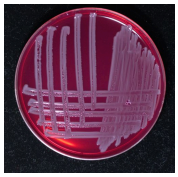
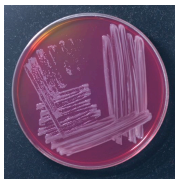
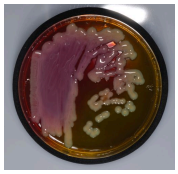
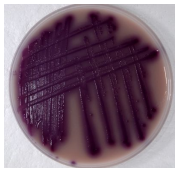
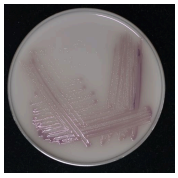
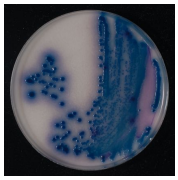
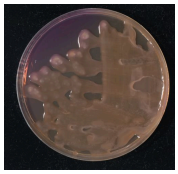
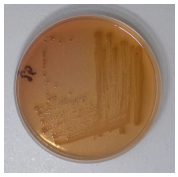
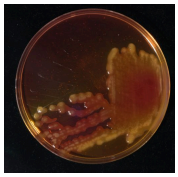
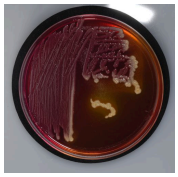
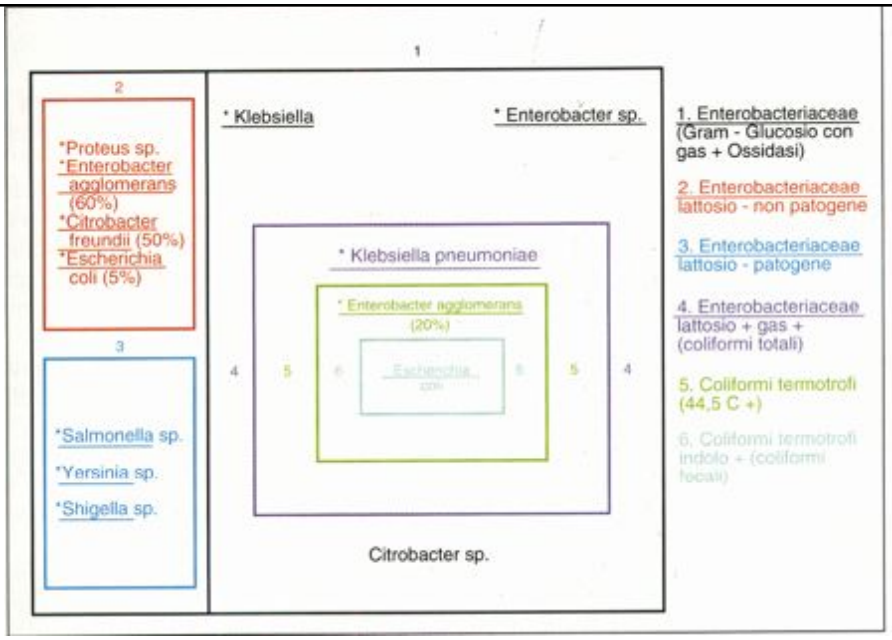
terreno	Salmonella	E. coli	Proteus	Enterobacter sakazakii	Enterobacter cloacae
AS					
BGA					
Cromogeno					
VRBG					
VRBL					
XLD					

terreno	Salmonella	E. coli	Proteus	Enterobacter sakazakii	Enterobacter cloacae
Kliger					
TSI					
Ureasi					
BGB					
Indolo					



ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Escherichia coli

- Bastoncellari, mobili per ciglia o immobili
- Sviluppano su terreni comuni
- Colonie lisce, convesse, umide, facilmente emulsionabili (ma anche rugose, secche e difficilmente emulsionabili). Anche mucose.

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Metabolismo di *E. coli*

- Fermenta: glucosio, molti stipiti il lattosio, e altri zuccheri con produzione di piruvato → ac. lattico, acetico e formico.
- Produce indolo, beta-galattosidasi positivo, non produce H2S.
- Classificazione in base agli antigeni superficiali: O (somatico), K (capsulare(, H: flagellare)

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E. coli responsabili di forme enteriche

- i) colonizzazione della mucosa
- ii) evasione dalle difese dell'ospite
- iii) moltiplicazione
- iv) danno
- Stabilita la colonizzazione, le strategie di *E. coli* diarrogeni sono diverse.

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Diarrea: meccanismo di azione

- i) produzione di enterotossina (ETEC e EAEC)
- ii) invasione (EIEC)
- iii) adesione con i sistemi di comunicazione di membrana (EPEC e EHEC)
- ognuna ha almeno un fattore di virulenza su plasmide

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ENTEROTOXIGENIC ETEC

- elabora almeno una di due gruppi di enterotossine: ST (heat-stable) e LT (heat-labile)

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ENTEROPATHOGENIC EPEC

- in passato definiti solo sulla base del sierotipo O e H oggi sulla base del potere patogeno:
- attaching-and-effacing (adesione e cancellazione dei microvilli) (A/E) istopatologia (biopsie)
  - i) adesione
  - ii) trasmissione del segnale (il gene responsabile sta su una pathogenicity island: locus of enterocyte effacement, LEE)
  - iii) aderenza tramite intimina (codificata dal gene eae: *E. coli* attaching effacing)

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ENTEROHEMORRAGIC EHEC 1/2

- Nel 1983 focolaio di gastrenterite con crampi addominali, diarrea acquosa e poi emorragica. Fu definita colite emorragica (HC) e collegata al consumo di hamburger poco cotti. Isolato *E. coli* O157:H7. Una seconda segnalazione, sempre nel 1983) fu di HUS (hemolytic hemorragic syndrome) con insufficienza renale acuta, trombocitopenia e anemia emolitica microangiopatica) con produzione di citotossina

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ENTEROHEMORRAGIC EHEC 2/2

- inizialmente effetto citopatico su cellule Vero neutralizzato dall'antisiero per la tossina 1 di Shigella dysenteriae (Stx). Molti *E. coli* isolati successivamente producevano tossine simili (Shiga-like: SLT): è la stessa tossina.
- Famiglia di STX: Stx1, Stx2c, Stx2v, Stx2vhb, Stx2e, etc... oppure VT1, VT2c, etc...
- EAST1, enteroemolisina (gene ehxA che è simile a hlyA degli stipiti uropatogeni), eae e altri per adesione, pO157 plasmide.
- Per la PCR: stx, eae, pO15

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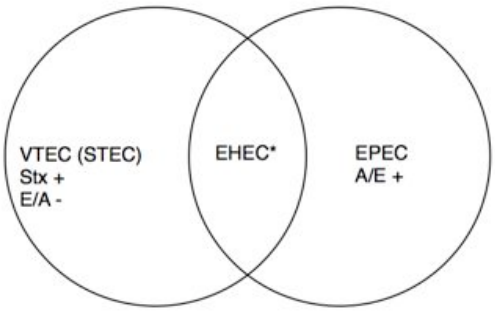
Enteropatogeni e enteroemorragici



* Molti O157:H7 non sono EHEC

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ENTEROAGGREGATIVE EAEC

- autoagglutinazione delle cellule una sull'altra: aggregativa
- diffusa
- mentre negli enteropatogeni è localizzata

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ENTEROINVASIVE EIEC

- i) penetrazione epiteliale
- lisi del vacuolo di endocitosi
- moltiplicazione intracellulare
- diffusione nel citoplasma
- passaggio a cellula adiacente

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DIFFUSELY ADHERENT DAEC

- mancano le microcolonie degli EAEC

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Terreno

- VRBG, VRBL agar
- BGB brodo
- Tryptone bile glucuronic medium (TBX)

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Terreno VRB (violet red bile)
pH 7,4

- Estratto di lievito 3 g/L
- Peptone 7 g/L
- Sodio Cloruro 5 g/L
- Sali biliari 1,5 g/L
- Lattosio (glucosio) 10g/L
- Rosso Neutro 0,03 g/L
- Cristalvioletto 0,002 g/L
- Agar 12 g/l

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Preparazione

- Sterilizzazione non richiesta
- Preparato di fresco
- Utilizzato entro 3 ore

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Terreno BGB (brilliant green bile broth) pH 7,4

- Peptone 10 g/l
- Lattosio 10 g/L
- Bile di bue 20 g/L
- Verde brillante 0,0133 g/L

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Preparazione

- Provette con campanelle Durham,
- Sterilizzare
- MPN

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Inoculo

- VRBG e VRBL: ml 1 di omogenato seminato per inclusione nell'agar; o col metodo dello strato superficiale;
- BGB: ml 1 di omogenato in tre provette per metodo MPN.

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Incubazione

- 30°C per 18-24h (VRB)

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Lettura

- VRB: colonie purpuree circondate da alone porpora. La fermentazione del lattosio (o del glucosio) produce un'acidificazione del terreno con viraggio dell'indicatore al rosso-viola e la precipitazione dei sali biliari.
- BGB: torbidità e gas nelle campanelle

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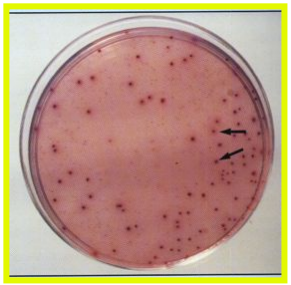
Enterobatteri su VRB



ET: enterotoxinogenic, EP: entero ve,
EI: enteroinvasive, DA: diffusely adherent

E. coli su Agar Sangue

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative,
EI: enteroinvasive, DA: diffusely adherent

Proteus

ET: enterotoxinogenic, EP
EI: enteroinvasive, DA: di

Enterobacter

ET: enterotoxinogenic, EP: ent
EI: enteroinvasive, DA: diffuse

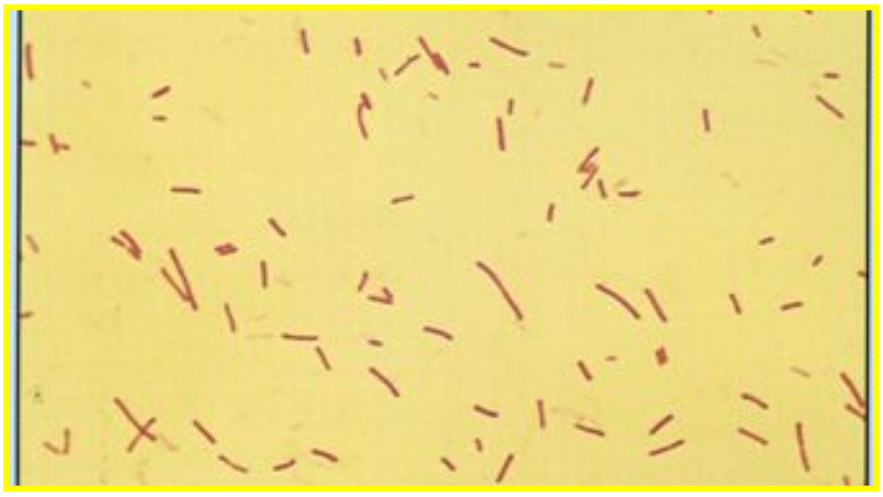
E. coli su TBX

ic, EA: enteroaggregative,

E. coli su cromogeno per Salmonella

ET: enterotoxinogenic, EP: entero
EI: enteroinvasive, DA: diffusely

Enterobatteri Gram



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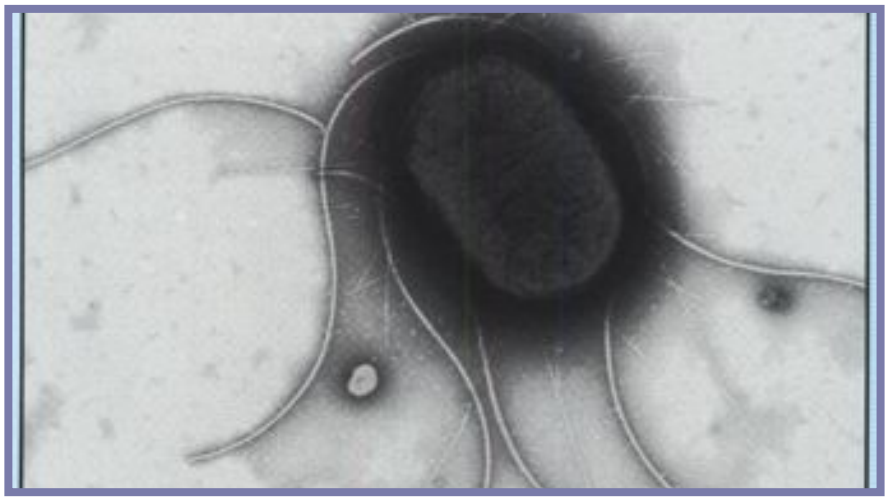
EI: enteroinvasive, DA: diffusely adherent

Enterobatteri microscopio elettronico



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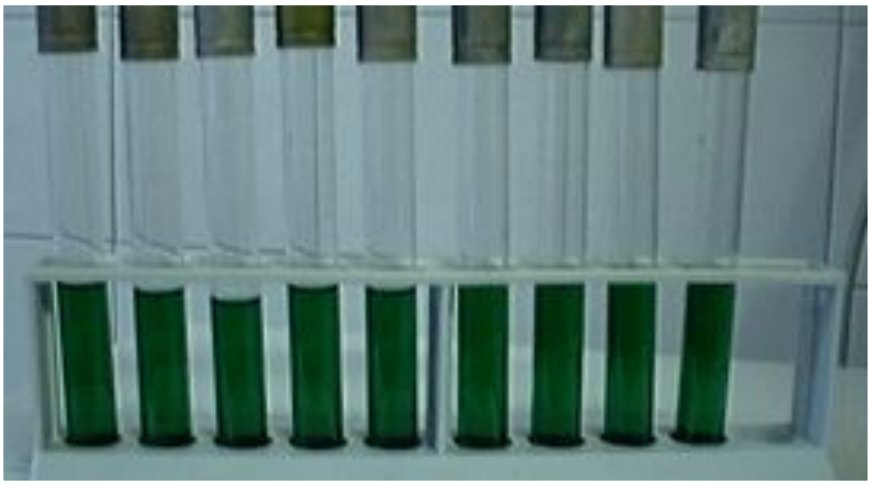
Enterobatteri su BGB

(prima dell'inoculo)



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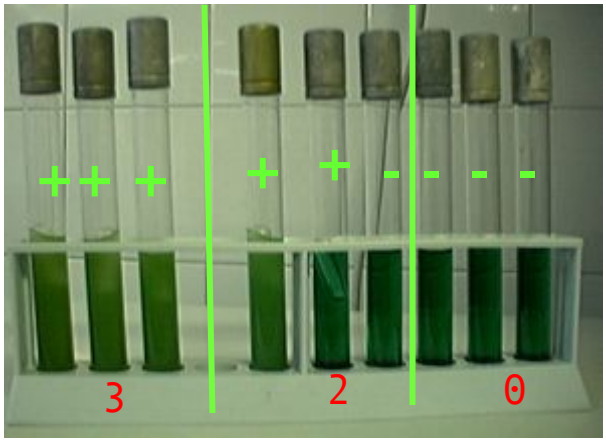
Enterobatteri su BGB



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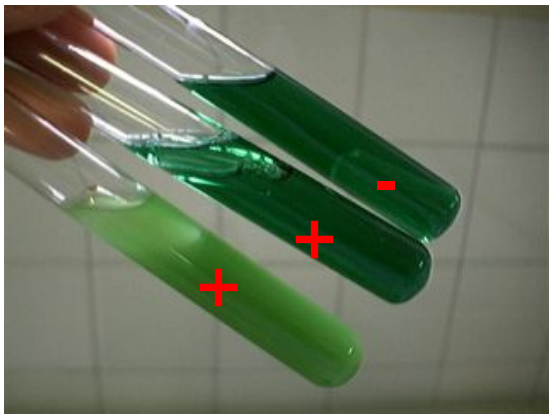
Enterobatteri su BGB



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Enterobatteri su BGB

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Metodo MPN

Inoculo corrispondente a 1 g	0,1 g	0,01 g	MPN/g
0	0	0	< 0,3
* 0	1	0	0,3
1	0	0	0,4
* 1	0	1	0,7
1	1	0	0,7
* 1	2	0	1,1
2	0	0	0,9
* 2	0	1	1,4
2	1	0	1,5
* 2	1	1	2
2	2	0	2,1
3	0	0	2,3
* 3	0	1	4
3	1	0	4
3	1	1	7
3	2	0	9
3	2	1	15
* 3	2	2	21
3	*3*	*0*	*20*
3	3	1	50
3	3	2	110
3	3	3	>110

ET: enterotoxinogenic, EP: enteropathogenic, EI eroaggregative, EI: enteroinvasive, DA: diffusely adherent

Sviluppo a 44°C e produzione di indolo

- Da tutte le provette positive a 30°C effettuare un passaggio su acqua peptonata e su BGB.

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Incubazione

- 44,5°C per 24.

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Lettura

- Aggiunta di Reattivo di Kovacs (dimetilamminobenzaldeide).
- Produzione di indolo: **anello rosso** in superficie.

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ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

BGA

Formula	gm/litre
Proteose peptone	10.0
Yeast extract	3.0
Lactose	10.0
Sucrose	10.0
Sodium chloride	5.0
Phenol red	0.08
Brilliant green	0.0125
Agar	12.0
pH 6.9 ± 0.2 @ 25°C	

Colonial Characteristics

Non-lactose/sucrose-fermenting organisms

Red-pink-white opaque coloured colonies surrounded by brilliant red zones in the agar - most probably salmonella (but not Salmonella typhi).

Proteus and Pseudomonas species

These may grow as small red colonies.

Lactose/sucrose-fermenting organisms (normally inhibited)

Yellow to greenish-yellow coloured colonies surrounded by intense yellow-green zones in the agar - Escherichia coli or Klebsiella/Enterobacter group.

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Cromogeno per salmonella 1/3

SALMONELLA CHROMOGENIC AGAR BASE

CODE: CM1007

Salmonella Chromogenic Medium is a selective and differential agar base the identification of Salmonella species from other organisms in the family Enterobacteriaceae.

Formula	gm/litre
Special Peptone	10.0
Chromogenic mix	26.0
Agar	12.0
pH 7.2 ± 0.2	

SALMONELLA SELECTIVE SUPPLEMENT

Code: SR0194

Vial contents (each vial is sufficient for 500 ml of medium)	**per vial**	**per litre**
Cefsulodin	6.0 mg	12.0 mg
Novobiocin	2.5 mg	5.0 mg

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Cromogeno per salmonella 2/3

Salmonella Chromogenic Medium is designed to identify Salmonella species based on their utilisation of one chromogenic substrate. Their inability to utilise another chromogenic substrate, that most other members of the family Enterobacteriaceae can utilise, enables rapid and reliable identification of Salmonella species. Traditionally, media used to differentiate Salmonella species from other members of the family Enterobacteriaceae depend upon the ability of Salmonella species to produce hydrogen sulphide coupled with their inability to ferment lactose[2,3]. These are, however, essentially inadequate methods, with a significant number of the 2000 plus species not exhibiting these characteristics. In recent times chromogenic media have been developed for the rapid and more reliable identification of Salmonella.

Salmonella Chromogenic Agar Base combines two chromogens for the detection of Salmonella sp., 5-Bromo-6-Chloro-3-Indolyl caprylate (Magenta-caprylate) and 5-Bromo-4-Chloro-3-Indolyl b-D galactopyranoside (X-gal). X-gal is a substrate for the enzyme b-D-galactosidase. Hydrolysis of the chromogen, Mag-caprylate, by lactose negative Salmonella species results in magenta colonies.

The medium contains bile salts to inhibit the growth of Gram-positive organisms and the addition of the Salmonella Selective Supplement SR0194 is recommended to increase the selectivity of the medium. This uses novobiocin to inhibit Proteus growth and cefsulodin to inhibit growth of Pseudomonads.

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Cromogeno per salmonella 3/3

Species	Colony Colour	Colony Diameter	Colony Morphology
Salmonella spp.	Magenta	1.0 mm	Raised, smooth
Salmonella typhi	Magenta	1.0 mm	Raised, smooth
Salmonella paratyphi	Magenta	1.0 mm	Raised, smooth
Salmonella arizonae	Magenta / blue †	1.5 mm	Raised, smooth
Salmonella gallinarum	Magenta	0.75 mm	Raised, smooth
Salmonella indiana	Blue †	1.0 mm	Raised, smooth
Escherichia coli	Blue	1.0 mm	Raised, smooth
Enterobacter spp.	Blue	1.5 mm	Raised, smooth
Klebsiella spp.	Blue	3.0 mm	Raised, mucoid
Citrobacter spp.	Blue	1.5 mm	Raised, mucoid
Proteus spp.	No growth / straw	0.25 mm	-
Pseudomonas spp.	No growth	-	-
Shigella sonnei	Blue	4.0 mm	Undulate
Shigella dysenteriae	Magenta	1.0 mm	Raised

† Colour of colonies is a presumptive identification as it is dependent on enzyme activity. Some strains of *Salmonella arizonae* and *Salmonella indiana* can appear as blue colonies. In addition, some *Shigella* spp. can appear as magenta colonies. Further confirmatory tests are required.

Citrobacter – Escherichia – Enterobacter
Salmonella – Proteus – Serratia - Pseudomonas

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XLD 1/4

Formula	gm/litre
Yeast extract	3.0
L-Lysine HCl	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5
pH 7.4 ± 0.2 @ 25°C	

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XLD 2/4

It relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide for the primary differentiation of shigellae and salmonellae from non-pathogenic bacteria. Rapid xylose fermentation is almost universal amongst enteric bacteria, except for members of the Shigella, Providencia and Edwardsiella genera. Xylose is thus included in the medium so that Shigella spp. may be identified by a negative reaction. almonella spp. are differentiated from non-pathogenic xylose fermenters by the incorporation of lysine in the medium. Salmonellae exhaust the xylose and decarboxylate the lysine, thus altering the pH to alkaline and mimicking the Shigella reaction. However, the presence of Salmonella and Edwardsiella spp. is differentiated from that of shigellae by a hydrogen sulphide indicator. The high acid level produced by fermentation of lactose and sucrose, prevents lysine-positive coliforms from reverting the pH to an alkaline value, and non-pathogenic hydrogen sulphide producers do not decarboxylate lysine. The acid level also prevents blackening by these micro-organisms until after the 18-24 hour examination for pathogens. Sodium desoxycholate is incorporated as an inhibitor in the medium. The concentration used allows for the inhibition of coliforms without decreasing the ability to support shigellae and salmonellae.

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XLD 3/4

Colonial Appearances

Organism	Appearance
Salmonella, Edwardsiella	Red colonies with black centres
Shigella, Providencia, H_2S-negative *Salmonella* (e.g. *S. paratyphi A*)	Red colonies
Escherichia, Enterobacter, Klebsiella, Citrobacter, Proteus, Serratia	Yellow, opaque colonies

Note
Red colonies may occur with some *Proteus* and *Pseudomonas* species.

Salmonella
Escherichia, Enterobacter, Citrobacter, Proteus
Pseudomonas

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XLD 4/4
spiegazione

fermentazione xilosio → <pH: giallo
decarbossilazione lisina →> pH: rosso
produzione di H2S → nero

Salmonella esaurisce lo xilosio e poi usa la lisina → pH alcalino (di fatto mima il comportamento di Shigella che non usa lo xilosio).
Produce H2S e si differenzia da Shigella per avere il centro della colonia nero.
I coliformi, invece determinano una imponente acidificazione (eventuali stipiti che usano la lisina non riescono ad alcalinizzare e il terreno resta giallo). Eventuali stipiti H2S + non usano la lisina (la forte acidità impedisce l'annerimento per 24h). Oltre le 24 h il neo di H2S si dissolve

ET: enterotoxinogenic, EP: enteropathogenic, EH: enterohemorragic, EA: enteroaggregative, EI: enteroinvasive, DA: diffusely adherent

Material Safety Data Sheet

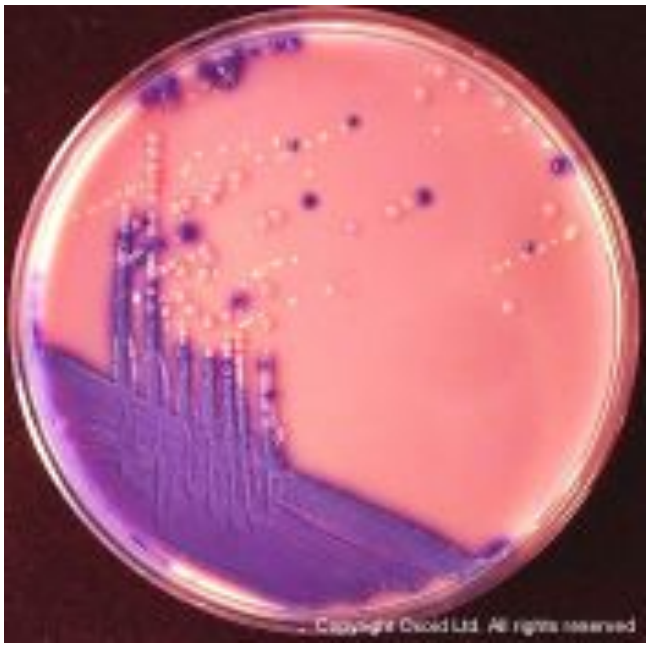
Click here to download Material Safety Data Sheets for this product.

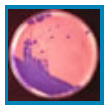
Organisms

Organisms this product works with:

Escherichia coli

Dehydrated Culture Media





close image gallery

Featured Organism: Klebsiella

***BRILLIANCE* E. COLI/COLIFORM AGAR**

Code: CM0956

Brilliance™ E. coli/coliform Agar (formerly Chromogenic E. coli/coliform Agar) is a chromogenic medium to aid differentiation between Escherichia coli and other coliforms in cultures produced from food and environmental samples.

Typical Formula*	**gm/litre**
Chromogenic mix	20.3
Yeast extract	3.0
Peptone	5.0
Lactose	2.5
Sodium chloride	5.0
Di-sodium hydrogen phosphate	3.5
Potassium di-hydrogen phosphate	1.5
Neutral red	0.03
Agar	15.0
pH 7.0 ± 0.2 @ 25°C	

*** Adjusted as required to meet performance standards**

Directions
Suspend 55.8g of *Brilliance* E.coli/coliform agar in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C. Mix well and pour and into sterile Petri dishes.

Description
Brilliance E.coli/coliform agar is a differential agar used for the presumptive identification of *Escherichia*

coli and coliforms from food and environmental samples. The agar base uses two enzyme substrates to differentiate between *Escherichia coli* and other coliforms. One chromogenic substrate is cleaved by the enzyme glucuronidase, which is specific for *Escherichia coli* and produced by approximately 97% of strains[1,2,3]. The second chromogenic substrate is cleaved by galactosidase, an enzyme produced by the majority of coliforms. This results in purple *Escherichia coli* colonies, as they are able to cleave both chromogenic substrates, and pink coliform colonies, as they are only able to cleave the galactosidase chromogen.

Technique
Dry the surface of the medium. Prepare the food sample by diluting as appropriate with 0.1% sterile peptone water (CM0009) and homogenise in a stomacher or laboratory blender. Pipette 0.5ml or 1.0ml of the homogenate, as appropriate, on to the plate, and spread over the surface with a glass spreader. Incubate the plates for 18-24 hours at 37°C.

Calculate the total number of coliforms per gram by multiplying purple and pink colonies by the dilution factor. The number of presumptive *Escherichia coli* is obtained by multiplying the number of purple colonies by the dilution factor.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C, and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium; Straw to straw/pink free flowing powder
Prepared medium; Opaque pink coloured gel.

Positive controls:	**Expected results**
Escherichia coli ATCC®25922*	Good growth; purple colonies
Klebsiella pneumoniae ATCC®13883*	Good growth; pink colonies
Negative control:	
Pseudomonas aeruginosa ATCC®27853*	Good growth; straw colonies

*** This organism is available as a Culti-Loop®.**

Precautions
Refer to the material safety data sheet before handling this product.
Some pathogenic strains, typically *Escherichia coli* O157:H7 are usually glucuronidase negative and therefore will not give purple colonies on this medium.

References
1. Kilian M. and Bulow P. (1976). *Acta. Pathol. Microbiol. Scand*. Sect. B 84: 245-251.
2. Kilian M. and Bulow P. (1979). *Acta. Pathol. Microbiol. Scand*. Sect. B 87: 271-276.
3. Frampton E.W., Restaino L. and Blaszko N. (1988). *J. Food Prot*. 51(5): 402-404.

Material Safety Data Sheet

Click here to download Material Safety Data Sheets for this product.

Organisms

Organisms this product works with:

Escherichia coli

For this Organism

Other products used in the isolation of Escherichia coli:

BR0050
Basic Fuchsin Indicator

DD0029
Cefpodoxime Combination Disc Kit

BR0071
MUG Supplement

CM0031
Brilliant Green Bile 2% Broth

CM0043
MRVP Medium (Clarks and Lubs Medium)

CM0451
Lauryl Tryptose Broth (Lauryl Sulphate Broth)

CM0505
MacConkey Broth Purple (US formulation)

CM0595
Tryptone Bile Agar

CM0607
Minerals Modified Glutamate Base

CM0853
E. C. Broth

CM0945
TBX Agar

CM0956
Brilliance E. coli/coliform Agar

CM0967
Modified Lauryl Tryptose Broth with MUG and added Tryptophan

CM0968
VIOLET RED BILE LACTOSE AGAR (ISO)

Dehydrated Culture Media

close image gallery

Featured Organism: Escherichia coli

TRYPTONE BILE X-GLUCURONIDE MEDIUM (TBX)

Code: CM0945

A selective, chromogenic medium for the detection and enumeration of Escherichia coli in food.

Formula	gm/litre
Tryptone	20.0
Bile Salts No. 3	1.5
Agar	15.0
X-glucuronide	0.075
pH 7.2 ± 0.2	

Directions

Suspend 36.6 g of TBX Medium in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour the medium into sterile Petri dishes.

Description

TBX Medium is based on Tryptone Bile Agar CM0595. Tryptone Bile Agar was originally formulated to improve on earlier methods used to detect *Escherichia coli* in foods[1,2] in terms of speed, reliability, better recovery from frozen samples and the detection of poor lactose fermenters.

TBX Medium builds on these advantages through the addition of a chromogenic agent - X-glucuronide - which detects glucuronidase activity. This is the same enzyme detected by MUG reagent[3], and has been shown to be highly specific for *Escherichia coli*[4]. However, approximately 3-4% of *Escherichia coli* are glucuronidase negative, notably *Escherichia coli* O157 strains[5].

Unlike MUG, where the flurophore leaches out of the cell into the surrounding agar, the released chromophore in TBX Medium is insoluble and accumulates within the cell. This ensures that coloured target colonies are easy to identify.

Most *Escherichia coli* strains can be differentiated from other coliforms by the presence of the enzyme

CM0979
E. C. Broth with MUG

CM0980
Lauryl Tryptose Broth with MUG

CM1031
Membrane Lactose Glucuronide Agar (MLGA)

CM1046
Brilliance E. coli/coliform Selective Agar

CM1115
Enterobacteria Enrichment Broth-Mossel (EP/USP/JP/BP)

CM1153
MRS (ISO) AGAR (DE MAN, ROGOSA and SHARPE)

LT0620
Foodborne Pathogens, Monograph Number 5 - E. coli Shigella

Q7085C
Escherichia coli ATCC 8739

DR0300
Dryspot Seroscreen

TD0920
VET-RPLA

TD0960
VTEC-RPLA

QB3648
BAX® REAL-TIME ESCHERICHIA COLI O157:H7 ASSAY

BO0394
MEMBRANE LAURYL SULPHATE BROTH

PO0148
MACCONKEY AGAR WITHOUT SALT

PO0149
MACCONKEY AGAR

PO0727
TBX MEDIUM

PO0745
BRILLIANCE E. COLI / COLIFORM AGAR

PO1142
MACCONKEY AGAR (EP,USP,JP,BP)

PO1226
Brilliance CRE Agar

glucuronidase. The chromogen in TBX Medium is 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (X-glucuronide), and is targeted by this enzyme. *Escherichia coli* cells are able to absorb this complex intact and intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore is coloured and builds up within the cells, causing *Escherichia coli* colonies to be coloured blue/green.

Technique

Dry the surface of the medium in the prepared plates. Dilute the food sample according to the method being followed e.g. 1:10 with Maximum Recovery Diluent CM0733. Homogenise in a stomacher or a laboratory blender.

The following incubation techniques may be used (consult the relevant standard for the complete method):

1. Pipette 0.1 ml of the homogenate on to the plate and spread over the surface with a sterile glass spreader. Incubate the plates for 24 hours at 37°C[6].

2. Pipette 0.5 ml of the homogenate on to the plate and spread over the surface with a sterile glass spreader. Incubate the plates for 4 hours at 30°C, then 18-24 hours at 44°C[7].

3. Place a cellulose membrane on to the surface of a Minerals Modified Glutamate Medium CM0607 prepared plate. Pipette 1 ml of the homogenate on to the membrane. Incubate for 4 hours at 37°C. Transfer the membrane to a TBX prepared plate and incubate for 18-24 hours at 44°C[8].

4. Pipette 1 ml of the homogenate into a sterile Petri dish. Add TBX Medium, cooled to 45°C. Mix well and allow to set. Incubate for 18-24 hours at 44°C. If the presence of stressed cells is suspected pre-incubate the plates for 4 hours at 37°C[9].

Multiply the numbers of blue/green colonies by the dilution factor and express the result as the number of *Escherichia coli* per gram of food.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.
Prepared medium: Straw coloured gel.

Quality control

Positive controls:	**Expected results**
Escherichia coli ATCC® 25922 *	Good growth; blue/green coloured colonies.
Negative control:	
Klebsiella pneumoniae ATCC® 29665	Good growth; straw coloured colonies.

* This organism is available as a Culti-Loop®

References

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5. Ratnam S., March S.B., Almed R., Bezanson G.S. and Kasatiya S. (1988) *J. Clin. Microbiol*. 26. 2006-2012.
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8. ISO 16649-1: 2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli* . Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indoyl-beta-D-glucuronide.
9. ISO 16649-2: 2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of ß-glucuronidase-positive *Escherichia coli* . Part 2: Colony-count technique a 44°C using 5-bromo-4-chloro-3-indoyl-beta-D-glucuronide.

Material Safety Data Sheet

Click here to download Material Safety Data Sheets for this product.

Optional Products

SR0172
C-T Supplement

Organisms

Organisms this product works with:

Escherichia coli O157

For this Organism

Other products used in the isolation of Escherichia coli O157:

CM0813
Sorbitol MacConkey Agar

CM0981
Sorbitol MacConkey Agar (SMAC) with BCIG

CM0989
Modified Tryptone Soya Broth

CM0990
E. C. Broth (Reduced Bile Salts)

CM1005
Cefixime Rhamnose Sorbitol MacConkey Agar Base

SR0172
C-T Supplement

SR0181
Novobiocin Supplement

SR0191
Cefixime Supplement

DR0120
Dryspot E. coli O157

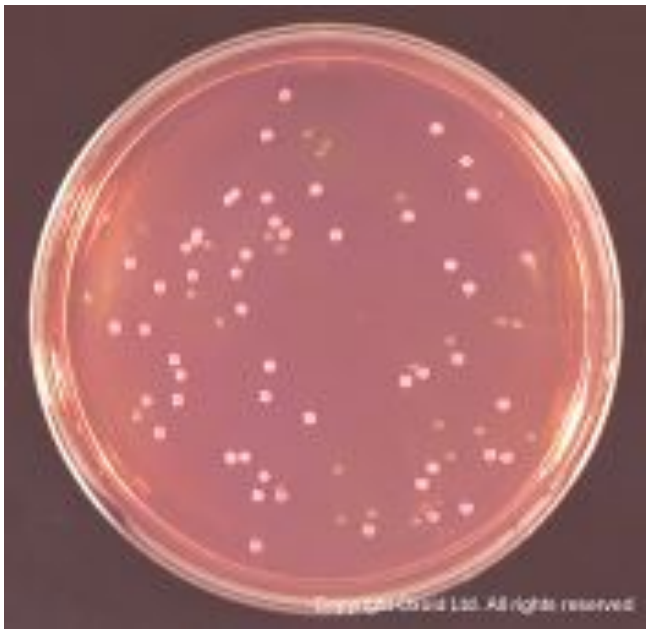
DR0300
Dryspot Seroscreen

DR0620
Escherichia coli O157 Latex Test

PO0232
SORBITOL MACCONKEY AGAR

PO0702
SORBITOL MACCONKEY AGAR WITH CEFIXIME TELLURITE

Dehydrated Culture Media



close image gallery

Featured Organism: Escherichia coli O157

SORBITOL MacCONKEY AGAR

Code: CM0813

a selective and differential medium for the detection of Escherichia coli O157

Typical Formula*	**gm/litre**
Peptone	20.0
Sorbitol	10.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
pH 7.1 ± 0.2 @ 25°C	

*** Adjusted as required to meet performance standards**

CEFIXIME-TELLURITE SUPPLEMENT

Code: SR0172

a freeze-dried supplement for use with Sorbitol MacConkey Agar for the selective isolation of Escherichia coli O157

Vial contents	per vial	per litre
Potassium tellurite	1.25mg	2.5mg

Cefixime	0.025mg	0.05mg

Directions
Suspend 51.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C. Pour into sterile Petri dishes.

If required, reconstitue one vial of C-T supplement per 500ml medium, according to instruction in the product leaflet. Aseptically add this to to cooled medium before pouring into sterile Petri dishes

Description
Escherichia coli O157 is recognised as a cause of haemorrhagic colitis, an illness characterised by bloody diarrhoea and severe abdominal pain, and haemolytic uraemic syndrome (HUS)[1, 2, 3, 4, 5], and as such, it a significant human pathogen.

Sorbitol MacConkey Agar is recommended for the isolation of pathogenic *E. coli* O157. The formulation, based on that described by Rappaport and Henig[6], is identical to MacConkey Agar No.3, except that lactose has been replaced with sorbitol. *E.coli* O157 does not ferment sorbitol and, therefore, produces colourless colonies. In contrast, most *E. coli* strains ferment sorbitol and form pink colonies. The efficiency of Sorbitol MacConkey Agar has been confirmed by March and Ratnam[7]. These workers reported a sensitivity of 100% and a specificity of 85%, and recommended the medium as a simple, inexpensive, rapid and reliable means of screening for *E. coli* O157.

Chapman and co-workers[8] added cefixime and potassium tellurite to Sorbitol MacConkey Agar to improve the selectivity of the medium. The level of potassium tellurite selects serogroup O157 from other *E. coli* serogroups and inhibits *Providencia* and *Aeromonas* species. Cefixime is inhibitory to *Proteus* spp.

The use of cefixime and tellurite in Sorbitol MacConkey Agar for isolation of *E. coli* O157:H7 is described in the FDA Bacteriological Analytical Manual [9].

Technique
1. Make up the agar according to the directions and pour into plates. If necessary dry the surface of the agar.
2. Inoculate the plates with a suspension of the test substance (food, faeces, etc) to produce separated colonies.
3. Incubate at 35°C for 24 hours. Doyle and Schoeni [10] reported that 35-37°C is the optimal temperature for growth of *Escherichia coli* O157. At 44-45.5°C growth is poor, even after 48 hours incubation.

Delay in reading plates beyond 24 hours should be avoided because the colour intensity of sorbitol-fermenting colonies fades, reducing the contrast with non-fermenting colonies. Other Gram-negative organisms including *Pseudomonas, Proteus* and *Klebsiella* species are able to grow on Sorbitol MacConkey Agar but may generally be differentiated by the appearance of their colonies.

A diagnostic reagent *Escherichia coli* O157 latex test (DR0620) is available so that instant confirmatory tests can be made from suspicious colonies.

Storage conditions and Shelf life
Dehydrated Sorbitol MaConkey Agar must be stored tightly capped in the original container at 10-30ºC.

Cefixime-Tellurite Supplement (SR0172) should be stored in the dark at temperatures below 0°C.

Oxoid Sorbitol MacConkey and Cefixime Tellurite Sorbitol MacConkey plates should be stored in the original packaging, at the temperature stated on the pack or product specification, and protected from direct light. When stored as directed, the unopened product will remain stable until the expiry date on the label.

Locally prepared media can be stored for up to 2 weeks when made from CM0813 and SR0172 according to the manufacturer's instructions and stored at 2-8ºC, out of direct sunlight and protected from dessication. A longer shelf life may be attainable, but should be validated under the relevant, local manufacturing and storage conditions.

Appearance
Dehydrated medium: Straw/pink coloured, free-flowing powder
Prepared medium: Dark red gel

Quality control

Unsupplemented

Positive control:	**Expected results**

Escherichia coli O157:H7 Non-toxigenic NCTC12900 *	Good growth; 1-2mm straw colonies
Negative control:	
Escherichia coli ATCC® 25922 *	Good growth; 1-2mm pink colonies

Supplemented with SR0172

Positive control:	**Expected results**
Escherichia coli O157:H7 Non-toxigenic NCTC12900 *	Good growth; 1-2mm straw colonies
Negative control:	
Escherichia coli ATCC® 25922 *	No growth/pinpoint-0.25mm pink colonies

*** This organism is available as a Culti-Loop®**

Precautions
Although the great majority of *Escherichia coli* O157 strains have a typical appearance on Sorbitol MacConkey Agar, some strains are atypical [11]. Sorbitol MacConkey Agar cannot be used solely to detect VTEC strains of *Escherichia coli* as some non-toxic strains will not ferment sorbitol [12].

Sorbitol MacConkey Agar and Cefixime Tellurite Sorbitol MacConkey Agar are *in vitro* diagnostic use only, by experienced microbiologists. They must not be used beyond the stated expiry date, or if the product shows any sign of deterioration.

Sterilise specimens, equipment and media properly after use.

Limitations
Micro-organisms with atypical enzyme patterns may give anomalous results.

References
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