in human myocardium. Some researchers attribute it in part to the heterogeneity of disease states present in a given patient population. The key to overcoming these problems is a very careful selection of antibodies, the matrix for stabilizing the cTnI and the cTnI protein used as the standard itself. Monobind has been able to successfully put together an assay that promises to have overcome those issues.

In this method, Troponin-I calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes *of Troponin-I* are added and the reactants mixed. Reaction between the various *Troponin-I* antibodies and native *Troponin-I* forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme- Troponin-I antibody bound conjugate is separated from the unbound enzyme-Troponin-I conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known Troponin-I levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with Troponin-I concentration.

3.0 PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of x-cTnI antibody coated on the well.

Upon mixing the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{cTnI} + Ab_{(well)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{cTnI} - Ab_{(well)}$$

$Ab_{(well)}$ = Antibody coated on well (Excess Quantity)
Ag_{cTnI} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{cTnI} - Ab_{(well)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

After sufficient time results, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. cTnI Calibrators – 1.0 ml/vial (Lyophilized) – Icons [A-F]
Six (6) vials of references for *Troponin-I* antigen at levels of 0(**A**), 0.4(**B**), 1.25(**C**), 2.5(**D**), 7.5(**E**), and 20(**F**) ng/ml. A preservative has been added. ***Reconstitute each vial with 1.0ml of distilled or deionized water.***
The reconstituted calibrators are stable for 24 hours at 2-8°C. In order to store for a longer period of time aliquot the reconstituted calibrators in cryo vials and store at -20°C. ***DO NOT FREEZE THAW MORE THAN ONCE.***
Note: The calibrators, human serum based, were calibrated using NIST standard for cTnI # 2921.

B. cTnI Enzyme Reagent – 13 ml/vial – Icon
One (1) vial containing enzyme labeled affinity purified antibody labeled monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. cTnI Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with x-cTnI antibody, packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7.0ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7.0ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8.0ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 25µl and 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for 1 year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate calibrators, controls and samples into the assigned wells
3. Add 0.100 ml (100µl) of the Troponin-I Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
Note: Use a multichannel pipet to quickly dispense the Enzyme Reagent to avoid drift if the dispensing is to take more than a few minutes.
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 15 minutes at room temperature (20-27°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350 (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

NOTE: Always add reagents in the same order to minimize reaction time differences between wells.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Troponin I in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1
2. Plot the absorbance for each duplicate serum reference versus the corresponding cTnI concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of cTnI for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the

average absorbance (0.322) intersects the dose response curve at 3.61ng/ml cTnI concentration (See Figure 1).

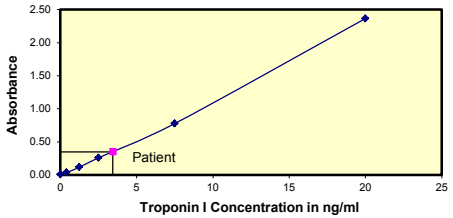
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.007	0.007	0
	B1	0.008		
Cal B	C1	0.035	0.037	0.4
	D1	0.040		
Cal C	E1	0.112	0.116	1.25
	F1	0.119		
Cal D	G1	0.257	0.260	2.5
	H1	0.264		
Cal E	A2	0.777	0.777	7.5
	B2	0.776		
Cal F	C2	2.362	2.365	20
	D2	2.368		
Ctrl 1	E2	0.050	0.051	0.43
	F2	0.051		
Ctrl 2	G2	1.256	1.245	12.75
	H2	1.233		
Patient	A3	0.320	0.322	3.61
	B3	0.324		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'A' should be ≤ 0.07.
2. The absorbance (OD) of calibrators 'F' should be ≥ 1.3.
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient samples with Troponin-I concentrations above 30 ng/ml may be diluted with the zero calibrator or Troponin-I free pooled human serum or urine and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The reagents for the procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33)*. For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.

13.0 EXPECTED VALUES

Troponin-I values are different in plasma and serum. In addition, plasma sample may be influenced by the additives used. For example, heparin minimally affects the results but oxalate and EDTA have significant effect. A serum sample that has been quickly separated from red cells is preferred.

Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned. **These ranges should be used as guidelines only:**

Adult (Normal)	≤ 0.4 ng/ml

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the cTnI AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	0.44	0.014	3.3%
Pool 2	20	3.55	0.072	2.0%
Pool 3	20	12.75	0.311	2.4%

TABLE 3
Between Assay Precision* (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	10	0.48	0.038	7.9%
Pool 2	10	3.68	0.242	6.6%
Pool 3	10	13.56	0.745	5.5%

*As measured in ten experiments in duplicate over ten days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the LoB and LoD. The LoB is 0.0639 ng/ml and the LoD is 0.0759 ng/ml.

14.3 Accuracy

The cTnI AccuBind® ELISA Test System was compared with a predicate radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from N/D – 18.1 ng/ml.) The total number of such specimens was 151. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Linear Square Regression Analysis	Correlation Coefficient
Monobind (y)	3.04	y = 0.3500+0.9266(x)	0.950
Reference(x)	*2.92*		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the Troponin-I ELISA method to selected substances was evaluated by adding the interfering compounds to a serum matrix at the very high concentration(s).

SUBSTANCE	Cross Reactivity
Hemoglobin	ND
CK-MB	ND
TnT	ND
FABP	ND

The presence of lipemia (25 mg/ml); hemoglobin (4.0 mg/ml) and bilirubin (2.5 mg/ml) did not affect the assay precision.

14.5 Hook Effect

Human serum samples spiked with concentrations up to 10,000 ng/ml of cTnI did not show any hook effect with cTnI AccuBind® ELISA Test System.

15.0 REFERENCES

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MP3825 **Product Code: 3825-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity