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HiCrome™

Single Streak Rapid Differentiation Series

Single streak
24hr
Results

Confirm your identity

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5th generation Chemically Defined Microbiology Media

HiCynth™

5th Gen Chemically Defined Microbiology Media
Free from TSE / BSE / GMO

4th Gen Vegetable based dehydrated culture media
Free from TSE / BSE risk

3rd Gen Animal based dehydrated culture media

2nd Gen Dehydrated culture media prepared using raw materials

1st Gen Media preparation in lab using meat and other ingredients

HiCrome™
Single Streak Rapid Differentiation Series

Index

HiCrome™
Single Streak Rapid Differentiation Series

28 Chromogenic Media are also available in the HiCromeVeg Category where animal based nutrients have been substituted with Vegetable based nutrients

HiCrome™
Single Streak Rapid Differentiation Series

HiCromeVeg™
Single Streak Rapid Differentiation Series

Corresponding lists of Chromogenic media containing Animal peptone and HiVeg™ peptones

HiCrome™ Animal Peptone Based Media	HiCrome™ Veg-Peptone Based media
M1651 - HiCrome™ Bacillus Agar	#MCD1651 - HiCrome™ Bacillus HiCynth™ Agar
M1297A- HiCrome™ Candida Differential Agar	#MV1297A- HiCrome™ Candida Differential HiVeg™ Agar
M1456A- HiCrome™ Candida Differential Agar Base, Modified	MV1456A- HiCrome™ Candida Differential HiVeg™ Agar Base, Modified
M1991I - HiCrome™ Chromogenic Coliform Agar (CCA Agar)	#MCD1991I - HiCrome™ Chromogenic Coliform HiCynth™ Agar
M1300 - HiCrome™ Coliform Agar w/ SLS	MV1300 - HiCrome™ Coliform HiVeg™ Agar w/SLS
M1293 - HiCrome™ ECC Agar	MV1293 - HiCrome™ ECC HiVeg™ Agar
M1294 - HiCrome™ ECC Selective Agar Base	MV1294 - HiCrome™ ECC Selective HiVeg™ Agar Base
M1488 - HiCrome™ ECD Agar w/MUG	MV1488 - HiCrome™ ECD HiVeg™ Agar w/ MUG
M1295 - HiCrome™ E. coli Agar	MV1295 - HiCrome™ E. coli HiVeg™ Agar
M1575A - HiCrome™ EC 0157:H7 Selective Agar Base	MV1575A - HiCrome™ EC 0157:H7 Selective HiVeg™ Agar Base
M1577- HiCrome™ Enterobacter sakazakii Agar	MV1577- HiCrome™ Enterobacter sakazakii HiVeg™ Agar
M1641 - HiCrome™ Enterobacter sakazakii Agar, Modified	MV1641 - HiCrome™ Enterobacter sakazakii HiVeg™ Agar, Modified
M1376 - HiCrome™ Enterococci Broth	MV1376 - HiCrome™ Enterococci HiVeg™ Broth
M1580- HiCrome™ Enterococcus faecium Agar Base	MV1580- HiCrome™ Enterococcus faecium HiVeg™ Agar Base
M1466 - HiCrome™ Improved Salmonella Agar	#MV1466 - HiCrome™ Improved Salmonella HiVeg™ Agar
M1573 - HiCrome™ Klebsiella Selective Agar Base	MV1573 - HiCrome™ Klebsiella Selective HiVeg™ Agar Base
M1393 - HiCrome™ MM Agar	MV1393 - HiCrome™ MM HiVeg™ Agar
M1674 - HiCrome™ MeReSa Agar Base	MV1674 - HiCrome™ MeReSa HiVeg™ Agar Base
M1712 - HiCrome™ Nickels and Leesment Agar Base	MV1712 - HiCrome™ Nickels and Leesment HiVeg™ Agar Base
M1296 - HiCrome™ Salmonella Agar	MV1296 - HiCrome™ Salmonella HiVeg™ Agar
M1353 - HiCrome™ UTI Agar	#MV1353 - HiCrome™ UTI HiVeg™ Agar
M1418 - HiCrome™ UTI Agar, Modified	MV1418 - HiCrome™ UTI HiVeg™ Agar, Modified
M1505 - HiCrome™ UTI Selective Agar	MV1505 - HiCrome™ UTI Selective HiVeg™ Agar
M1682 - HiCrome™ Vibrio Agar	#MV1682 - HiCrome™ Vibrio HiVeg™ Agar
M1469 - HiFluoro Pseudomonas Agar Base	MV1469 - HiFluoro Pseudomonas HiVeg™ Agar Base
M1826- Coliform Broth w/SLS	MV1826 - Coliform HiVeg™ Broth w/SLS
M1540 - L.mono Differential Agar Base	#MV1540 - L.mono Differential HiVeg™ Agar Base
M1354 - M-CP Agar Base	MV1354 - M-CP HiVeg™ Agar Base
M1465 - Rapid HiColiform Agar	MV1465 - Rapid HiColiform HiVeg™ Agar
M1453 - Rapid HiColiform Broth	MV1453 - Rapid HiColiform HiVeg™ Broth
M1082 - Salmonella Differential Agar, Modified (Twin Pack)	MV1082 - Salmonella Differential HiVeg™ Agar, Modified (Twin Pack)
M1078 - Salmonella Differential Agar (Twin Pack) (RajHans Medium)	MV1078 - Salmonella Differential HiVeg™ Agar (Twin Pack) (RajHans Medium)

HiCrome™ HiCynth™ Media available

HIMEDIA®

Conventional culture media procedures, though a reliable way of detecting micro-organisms lack speed and accuracy of isolation and differentiation so critical in dealing with pathogenic microorganisms.

HiMedia's HiCrome™ range of culture media employing the chromogen technology of visual identification significantly removes the guesswork out of identification and differentiation, thereby obviating the need for subculturing, thus saving time.

The methodology is simple and precisely designed for each bacteria. Organisms are identified through simple enzymatic reactions specific to their species, yielding visually distinct colours.

Over the past decade chromogenic media have been well researched and documented to merit incorporation in standard microbiology lab protocols.

HiMedia have been a part of this global revolution in diagnostic microbiology where we have developed the largest range of chromogenic media, and the research continues.

We also offer the range of these HiCrome™ media in the HiCromeVeg™ and HiCrome™ HiCynth™ version, wherein the animal based nutrients have been substituted with their veg-based counterparts or chemically defined peptones.

Chromogenic Media Index / Cross References

Equivalent Media of various Brands

Organisms	Page No.	Code	HiMedia	Chromagar	Merck	Oxoid	Remel
E.coli and Total coliforms	6	M1293	HiCrome™ ECC Agar	CHROMagar™ ECC	—	Brilliance™ E. coli/ coliform Agar	—
	7	M1294	HiCrome™ ECC Selective Agar	—	Chromocult Coliform Agar	Brilliance™ E. coli/coliform Selective Agar	—
	8	M1300	HiCrome™ Coliform Agar w/ SLS	—	ChromoCult® Coliform Agar es	—	—
	9	M1826	HiCrome™ Coliform Broth w/ SLS	—	—	—	—
	10	M1951	HiCrome™ M-Coliform Differential Agar Base	—	—	—	—
	11	M1426	M-E. coli Broth	—	—	—	—
	12	M1569	HiCrome™ M-Lauryl Sulphate Agar	—	—	—	—
	13	M1991I	HiCrome™ Chromogenic Coliform Agar (CCA Agar)	CHROMagar™ CCA	ChromoCult® Coliform Agar		
	15	M1832	HiCrome™ Coliform Agar Modified	—	—	—	—
	64	M1663	HiCrome™ PA Broth	—	—	—	—
	65	M1850	HiColiform™ Broth, Modified	—	—	—	—
	66	M1465 / M1453	Rapid HiColiform Agar / Broth	AquaCHROM™ ECC	Fluorocult LMX Broth Modified (Manafi and Ossmer)	—	—
	16	M1571 / M1713	HiCrome™ M-TEC Agar / HiCrome™ M-TEC Broth	m-TEC Agar	—	—	—
E.coli	14	M1295 / M1295I	HiCrome™ E. coli Agar	CHROMagar™ E. coli / CHROMagar™ TBX	ChromoCult® TBX-Agar	Tryptone Bile X-Glucuronide Medium (TBX)	—
	67	M1488	HiCrome™ ECD Agar w/ MUG	—	—	—	—
Escherichia coli O157:H7	17	M1340	HiCrome™ MacConkey Sorbitol Agar Base	CHROMagar™ .0157	—	MacConkey Sorbitol Agar (with chromogenic substrate)	Sorbitol MacConkey Agar w/BCIG
	18	M1574A	HiCrome™ EC 0157:H7 Agar	CHROMagar™ .0157	—	—	—
	19	M1575A	HiCrome™ EC 0157:H7 Selective Agar Base*	—	—	—	—
	20	M1598	HiCrome™ Enrichment Broth Base for EC 0157:H7	—	—	—	—
	21	M1862	HiCrome™ Modified EC0157:H7 Selective Agar Base	—	—	—	—
Enterobacter sakazzakii	22	M1577 M1641	HiCrome™ Enterobacter sakazakii Agar, Modified (HiCrome™ Cronobacter sakazakii Agar, Modified)	CHROMagar™ E.sakazakii	ChromoCult® Enterobacter sakazakii Agar	Brilliance Enterobacter sakazakii Agar	Chromo E. sakazakii Medium
Salmonella species	23	M1078 / M1082	Salmonella Differential Agar (RajHans Medium / Modified) (Twin pack)	Rambach Agar	Rambach Agar	—	—
	24	M1633/ M1634	HiCrome RajHans Medium/ Modified (Salmonella Agar/ Modified)	Rambach Agar	Rambach Agar	Rambach Agar	—
	25	M1296 / M1466	HiCrome™ Salmonella Agar / HiCrome™ Improved Salmonella Agar	CHROMagar™ Salmonella	—	Salmonella Chromogenic Agar Base	Salmonella Chromogenic Agar
	26	M1842	HiCrome™ Selective Salmonella Agar Base	CHROMagar™ Salmonella Plus	—	Brilliance Salmonella Agar Base	
	27	M1393	HiCrome™ MM Agar	—	—	—	—
	28	M1816	HiCrome™ MM Agar Modified	—	—	—	—
Klebsiella species	29	M1573	HiCrome™ Klebsiella Selective Agar Base	—	—	—	—
ESBL/ Carbapenem resistant	30	M1829	HiCrome™ ESBL Agar Base	CHROMagar™ ESBL	—	Brilliance ESBL Agar	—

Organisms	Page No.	Code	HiMedia	Chromagar	Merck	Oxoid	Remel
	31	M1831	HiCrome™ KPC Agar Base	CHROMagar™ KPC	—	Brilliance CRE Agar	—
Vibrio species	32	M1682	HiCrome™ Vibrio Agar	CHROMagar™ Vibrio	—	—	—
UTI Infections	33	M1418	HiCrome™ UTI Agar, modified	—	—	—	—
	33	M1505	HiCrome™ UTI Selective Agar	—	—	—	—
	34	M1353 / M1353R	HiCrome™ UTI Agar	CHROMagar™ Orientation	—	Brilliance UTI Clarity Agar Brilliance UTI Agar	Chromogenic UTI Medium
	35	M1600	HiCrome™ Universal Differential Medium	—	—	—	—
	36	M2010	HiCrome Mueller Hinton Agar	CHROMagar™ Orientation	—	—	—
Enterococcus	37	M1414 / M1376	HiCrome Enterococci Agar / Broth	Aquachrom™ Enterococcus	Chromocult Enterococci Broth / ChromoCult® enterococci Agar	—	—
	38	M1580	HiCrome™ Enterococcus faecium Agar Base	—	—	—	—
	39	M1840	HiCrome™ Strep B Selective Agar Base	CHROMagar™	—	Brilliance GBS Agar	—
	40	M1966	HiCrome™ Strep B Selective Agar Base, Modified	CHROMagar™ Strep B			
VRE	41	M1830	HiCrome™ VRE Agar Base	CHROMagar™ VRE blue	—	Brilliance VRE Agar	—
	42	M1925	HiCrome™ VRE Agar Base, Modified	CHROMagar™ VRE	—	—	—
Listeria species	43	M1417/ M1417F	HiCrome™ Listeria Agar Base, Modified	—	—	—	—
	44	M1540	L.mono Differential Agar Base	CHROMagar™™ ALOA	ChromoCult® listeria Selective Agar (ALOA®)	—	—
	45	M1924	HiCrome™ L. mono Rapid Differential Agar Base	CHROMagar™ Listeria	Chromocult Listeria Selective Agar	Brilliance™ Listeria Agar	—
	46	M2009	HiCrome™ L.mono Differential Agar Base	—	—	Brilliance™ Listeria Agar Base	—
Staphylococcus	47	M1468	HiCrome™ Aureus Agar Base	—	—	—	—
	48	M1837	HiCrome™ Staph Agar Base, Modified	CHROMagar™ Staph aureus	—	Brilliance Staph 24	—
	49	M1931	HiCrome™ Staph Selective Agar	—	—	—	—
MRSA/ MRSE	50	M1674	HiCrome™ MeReSa Agar Base	CHROMagar™ MRSA	—	—	—
	51	M1953	HiCrome™ MRSA Agar Base, Modified	—	—	Brilliance MRSA Agar	—
	52	M1974	HiCrome™ Rapid MRSA Agar Base			Brilliance MRSA 2 Agar	
Bacillus	53	M1651	HiCrome™ Bacillus Agar	CHROMagar™ B.cereus	—	Brilliance Bacillus Cereus Agar	—
Clostridium species	54	M1354	M-CP Agar Base	—	—	—	—
Lactic acid bacteria	55	M1712	HiCrome™ Nickels and Leesment Medium	—	—	—	—
Bifidobacterium species	56	M1960	HiCrome™ Bifidobacterium Agar				
Acinetobacter	57	M1938	HiCrome™ Acinetobacter Agar Base	CHROMagar™ Acinetobacter	—	—	—
Yeasts and Moulds	58	M1297A / M1456A	HiCrome™ Candida Differential Agar / Base, Modified	CHROMagar™ Candida	—		
	59	M1297AR	HiCrome™ Candida Differential Agar Base			Brilliance Candida Agar	Chromogenic Candida Agar
	60	M1467	HiCrome™ OGYE Agar Base	—	—	—	—
	61	M1985	HiCrome™ Malassezia Agar Base (Twin Pack)	CHROMagar™ Malassezia			
Pseudomonas	68	M1469	HiFluoro™ Pseudomonas Agar Base	—	—	—	—

HiCrome™ ECC Agar

Recommended as a differential medium for presumptive identification of *Escherichia coli* and other coliforms in food and environmental samples other coliforms in food and environmental samples

M1293

Composition **

Ingredients	Grams/Litre
Peptone, special	5.00
Yeast extract	3.00
Lactose	2.50
Disodium hydrogen phosphate	3.50
Potassium dihydrogen phosphate	1.50
Sodium chloride	5.00
Chromogenic mixture	20.30
Neutral red	0.03
Agar	15.00

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.83 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Escherichia coli, a member of the family *Enterobacteriaceae* is a part of normal flora of the intestinal tract of humans and a variety of animals. Although most of *E. coli* does not cause gastrointestinal illnesses, certain groups of *E. coli* can cause life-threatening diarrhoea and severe sequelae or disability (1). HiCrome™ ECC Agar is a differential medium recommended for the presumptive identification of *E. coli* and other coliforms in food and environmental samples (2). The medium contains two chromogens. One of the chromogen is cleaved by the enzyme glucuronidase produced by *E. coli* to give blue to purple coloured colonies whereas the other chromogen is cleaved by the enzyme galactosidase, produced by majority of coliforms, resulting in the formation of rose-pink coloured colonies (3, 4).

Peptone special, yeast extract provide nitrogenous substances, carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Lactose is the fermentable carbohydrate, which aids in detecting lactose fermenters with neutral red as an indicator. Disodium hydrogen phosphate and potassium dihydrogen phosphate buffers the medium well. Sodium chloride maintains the osmotic equilibrium. Dry the surface of plate medium before use.

Dilute the food sample 1: 5 or 1: 10 with 0.1% sterile Peptone Water (M028) and homogenize in a blender or a stomacher. Spread 0.5 ml or 1.0 ml of the homogenate over the agar surface with a sterile glass spreader and incubate the plates at 35-37°C for 18-24 hours. Count the blue/purple colonies and multiply with the dilution factor. The number of *E. coli* is reported per gram of food. The medium should be used only for in-vitro diagnostic purpose. Wear mask while handling the dehydrated product and avoid contact with eyes.

Quality Control

Appearance of Powder : Light yellow to pink coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Reddish pink coloured, opaque gel forms in Petri plates.

Reaction : Reaction of 5.58% w/v aqueous solution at 25°C. pH: 6.8 ± 0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum CFU)	Growth	Recovery of colony	Colour
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=70%	blue / purple
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=70%	rose / pink
Pseudomonas aeruginosa (27853) (00025*)	50-100	good - luxuriant	>=70%	straw
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	>=70%	pink

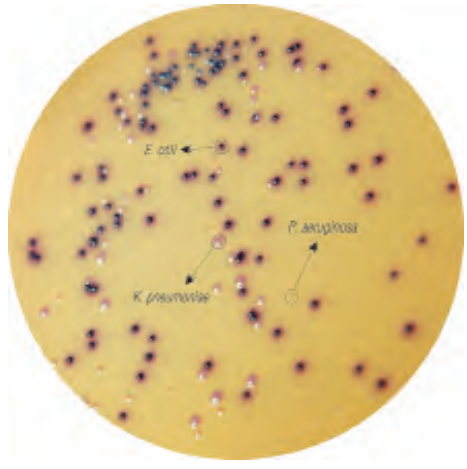
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Doyle M. P., (Ed.), 1989, Foodborne Bacterial Pathogens, Marcel Dekker, New York
2. Frampton E.W., Restaino L. and Blaszko N., 1988, J. Food Prot., 51:402.
3. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
4. Kilian M. and Bülow P., 1979, Acta. Pathol. Microbiol. Scand., Sect. B, 87:271.



M1293 – HiCrome™ ECC Agar

HiCrome™ ECC Selective Agar Base

Recommended for detection of *Escherichia coli* and coliforms in water and food samples.

M1294

Composition **

Ingredients	Grams/Litre
Peptone, special	6.00
Tryptone	3.30
Sodium dihydrogen phosphate	0.60
Disodium hydrogen phosphate	1.00
Sodium chloride	2.00
Sodium pyruvate	1.00
L-Tryptophan	1.00
Sorbitol	1.00
Tergitol-7® (Sodium heptadecyl sulphate)	0.15
Chromogenic mixture	0.43
Agar	10.00

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.48 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, selective medium can be prepared by aseptically adding the rehydrated contents of 1 vial of HiCrome™ ECC Selective Supplement (FD190). Mix well and pour into sterile Petri plates. Medium may show haziness, but it does not affect the performance of the medium.

Principle and Interpretation

HiCrome™ ECC Selective Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (1, 3). The chromogenic mixture contains two chromogenic substrates. The enzyme β-D-galactosidase produced by coliforms cleaves one of the chromogen to form salmon to red coloured colonies (4). The enzyme β-D-glucuronidase produced by *E. coli,* cleaves X-glucuronide, the other chromogen (5). *E. coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. Addition of L- Tryptophan improves the indole reaction, thereby increasing the detection reliability.

Peptone special, Tryptone and sodium pyruvate provide nitrogenous substances carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients for the organisms. Sorbitol is the fermentable carbohydrate. Phosphates buffer the medium. The media formulation helps even the sublethally injured coliforms to recover and grow rapidly. Tergitol inhibits gram-positive as well as some gram-negative bacteria other than coliforms (3). Addition of HiCrome™ ECC Selective Supplement (FD190) helps to inhibit the accompanying heterogenous microflora.

The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. Other gram negative bacteria forms colourless colonies, except some organisms which are β-glucuronidase positive. β-glucuronidase positive organisms gives light blue to turquoise colonies. Glucuronidase is present in 94–96% of *E. coli* strains and in some *Salmonella, Shigella* and *Yersinia* spp (2). To confirm *E. coli*, add a drop of Kovac's reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Quality Control

Appearance of powder	:	Light yellow to pink coloured, homogeneous, free flowing powder.
Gelling	:	Firm, comparable with 1.0% Agar gel.
Colour and Clarity of prepared medium	:	Light pink coloured, clear to slightly opalescent gel forms in Petri plates
Reaction	:	Reaction of 2.65% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2.
Cultural Response	:	Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	Indole
Escherichia coli (25922) (00013*)	50-100	good-luxuriant	>=50%	dark blue to violet	+
Escherichia coli O157:H7 (NCTC 12900)	50-100	luxuriant	>=50%	salmon to red	+
Enterobacter aerogenes (13048) (00175*)	50-100	luxuriant	>=50%	salmon to red	-
Citrobacter freundii (8090)	50-100	luxuriant	>=50%	salmon to red (big)	-
Salmonella Enteritidis (13076) (00030*)	50-100	good	40-50%	colourless	-
Shigella flexneri (29508)	50-100	good	40-50%	light blue to turquoise	-
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%		

Key : + : positive reaction, confirmation of red colour around the colony by addition of Kovac's reagent (R008) - : negative reaction.

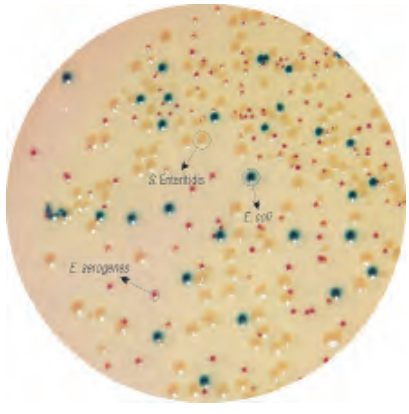
* : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Frampton E. W., Restaino L. and Blaszko N., 1988, J. Food Prof., 51:402.
2. Hartman, P.A., 1989 Turano, A. (Ed.), Brixia Academic Press, Brescia, Italy, pp. 290–308.
3. Kilian M. and Bulow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
4. LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur 102:267.



M1294 – HiCrome™ ECC Selective Agar Base

HiCrome™ Coliform Agar w/ SLS

Recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples.

M1300

Composition **

Ingredients	Grams/Litre
Peptone, special	3.00
Sodium chloride	5.00
Dipotassium hydrogen phosphate	3.00
Potassium dihydrogen phosphate	1.70
Sodium pyruvate	1.00
L-Tryptophan	1.00
Sodium lauryl sulphate	0.10
Chromogenic mixture	0.20
Agar	12.00

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Add 5mg/ l novobiocin before autoclaving the medium, when a high number of gram-positive accompanying bacteria are expected. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Coliform Agar w/ SLS is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (5). Peptone special and Sodium pyruvate provides nitrogenous, compounds, carbonaceous, long chain amino acid and other essential growth nutrients. The phosphates buffer the medium well. The medium composition helps even the sublethally injured coliforms to grow rapidly. Sodium lauryl sulphate inhibits gram-positive organisms.

The chromogenic mixture contains two chromogenic substrates. The enzyme β-galactosidase produced by coliforms cleaves one chromogen, resulting in the salmon to red colouration of coliform colonies. The enzyme β -glucuronidase produced by *E. coli*, cleaves X-glucuronide. *E. coli* forms dark blue to violet coloured colonies due to cleavage of both the chromogens (1, 3, 4). The addition of L-Tryptophan improves the indole reaction, thereby increasing detection reliability in combination with the two chromogens. Other gram negative bacteria forms colourless colonies, except some organisms which are β-glucuronidase positive. β-glucuronidase positive organisms gives light blue to turquoise colonies. GUD is present in 94–96% of *E. coli* strains and in some *Salmonella, Shigella* and *Yersinia* spp (2). To confirm *E. coli,* add a drop of Kovac's reagent (R008) on the dark-blue to violet colony. Formation of cherry-red colour indicates positive reaction.

Quality Control

Appearance of powder : Light yellow to beige coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium : Colourless, clear to very slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 2.70% w/v aqueous solution at 25°C. pH:6.8 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24 hours (48 hours if necessary).

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	Indole
Escherichia coli (25922) (00013*)	50-100	good - luxuriant	>=50%	dark blue /violet	+
Enterobacter cloacae (23355)	50-100	good - luxuriant	>=50%	salmon to red	-
Citrobacter freundii (8090)	50-100	good - luxuriant	>=50%	salmon to red	-
Klebsiella pneumoniae (13883)	50-100	good - luxuriant	>=50%	light pink	-
Salmonella Enteritidis (13076) (00030*)	50-100	good	40-50%	colourless	-
Shigella flexneri (12022)	50-100	good	40-50%	colourless	-
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	-	-

Key : + : positive reaction, confirmation of red colour around the colony by addition of Kovac's reagent (R008) - : negative reaction.

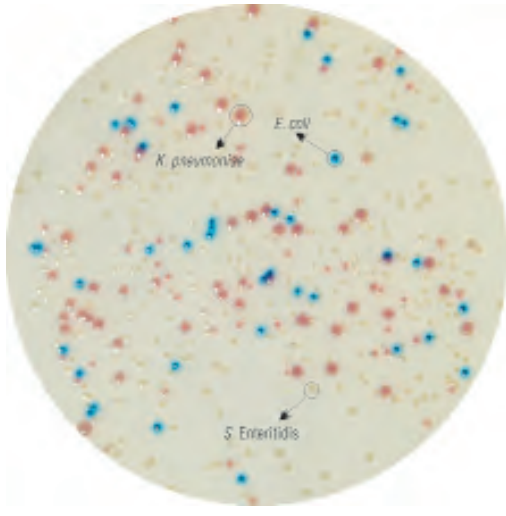
* : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Frampton E.W., Restaino L. and Blaszko N., (1988), J. Food Prot., 51:402.
2. Hartman, P.A., 1989 Turano, A. (Ed.), Brixia Academic Press, Brescia, Italy, pp. 290–308.
3. Kilian M. and Bülow P., (1976), Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
4. LeMinor L. and Hamida F., (1962), Ann. Inst. Pasteur (Paris), 102:267.
5. Manafi M. and Kneifel W., (1989), Zentralbl. Hyg., 189:225.



M1300 HiCrome™ Coliform Agar w/SLS

Coliform Broth w/SLS

Recommended for detection of *E.coli* and other *Enterobacteriaceae* in water samples.

M1826

Composition **

Ingredients	Grams/Litre
Special peptone	3.00
Sodium chloride	5.00
Dipotassium hydrogen phosphate	3.00
Potassium dihydrogen phosphate	1.70
Sodium pyruvate	1.00
L-Tryptophan	1.00
Sodium lauryl sulphate	0.10
Chromogenic mixture	0.30

Final pH (at 25°C) 6.8 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.10 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle and Interpretation

Coliform Broth w/SLS is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (4).

Special peptone serves as source of nitrogen, carbon and long chain amino acids and essential growth nutrients to the organisms. The phosphates in the medium buffers the medium. Sodium chloride maintains the osmotic balance. Sodium lauryl sulphate inhibits the gram positive organisms. L-Tryptophan in the medium improves the indole reaction. The enzyme β-glucuronidase produced by *E.coli* cleaves X-glucuronide thus imparting blue colour to the medium (1, 2, 3). Formation of cherry red colour after addition of few drops (0.5ml) of Kovac's reagent (R008) to the medium indicates the presence of *E.coli*

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium : Cream, clear to slightly opalescent solution, may have slight precipitate.

Reaction : Reaction of 1.51% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Colour of Medium
Citrobacter freundii (8090)	50-100	luxuriant	colourless
Escherichia coli (25922) (00013*)	50-100	luxuriant	blue
Escherichia coli (35218)	50-100	luxuriant	blue
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	—
Klebsiella pneumoniae (13883)	50-100	luxuriant	colourless
Salmonella Enteritidis (13076) (00030*)	50-100	good	colourless
Shigella flexneri (12022)	50-100	luxuriant	colourless
Staphylococcus aureus (25923)	>=10^3	inhibited	—
Staphylococcus aureus (6538)	>=10^3	inhibited	—

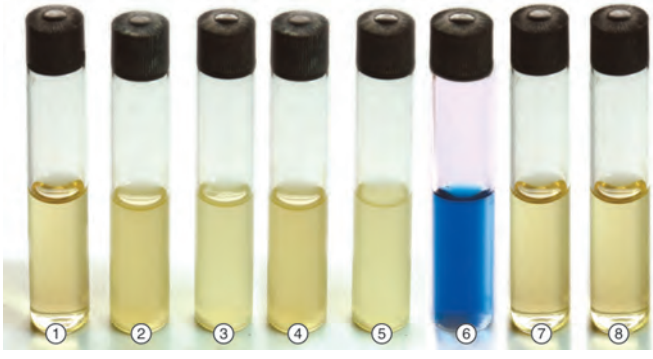
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Frampton E. W., Restaino L. and Blaszko N., 1988, J. Food Prot., 51:402.
2. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
3. LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur (Paris), 102:267.
4. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.



M1826 Coliform Broth w/SLS
1. Control
2. *Citrobacter freundii* ATCC 8090
3. *Klebsiella pneumoniae* ATCC 13883
4. *Salmonella* Enteritidis ATCC 13076
5. *Shigella flexneri* ATCC 12022
6. *Escherichia coli* ATCC 25922
7. *Enterococcus faecalis* ATCC 29212
8. *Staphylococcus aureus* ATCC 25923

HiCrome™ M-Coliform Differential Agar Base

Selective and differential agar recommended for the detection of coliform bacteria using membrane filtration technique.

M1951

Composition **

Ingredients	Grams/Litre
Peptone	5.00
Tryptone	10.00
Yeast extract	3.00
Lactose	12.50
Sodium deoxycholate	0.15
Aniline Blue	0.10
Chromogenic substrate	0.50
Agar	15.00

Final pH (at 25°C) 7.2 ± 0.2
** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45- 50°C. Aseptically add the rehydrated contents of one vial of Monensin Selective supplement (FD309). Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ M-Coliform Differential Agar Base is based on coliform enumeration medium, M-FC Agar (1). This medium was modified for detection and enumeration of total coliforms by addition of Monensin supplement to improve the recovery of injured coliforms (2).

Peptone and Tryptone provides nitrogeneous compounds, carbonaceous compounds, long chain amino acids and other growth nutrients and vitamins. Yeast extract provides the essential nutrients and vitamins. Lactose is the fermentable carbohydrate. Monensin and sodium deoxycholate acts as selective agents, inhibiting Gram-positive bacteria. Aniline blue forms the indicator system of the medium. The chromogenic substrate is utilized by *E.coli* which detects the presence of β-glucuronidase. The medium helps injured coliforms to grow in the presence of selective agents.

Quality Control

Appearance of Powder : Light yellow to greyish yellow homogeneous free flowing powder
Gelling : Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium : Light yellow, clear to slightly opalescent gel forms in Petri plates
Reaction : Reaction of 4.63% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2
Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony (On memberane filter)
Escherichia coli (25922) (00013*)	50-100	good-luxuriant	>=50%	blue
Proteus vulgaris (13315)	50-100	good-luxuriant	>=50%	tan
Bacillus subtilis (6633)	>=10^3	inhibited	0%	

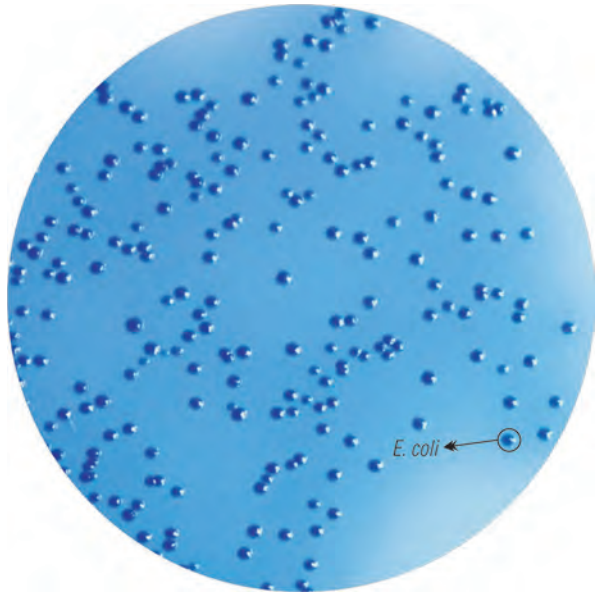
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Brodsky, M. H., P. Entis, A. N. Sharpe, and G. A. Jarvis. 1982.Enumeration of indicator organisms in foods using the automated hydrophobic membrane filter technique.J. Food Prod. 45:292-296.
2. Entis, P., and P. Boleszczuk. 1990. Direct enumeration of coliforms and *Escherichia coli* by hydrophobic grid membrane filter in 24 hours using MUG. J. Food Prot. 53:948-952.



M1951 – HiCrome™ M-Coliform Differential Agar Base

HiCrome™ Coliconfirm Broth Base

Recommended for detection of *E.coli* and other *Enterobacteriaceae* in water samples.

M2064

Composition **

Ingredients	Grams/Litre
Special peptone	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	3.000
Potassium dihydrogen phosphate	1.700
Sodium pyruvate	1.000
L-Tryptophan	1.000
Sodium lauryl sulphate	0.100
Chromogenic mixture	0.30

Final pH (at 25°C) 6.8 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.10 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle and Interpretation

Coliform Broth w/SLS is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (4).

Special peptone supplies the essential growth nutrients to the organisms. The phosphates in the medium buffers the medium. Sodium chloride maintains the osmotic balance. Sodium lauryl sulphate inhibits the gram positive organisms. L-Tryptophan in the medium improves the indole reaction. The enzyme -glucuronidase produced by *E.coli* cleaves X-glucuronide thus imparting blue colour to the medium (1,2,3). Formation of cherry red colour after addition of few drops (0.5ml) of Kovac's reagent (R008) to the medium indicates the presence of *E.coli*

Quality Control

Appearance of Powder: Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium : Cream, clear to slightly opalescent solution, may have slight precipitate.

Reaction : Reaction of 1.51% w/v aqueous solution at 25°C. pH : 6.8±0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Colour of Medium
Citrobacter freundii (8090)	50-100	luxuriant	colourless
Escherichia coli (25922) (00013*)	50-100	luxuriant	blue
Escherichia coli (35218)	50-100	luxuriant	blue
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	—
Klebsiella pneumoniae (13883)	50-100	luxuriant	colourless
Salmonella Enteritidis (13076) (00030*)	50-100	good	colourless
Shigella flexneri (12022)	50-100	luxuriant	colourless
Staphylococcus aureus (25923)	>=10^3	inhibited	—
Staphylococcus aureus (6538)	>=10^3	inhibited	—

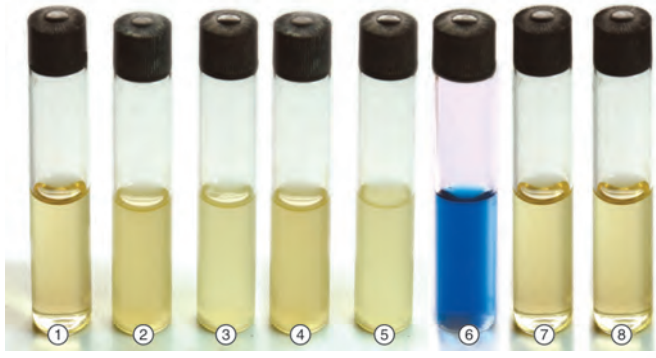
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Frampton E. W., Restaino L. and Blaszko N., 1988, J. Food Prot., 51:402.
2. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
3. LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur (Paris), 102:267.
4. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.



M1826 Coliform Broth w/SLS

1. Control
2. *Citrobacter freundii* ATCC 8090
3. *Klebsiella pneumoniae* ATCC 13883
4. *Salmonella* Enteritidis ATCC 13076
5. *Shigella flexneri* ATCC 12022
6. *Escherichia coli* ATCC 25922
7. *Enterococcus faecalis* ATCC 29212
8. *Staphylococcus aureus* ATCC 25923

M-E. coli Broth

M1426

For the detection, differentiation and enumeration of *Escherichia coli* and coliforms in water samples by membrane filtration technique.

Composition **

Ingredients	Grams/Litre
Tryptone	20.00
Bile salts mixture	1.50
Chromogenic mixture	0.175

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.67 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Aseptically add desired quantity (2 to 5 ml) of broth on sterile absorbent cotton pad or sterile filter paper for saturation. The medium should be used within 24 hours of rehydration.

Principle and Interpretation

M-E.coli Broth is used for detection and differentiation of *Escherichia coli* and coliforms in water samples using membrane filter technique. It is based on Tryptone Bile Agar used for detection of *Escherichia coli* in foods (1) where recovery of *Escherichia coli* is faster, more reliable and accurate.

The water sample is filtered through membranes and then placed on pad saturated with M-E.coli Broth and incubated at 37°C in sealed Petri plates. Glucuronidase test is used increasingly for detection of *E. coli* in water and food microbiology as *E. coli* is an important indicator of fecal contamination in samples from the food processing and water purification plants. Other *Escherichia* spp. do not produce this enzyme (3). The medium contains chromogenic mixture which helps to detect glucuronidase activity of *Escherichia coli* (2). This specific enzyme differentiates *Escherichia coli* from other coliforms. *Escherichia coli* cells split the chromogenic mixture with the help of the enzyme glucuronidase to give blue to green colouration to the colonies. Coliforms other than *Escherichia coli* turn red as they reduce TTC (2,3,5-triphenyl tetrazolium chloride). Thus, the resulting colour distinction allows simple interpretation of test without further confirmation.

Tryptone provides nitrogen and carbon source, long chain amino acids, vitamins and other essential growth nutrients to the organisms. Bile salt mixture inhibit gram-positive organisms.

Quality Control

Appearance of Powder : Light yellow to beige coloured, homogeneous, free flowing powder.

Colour and Clarity of prepared medium : Light yellow coloured, clear solution. without any precipitate

Reaction : Reaction of 2.17% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of colony on membrane filter
Escherichia coli (25922) (00013*)	50-100	luxuriant	blue
Enterobacter aerogenes (13048) (00175*)	50-100	luxuriant	red
Staphylococcus aureus (25923)	>=10^3	inhibited	—

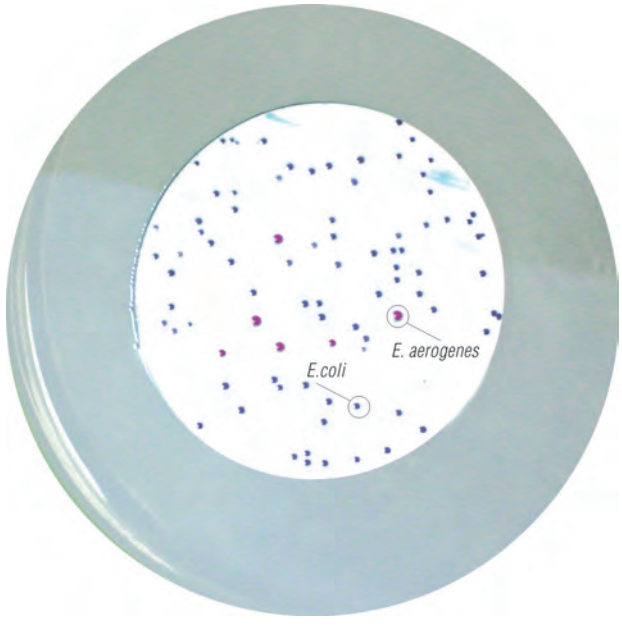
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Anderson J. M. and Baird Parker A.C., (1975), J. Appl. Bact., 39:111.
2. Hansen W. and Yourassawsky E., (1984), J. Clin. Microbiol. 20:1177.
3. Rice, E.W., Allen, M.J., Brenner, D.J., Edberg, S.C., 1991. Appl. Environ. Microbiol. 57, 592–593.



M1426 M-E. coli Broth

HiCrome™ M-Lauryl Sulphate Agar

Recommended for the differentiation and enumeration of *Escherichia coli* and other coliforms by a single membrane filtration technique

M1569

Composition **

Ingredients	Grams/Litre
Peptone	40.00
Yeast extract	6.00
Lactose	30.00
Phenol red	0.20
Sodium lauryl sulphate	1.00
Sodium pyruvate	0.50
Chromogen	0.20
Agar	10.00

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 87.9 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45- 50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ M-Lauryl Sulphate Agar is a modification of the Lauryl Tryptose Broth, formulated by Mallman and Darby, (1). This chromogenic medium is recommended for the presumptive identification and differentiation of *Escherichia coli* and other coliforms by a single membrane filtration technique (2, 3). The incorporation of chromogen X-glucuronide and the dye phenol red favours the differentiation of *E.coli* and other coliforms on the basis of colour.

Peptone provide nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients to the organisms. Yeast extract serves as a source of vitamins especially group B vitamins. Lactose acts as a source of fermentable sugar while sodium lauryl sulphate inhibits gram positive organisms. The enzyme β-D-glucuronidase produced by *E.coli,* cleaves X-glucuronide, imparting a green colour to the colonies. Lactose fermentation is detected by phenol red indicator. Other lactose fermentors not possesing β-D-Glucuronidase enzyme will show yellow colonies, whereas lactose non-fermentors will exhibit pink coloured colonies.

Quality Control

Appearance of Powder : Light yellow to pink coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium : Red coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 8.8% w/v aqueous solution at 25°C. pH:7.4 ± 0.2 .

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	green
Klebsiella pneumoniae (13883)	50-100	good	yellow, mucoid
Staphylococcus aureus (25923)	>=10^3	inhibited	-
Salmonella Enteritidis (13076) (00030*)	50-100	good	pink

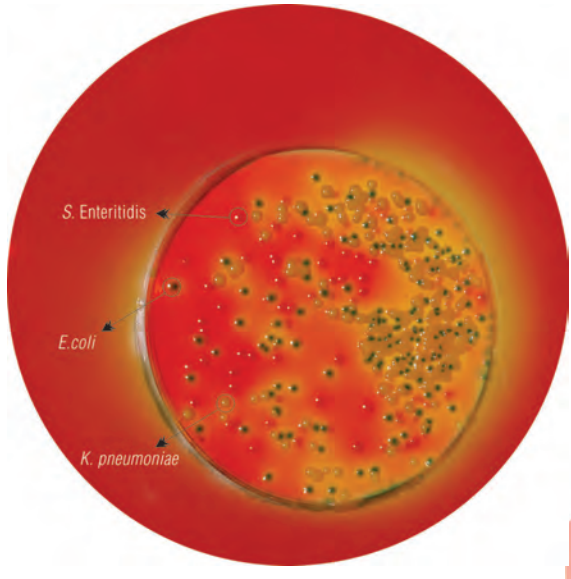
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Mallman and Darby, 1941, Am. J. Public Health, 31:127.
2. Methods for Examination of Waters and Associated Materials, Environment Agency, 1998, Standing Committee of Analysts.
3. Sartory D.P. and Howard L, 1992, Lett Appl. Microbiol. 15:273-276.



M1569 – HiCrome™ M-Lauryl Sulphate Agar

HiCrome™ Chromogenic Coliform Agar (CCA)

M1991I

Rrecommended for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014. samples.

Composition **

Ingredients	Grams/Litre
Enzymatic digest of casein	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, 2H2O	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexamine ammonium salt, monohydrate	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.100
Agar	15.000

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.92 grams(the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. DO NOT OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Chromogenic Coliform Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water samples (1). The medium contains three chromogenic substrates. The enzyme β-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxyl-β-D-galactopyranoside to form pink to red coloured colonies (3). The enzyme β-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxyl-β-D-glucuronic acid (2). Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Enzymatic digest of casein, yeast extract, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sublethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (3).

The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli*, add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light yellow coloured opalescent gel forms in Petri plates

Reaction : Reaction of 3.09% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2

Cultural Response : Cultural characteristics observed after an incubation at 34-38°C for 24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony*
Escherichia coli (10536)	50-100	good-luxuriant	>=50%	dark blue/violet
Citrobacter freundii (8090)	50-100	luxuriant	>=70 %	pink to red
Enterobacter aerogenes (13048) (00175*)	50-100	luxuriant	>=70%	pink to red
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=70%	dark blue to violet
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	-
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	>=70%	colourles

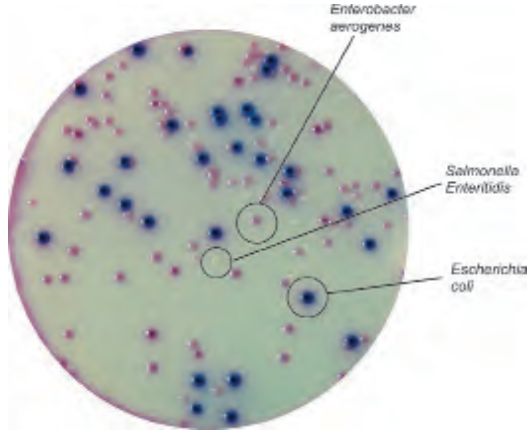
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label

References

1. International Organization for Standardization. Water quality: Enumeration of E.coli and coliform bacteria. Part IMembrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
2. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
3. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.



M1991I– HiCrome™ Chromogenic Coliform Agar (CCA)

HiCrome™ E. coli Agar

Recommended for the detection and enumeration of *Escherichia coli* in foods without further confirmation on membrane filter or by indole reagent.

M1295/ M1295I

Composition **

	M1295	M1295I
Ingredients	Grams/Litre	Grams/Litre
Tryptone	14.00	20.00
Peptone, special	5.00	—
Bile salts mixture	1.50	1.50
Disodium hydrogen phosphate	1.00	—
Sodium dihydrogen phosphate	0.60	—
Sodium chloride	2.40	—
X-Glucuronide	0.075	0.075
Agar	12.00	15.00

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.57 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ *E. coli* Agar is based on Tryptone Bile Agar to detect *Escherichia coli* in foods (1), where recovery of *E. coli* is faster, more reliable and accurate. Most of the *E. coli* strains can be differentiated from other coliforms by the presence of enzyme glucuronidase, which is highly specific for *E. coli* (2). Glucuronidase test is used increasingly for detection of *E. coli* in water and food microbiology as *E. coli* is an important indicator of fecal contamination in samples from the food processing and water purification plants. Other *Escherichia* spp. do not produce this enzyme (4). The chromogenic agent X-glucuronide used in this medium helps to detect glucuronidase activity of *E. coli*. *E. coli* cells absorb X-glucuronide and the intracellular glucuronidase enzyme splits the bond between the chromophore and the glucuronide. The released chromophore gives bluish green colouration to the *E. coli* colonies. Formulation of M1295I is in accordance with ISO (3).

Tryptone and peptone special provide the nitrogenous compounds, carbon, amino acids, vitamins and other essential growth nutrients to the organisms. Bile salts mixture inhibits gram-positive organisms. Sodium chloride and phosphates maintain osmotic balance and buffering action respectively.

The surface of the plated medium is dried before use. Dilute food samples 1:5 or 1:10 with 0.1% (w/v) sterile Peptone Water (M028) and homogenize in a blender or a stomacher. Pipette 0.5 ml or 1.0 ml of the homogenized food sample on to the plate and spread with sterile glass spreader. Incubate the plates at 30°C for 4 hours and then at 44°C

Quality Control

Appearance of powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.2% Agar gel of M1295 or 1.5% Agar gel of M1295I .

Colour and Clarity of prepared medium : Light yellow coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 3.66% w/v aqueous solution at 25°C. pH:7.2 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 44°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	bluish green
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

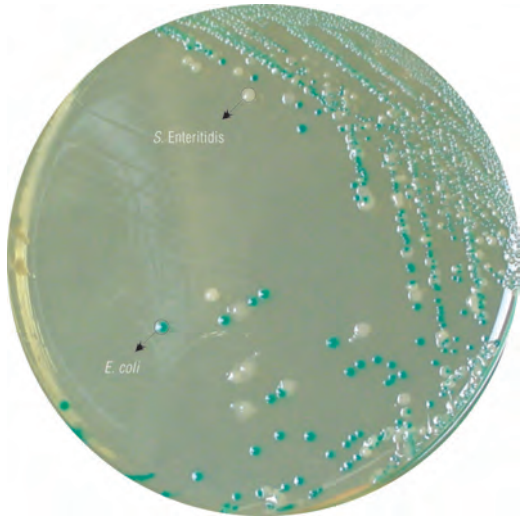
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Anderson J. M. and Baird-Parker A. C., 1975, J. Appl. Bacteriol., 39:111.
2. Hansen W. and Yourassawsky E., 1984, J. Clin. Microbiol., 20:1177.
3. International Standard ISO 166492:1999. Microbiology of food and animal feeding stuff - horizontal method for the enumeration of presumptive Escherichia coli; Part 2: Colony count technique at 44°C using 5-bromo-4-chloro-3indolyl-b-D-glucornic acid.
4. Rice, E.W., Allen, M.J., Brenner, D.J., Edberg, S.C., 1991. Appl. Environ. Microbiol. 57, 592–593.



M1295 – HiCrome™ E. coli Agar

HiCrome™ Coliform Agar Modified

Recommended for the simultaneous detection of *Escherichia coli* and thermotolerent coliforms in water, milk, dairy products and other food samples.

M1832

Composition **

Ingredients	Grams/Litre
Peptone, special	8.000
Sodium chloride	1.000
Yeast extract	3.000
Potassium dihydrogen phosphate	0.200
Dipotassium phosphate	0.600
Bile salts	0.800
Magnesium sulphate	0.200
Chromogenic mixture	0.200
Agar	10.000

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Coliform Agar Modified is a selective medium recommended for the simultaneous detection of *E.coli* and thermotolerant coliforms in water and food samples (4). Peptone special and yeast extract provide the neccssary nitrogen compound, carbon, vitamins and also some trace ingredients required for the growth of organisms. The phosphates buffer the medium well. Magnesium sulphate helps colour development. Bile salts inhibits gram-positive organisms. Sodium chloride maintains osmotic balance.

The chromogenic mixture contains two chromogenic substrates, which enables the detection of two specific enzymes, β-galactosidase and β-glucoronidase. β-galactosidase produced by coliforms cleaves one chromogen, resulting in the pink colouration of coliform colonies. The enzyme β-glucuronidase produced by *E. coli,* cleaves X-glucuronide. *E.coli* forms dark blue to violet coloured colonies due to cleavage of both the chromogens (1, 2, 3). *E.coli* strains that are β-glucoronidase negative (serotype O157:H7) produce pink coloured colonies. Other gram negative bacteria able to grow at (44±0.5)°C produce white or colourless colonies.

Transfer 1 ml of product to analyse and its tenfold dilutions to sterile Petri plates. Pour 12 ml of medium, mix well and allow to solidify. Overlay with 4 ml of medium, allow to solidify and incubate at 43-45°C for 18-24 hours.

Quality Control

Appearance of Powder : Light yellow to beige homogeneous free flowing powder

Gelling : Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium : Colourless clear to slightly opalescent gel forms in Petri plates

Reaction : Reaction of 2.4% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2

Cultural Response : Cultural characteristics observed after an ncubation at 43-45°C for 24 hours (48 hours if necessary).

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli (10536)	50-100	good-luxuriant	>=50%	dark blue/ violet
Escherichia coli (25922) (00013*)	50-100	good-luxuriant	>=50%	dark blue/ violet
Enterobacter cloacae (23355)	50-100	good-luxuriant	>=50%	pink
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	-
Klebsiella pneumoniae (13883)	50-100	good-luxuriant	>=50%	light pink
Staphylococcus aureus (25923)	>=10^3	inhibited	0 %	-

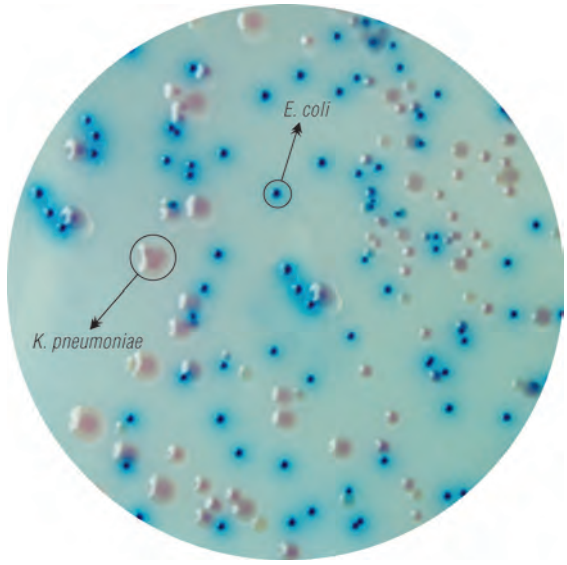
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Frampton E. W., Restaino L. and Blaszko N., 1988, J. Food Prot., 51:402.
2. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
3. LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur (Paris), 102:267.
4. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.



M1832 – HiCrome™ Coliform Agar Modified

Thermotolerant E. coli

HiCrome™ M-TEC Agar / HiCrome M-TEC Broth

M1571/ M1713

Recommended by the U.S. Environmental Protection Agency (USEPA) for differentiation and enumeration of thermotolerant *Escherichia coli* in water by the membrane filtration technique.

Composition **

	M1571	M1713
Ingredients	Grams/Litre	Grams/Litre
Proteose peptone	5.00	5.00
Yeast extract	3.00	3.00
Lactose	10.00	10.00
Sodium chloride	7.50	7.50
Dipotassium phosphate	3.30	3.30
Monopotassium phosphate	1.00	1.00
Sodium lauryl sulphate	0.20	0.20
Sodium deoxycholate	0.10	0.10
Chromogen	0.50	0.50
Agar	15.00	—

Final pH (at 25°C) 7.3 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.6 grams of M1571 or 30.6 grams of M1713 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C for M1571 Mix well & pour into sterile Petri plates. For M1713 Cool to 45-50°C. On cooling M1713 aseptically add desired quantity (2-5 ml broth) on sterile absorbent pad for saturation in a sterile Petri plate. The medium should be used within 24 hours after rehydration.

Principle and Interpretation

HiCrome™ M-TEC Agar/Broth are the chromogenic media used for detection and enumeration of thermotolerant *Escherichia coli* (TEC) in water by membrane filtration (2). HiCrome™ M-TEC Broth is a modification of the M-TEC Agar developed by Dufour (1). The modified medium contains the chromogen, 5-bromo-6-chloro-3-indolyl-β-D-glucuronide that is cleaved by enzyme β-D-glucuronidase to yield glucuronic acid, produced by *E.coli* strains. This imparts a purple-magenta colour to the colonies of *E. coli* only.

Proteose peptone and yeast extract provides nitrogenous compounds, amino acids and long chain peptides for the growth of microoganisms carbon compounds. Lactose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Monopotassium phosphate and dipotassium phosphate provide strong buffering system to control the pH in the presence of fermentative action. Sodium lauryl sulphate and sodium deoxycholate make the medium more selective by inhibiting gram-positive bacteria. Saturate a sterile cotton absorbent pad with about 2ml of HiCrome™ M-TEC Broth (M1713). Membrane filter through which water sample has been passed is aseptically placed on the saturated absorbent cotton pad face upwards. This absorbent pad is then incubated at 44.5 ± 0.2°C for 22-24 hours. Following incubation

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel of M1571

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates (M1571) / clear solution in tubes (M1713).

Reaction : Reaction of 4.56% w/v aqueous solution of M1571 and 3.06% w/v of M1713 at 25°C. pH : 7.3 ± 0.2 .

Cultural Response : Cultural characteristics observed after an incubation at 44.5 ± 0.2°C for 22-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of Colony
Escherichia coli (25922) (00013*)	50-100	good to luxuriant	purple / magenta
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	—
Proteus mirabilis (25933)	50-100	good	colourless-light brown
Klebsiella pneumoniae (13883)	50-100	good	colourless-tan

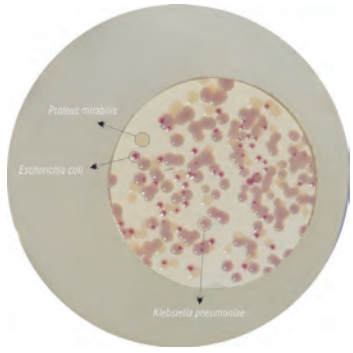
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

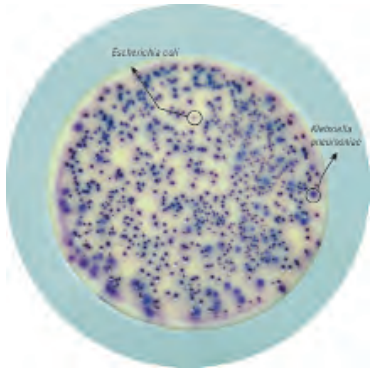
Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Dufour, Strickland and Cabelli, 1981, Appl. Environ. Microbiol. 41: 1152.
2. U.S. Environmental Protection Agency, 2002, Method 1603; Publication EPA-821-R-02-023.



M1571 – HiCrome™ M-TEC Agar



M1713– HiCrome™ M-TEC Broth

HiCrome™ Single Streak Rapid Differentiation Series

HiCrome™ MacConkey Sorbitol Agar Base

Recommended for the selective isolation of *Escherichia coli* 0157:H7 from food and animal feeding stuff.

M1340

Composition **

Ingredients	Grams/Litre
Tryptone	17.00
Proteose peptone	3.00
Sorbitol	10.00
Bile salts mixture	1.50
Sodium chloride	5.00
Crystal violet	0.001
Neutral red	0.03
B.C. Indicator	0.10
Agar	13.50

Final pH (at 25°C) 7.1 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.06 grams in 495 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. If desired rehydrated contents of 1 vial of Tellurite-Cefexime Supplement (FD147) may be added aseptically to 495 ml sterile molten, cooled (45-50°C) medium before pouring into sterile Petri plates.

Principle and Interpretation

Sorbitol MacConkey Agar is based on the formulation described by Rappaport and Henigh (4). The medium contains sorbitol instead of lactose and it is recommended for the detection of enteropathogenic strains of *Escherichia coli* O157:H7 that ferments lactose but does not ferment sorbitol (2) and hence produce colourless colonies. Sorbitol fermenting strains of *Escherichia coli* produce pink-red colonies. The red colour is due to production of acid from sorbitol, absorption of neutral red and a subsequent colour change of the dye when pH of the medium falls below 6.8. *Escherichia coli* O157:H7 has been recognised as a cause of hemorrhagic colitis (2). March and Ratnam (3) reported that the detection of *Escherichia coli* O157:H7 had a sensitivity of 100% and specificity of 85% on Sorbitol MacConkey Agar and they recommended this medium as reliable means of screening *Escherichia coli* O157:H7. B.C. indicator is added to detect the presence of the enzyme β-D-glucuronidase which is specific for *Escherichia coli.* (1). Strains of *Escherichia coli* fermenting sorbitol and possessing β-D-glucuronidase appear as blue - purple coloured colonies on the medium. Enteropathogenic strains of *Escherichia coli* O157:H7 do not possess β-D-glucuronidase activity (5) and thus produce colourless colonies.

Tryptone and proteose peptone provide carbonaceous, nitrogenous and other essential growth nutrients. Most of the gram-positive organisms are inhibited by crystal violet and bile salts. Sodium chloride maintains the osmotic equilibrium.

Addition of Tellurite-Cefixime Supplement makes the medium selective (6). Potassium Tellurite selects the serogroups and inhibits *Aeromonas* species and *Providencia* species. Cefixime inhibits *Proteus* species. *Pseudomonas* if present produces colourless colonies on this medium. For confirmation oxidase test may be performed with suspected colonies and results should be noted within 5-10 seconds.

Quality Control

Appearance of powder : Light yellow to pink coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium : Purplish red coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 5.01% w/v aqueous solution at 25°C. pH : 7.1 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (48 hours if necessary).

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	#Colour of colony	Oxidase
Escherichia coli O157:H7 (NCTC 12900)	50-100	good-luxuriant	>=50%	colourless	-
Escherichia coli (25922) (00013*)	50-100	good	40-50%	blue-green	-
Pseudomonas aeruginosa (27853) (00025*)	50-100	fair-good	30-40%	colourless	+
Klebsiella pneumoniae (13883)	50-100	good	40-50%	pink-red	-

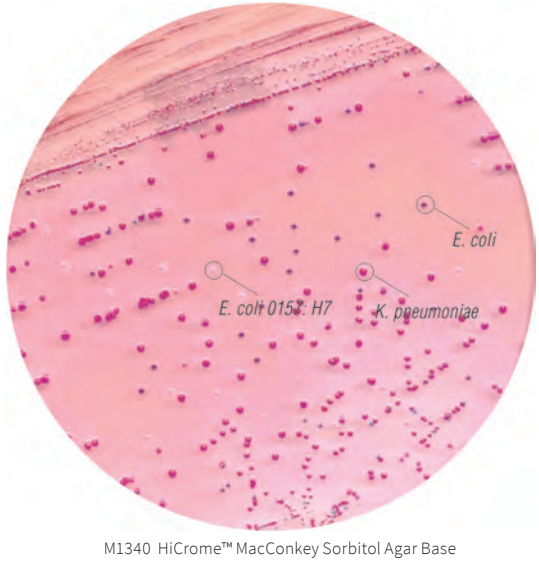
Key : # = Colour of the colony without addition of Tellurite-Cefixime Supplement (FD147)
+ = positive reaction deep-purple blue colour develops within 10 seconds
- = negative reaction
* = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Hansen W. and Yourassawsky E., (1984), J. Clin. Microbiol., 20:1177.
2. Karmali M.A., Petric M., Lim C., et al, (1985), J. Infect. Dis., 151-775.
3. March S.B. and Ratnam S., (1986) : J. Clin. Microbiol. 23, 869-872.
4. Rappaport F. and Henigh E., (1952), J. Clin. Path., 5:361.
5. Thompson et al. (1990). J. Clin. Microbiol. 29, 2165-2168.
6. Zadik P.M., Chapman P.A. and Siddons C.A., (1993), J. Med. Microbiol., 39, 155-158.



M1340 HiCrome™ MacConkey Sorbitol Agar Base

HIMEDIA®

HiCrome™ EC 0157: H7 Agar, Modified

For isolation and differentiation of *Escherichia coli* O157:H7 from food and environmental samples.

M1574A

Composition **

Ingredients	Grams/Litre
Yeast extract	3.000
Tryptone	5.000
Sorbitol	7.00
Bile salts mixture	1.50
SLS (Sodium Lauryl Sulphate)	0.10
Chromogenic mixture	0.25
Agar	12.00

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.85 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. This medium can be made more selective by aseptically adding 0.25 ml of rehydrated contents of one vial of 1% Potassium Tellurite Solution (FD052) to 1000 ml molten and cooled medium (45-50°C).

Principle and Interpretation

Escherichia coli O157:H7 belongs to the Enterohemorrhagic *Escherichia coli* (EHEC) group and it predominates as a food borne pathogen. *E. coli* O157: H7 was first recognized as a human pathogen in 1982 when two outbreaks of hemorrhagic colitis were associated with consumption of undercooked ground beef that has been contaminated with this organism (1).

HiCrome™ EC O157:H7 Agar, Modified is a chromogenic medium recommended for the isolation and differentiation of *E. coli* O157:H7 from food and environmental samples. HiCrome™ ECO157:H7 Agar, Modified is based on the formulation described by Rappaport and Henigh (2). The medium contains sorbitol and a proprietary chromogenic mixture instead of lactose and indicator dyes respectively, as is conventionally used. The chromogenic substrate is specifically and selectively cleaved by *E. coli* O157: H7 resulting in a dark purple to magenta coloured moiety. *E. coli* gives bluish green coloured colonies.

Tryptone and yeast extract provides carbonaceous, nitrogenous and growth nutrients. Bile salts mixture and SLS inhibits gram-positive organisms. Potassium tellurite selects the serogroups and inhibits *Aeromonas* species and *Providencia* species.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 2.88% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli O157:H7 12900)	50-100	luxuriant	>=50%	dark (NCTC purple-magenta
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	bluish green
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=50%	colourless mauve (mucoid)
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	>=50%	colourless
Bacillus subtilis (6633)	>=10^3	inhibited	0%	-
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

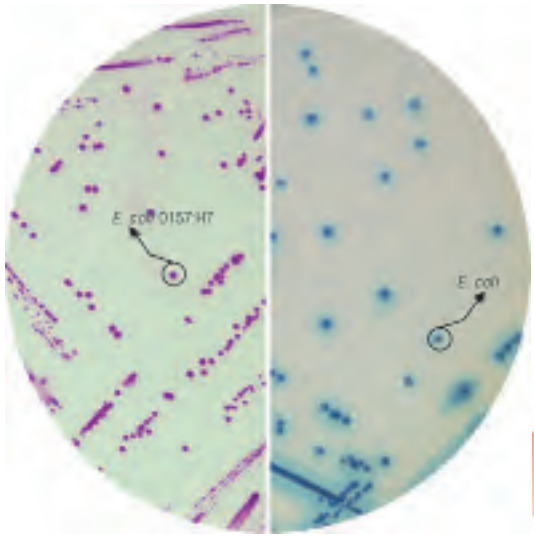
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
2. Rappaport F. and Henigh E., 1952, J. Clin. Pathol., 5:361.



M1574A HiCrome™ EC 0157: H7 Agar, Modified

HiCrome™ EC 0157:H7 Selective Agar Base, Modified

Recommended for selective isolation and easy detection of *Escherichia coli* O157:H7 from food samples.

M1575A

Composition **

Ingredients	Grams/Litre
Tryptone	5.00
Yeast extract	3.00
Sorbitol	7.00
Bile salts mixture	1.50
Sodium lauryl sulphate (SLS)	0.10
Chromogenic mixture	0.25
Agar	15.00

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.85 grams in 990 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Add rehydrated contents of 1 vial of HiCrome EC O157:H7 Selective Supplement (FD187) aseptically. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Enterohaemorragic *E. coli* strains are also termed as verocytotoxin-producing *E.coli* (VTEC/ EHEC). Although many different serotypes of *Escherichia coli* are known to produce verocytotoxin (2) those of *Escherichia coli* O157:H7 and O157:H are so far the common types causing human infections. O157 VTEC strains have several unusual biochemical characters that are exploited in methods for their laboratory identification. They belong to the minority of *E. coli* that are β-glucuronidase negative and do not ferment sorbitol or rhamnose within 24 hours. These can be isolated from faecal specimens by plating on media containing D-sorbitol instead of lactose.

HiCrome™ EC O157:H7 Selective Agar Base, Modified is based on the formulation described by Rappaport and Henigh (1). The medium contains sorbitol and a proprietary chromogenic mixture instead of lactose and indicator dyes respectively. The chromogenic substrate is specifically and selectively cleaved by *Escherichia coli* O157: H7 resulting in a dark purple to magenta coloured moiety. *E. coli* forms bluish green coloured colonies.

Tryptone provides carbonaceous, nitrogenous and growth nutrients. Addition of HiCrome™ EC O157:H7 Selective Supplement (FD187) makes the medium selective (3). Potassium tellurite selectively inhibits *Aeromonas* and *Providencia* species. Novobiocin inhibits gram-positive bacteria. Sodium lauryl sulphate helps to inhibit the accompanying gram-positive flora.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 3.18% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2.

Cultural Response : Cultural characteristics observed with added HiCrome™ EC O157:H7 Selective Supplement (FD187). after an incubation at 35-37°C for 18-24 hours

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli O157:H7 (NCTC 12900)	50-100	luxuriant	>=50%	dark purple-magenta
Escherichia coli (25922) (00013*)	50-100	none to poor	<=10%	bluish green
Pseudomonas aeruginosa (27853) (00025*)	50-100	fair to good	30-40%	colourless
Klebsiella pneumoniae (13883)	>=10^3	fair to good	30-40%	colourless -mauve (mucoid)

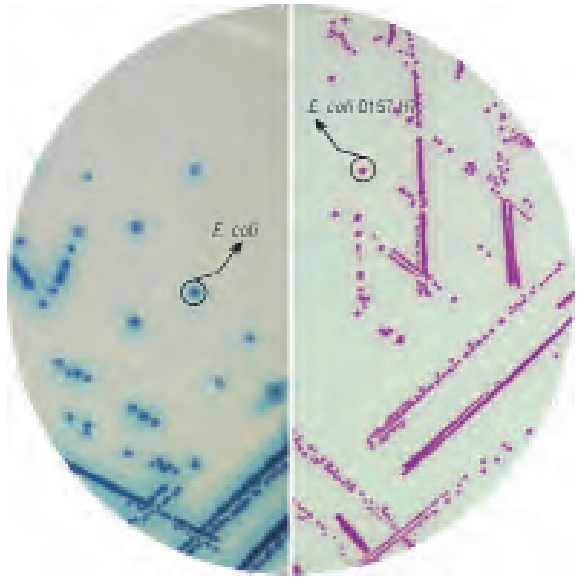
Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Rappaport F. and Henigh E., 1952, J. Clin. Pathol., 5:361.
2. Smith and Scottland, 1988, J. Med. Microbiol., 26:77-85.
3. Zadik P. M., Chapman P. A. and Siddons C. A., 1993, J. Med. Microbiol., 39, 155-158.



M1575A – HiCrome™ EC 0157:H7 Selective Agar Base, Modified

HiCrome™ Enrichment Broth Base for EC 0157:H7

Recommended for isolation and selective differentiation of *Escherichia coli* O157:H7 from food and environmental samples by chromogenic method.

M1598

Composition **

Ingredients	Grams/Litre
Tryptone	10.00
Sorbitol	10.00
Bile salts mixture	1.50
Chromogenic mixture	1.30

Final pH (at 25°C) 7.1 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 11.4 grams in 500 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. For selective isolation of *E.coli* O157:H7, aseptically add the rehydrated contents of 1vial of HiCrome™ ECO157:H7 Selective Supplement I (FD230). Mix well and dispense into sterile test tubes or flasks as desired.

Principle and Interpretation

March and Ratnam (1) reported the inability of *Escherichia coli* O157:H7 to ferment sorbitol while developing Sorbitol MacConkey medium. Subsequently Thomson et al (2) observed the absence of β-glucuronidase activity in *E. coli* O157:H7 from a variety of samples by direct culture.

The medium contains Tryptone that provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Sorbitol is the fermentable carbohydrate, bile salt mixture inhibits most of the gram-positive organisms. Addition of tellurite (FD230) makes the medium more specific and selective. The bluish colour development of *E. coli* and *Klebsiella* in the medium is due to the enzymes β-D-galactosidase and β-D-glucuronidase respectively that cleaves the chromogenic substrates present in chromogenic mixture. However *E. coli* O157:H7 gives a purple colour to the medium due to the absence of β-glucuronidase and its inability to ferment sorbitol.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Colour and Clarity of prepared medium : Light yellow coloured, clear solution without any precipitate.

Reaction : Reaction of 2.28% w/v aqueous solution at 25°C. pH : 7.1 ± 0.2.

Cultural Response : Cultural characteristics observed with added HiCrome™ EC O157:H7 Selective Supplement I (FD230) after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of Medium	Growth«	Colour of Medium«
Escherichia coli O157: H7 (NCTC 12900)	50-100	good-luxuriant	purple#	good-luxuriant	purple#
Escherichia coli (25922) (00013*)	50-100	good-luxuriant	blue#	inhibited	-
***Cronobacter sakazakii** (12868)	50-100	good-luxuriant	white#	none-poor	colourless#
Klebsiella pneumoniae (13883)	50-100	good-luxuriant	bluish green	good	bluish green#
Salmonella Enteritidis (13076) (00030*)	50-100	good-luxuriant	light green#	good	light green#
Shigella flexneri (12022)	50-100	good	colourless	inhibited	-
Enterococcus faecalis (29212) (00087*)	$>=10^3$	inhibited	-	inhibited	-
Staphylococcus aureus (25923)	$>=10^3$	inhibited	-	inhibited	-

KEY : « : after addition of HiCrome™ ECO157:H7 Selective Supplement I (FD230)

: may show slight precipitation of growth

** : Formerly known as *Enterobacter sakazakii*

* = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. March S. B. and Ratnam S., (1986), J. Clin. Microbiol. 23, 869 - 872.
2. Thompson et al. (1990), J. Clin. Microbiol. 29, 2165 - 2168.

M1598 HiCrome™ Enrichment Broth Base for EC 0157:H7

1. Control
2. *E. coli* 0157:H7 (NCTC 12900)
3. *Escherichia coli* (ATCC 25922)
4. *Cronobacter sakazakii* (ATCC 12868)
5. *Klebsiella pneumoniae* (ATCC 13883)

HiCrome™ M-Modified ECO157:H7 Selective Agar Base

Recommended for presumptive enumeration of *Escherichia coli* 0157:H7 by membrane filtration technique.

M1862

Composition **

Ingredients	Grams/Litre
Peptone	5.000
Yeast extract	3.000
Sodium chloride	5.000
Lysine	10.000
Sorbitol	20.000
Dextrose	2.500
Magnesium sulphate	1.500
Sodium deoxycholate	0.150
Sodium glucuronate	0.500
Phenol red	0.120
Chromogenic mixture	0.050
Agar	15.000

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 62.82 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add rehydrated contents of one vial of HiCrome™ ECO157: H7 Selective Supplement, Modified (FD295). Mix well and pour in to sterile Petri plates.

Principle and Interpretation

Escherichia coli O157:H7 belongs to the Enterohemorrhagic *Escherichia coli* (EHEC) group and it predominates as a food borne pathogen. *E.coli* O157:H7 was first recognized as a human pathogen in 1982 when two outbreaks of hemorrhagic colitis were associated with consumption of undercooked ground beef that has been contaminated with this organism (3) that results from the action of a shiga-like toxin (SLT) (1,7). This medium is recommended for isolation of enteropathogenic *Escherichia coli* O157:H7 in meats, poultry, dairy foods, infant formula, liquid eggs, mayonnaise and apple cider (4, 5). The medium is based on three differential biochemical reactions - lysine decarboxylase (positive for typical EHEC O157 strains), sorbitol fermentation and beta-glucuronidase (2). This medium is also used for the enumeration of β- glucuronidase-positive *E.coli* from foods (6).

Peptone and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Sodium chloride maintains the osmotic environment of the medium. Bacteria which were able to grow on this medium will ferment dextrose first. Once dextrose has been depleted, sorbitol positive bacteria will begin to ferment sorbitol, producing a drop in pH of medium, which produces yellow colour to the colony due to phenol red which is a pH indicator. Glucuronidase positive *E.coli* will break down X-Gluc, resulting in the production of an insoluble blue precipitate in the colony. This will combine with the colour of the pH indicator dye to produce a green colony in case of sorbitol positive or lysine negative bacteria. This medium also contains lysine, lysine positive organisms decarboxylates lysine which produces an increase in pH of medium, hence produces pink coloured colonies. Selectivity is achieved through the use of monensin (FD295) which inhibits gram positive bacteria and

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

Gelling : Firm, comparable with 1.5 % Agar gel.

Colour and Clarity of prepared medium : Red coloured, clear to slightly opalescent gel forms in Petri plates

Reaction : Reaction of 6.28% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2

Cultural Response : Cultural characteristics observed with added HiCrome™ ECO157:H7 Selective Supplement, Modified (FD295), after an incubation at 44 - 44.5°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony (On membrane filter)
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	green
Escherichia coli O157:H7 (NCTC 12900)	50-100	luxuriant	>=50%	pink
Klebsiella pneumoniae (13883)	50-100	fair	20-30%	yellow
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	-

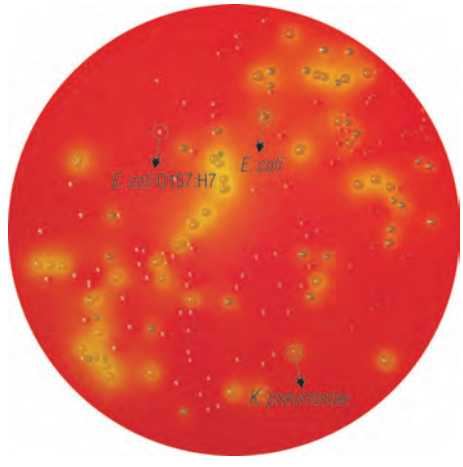
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Centre for Diseases Control, 1991, Morbid. Mortal, Weekly Rep 40:265.
2. Corry J.E.L, Curtis G.D.W., Baird R.M., Culture Media for Food Microbiology, Progress in Industrial Microbiology, Volume 37.
3. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., Public Health Association, Washington, D.C.
4. Entis, P.,and I. Lerner.1997. 24-hour presumptive enumeration of *Escherichia coli* O157:H7 in food using the ISO-GRID method with SD-39 agar.J.Food Prot. 60:883-890.
5. Entis, P.1998. Direct 24-hour presumptive enumeration of *Escherichia coli* O157:H7 in food using the hydrophobic grid membrane filter,followed by serological confirmation : collaborative study. J. AOAC Int. 81:403-418.
6. Entis, P., and I.Lerner. 1998. Enumeration of β-glucuronidase positive *E.coli* in foods by using the ISO-GRID method with SD-39 agar.J.Food Prot. 61:913-916.
7. March S. B. and Ratnam S., 1986, J. Clin. Microbiol., 23:869.



M1862 – HiCrome™ M-Modified ECO157:H7 Selective Agar Base

HiCrome™ Enterobacter sakazakii Agar / Modified

For the isolation and identification of *Enterobacter sakazakii* from food, milk and dairy products (*Enterobacter sakazakii* now referred as *Cronobacter sakazakii*)

M1577 / M1641

Composition **

	M1577	M1641
Ingredients	Grams/Litre	Grams/Litre
Tryptone	15.00	7.00
Soya peptone	5.00	-
Yeast extract	-	3.00
Sodium chloride	5.00	5.00
Sodium deoxycholate	0.50	0.60
Sodium thiosulphate	1.00	-
Chromogenic mixture	10.17	-
Chromogenic substrate	-	0.15
Crystal violet	-	0.002
Agar	15.00	15.00
Final pH (at 25°C)	7.3 ± 0.2	7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.67 grams of M1577 or 30.75 grams of M1641 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Enterobacter species are widely distributed in nature occurring in fresh water, soil, sewage, plants, vegetables, animal and human feaces.* *Cronobacter sakazakii* has been closely associated with neonatal meningitis and sepsis (3). The chromogenic substrate in HiCrome™ Enterobacter sakazakii Agar (M1577) is cleaved specifically (2) by the glucosidase enzyme possessed by *Enterobacter* species resulting in formation of blue-green colonies. Other organisms, which do not cleave this substrate, produce yellow coloured colonies. Incorporation of the chromogenic mixture in the media renders an intense blue colour to **C.sakazakii* colonies and light blue green colour to other *Enterobacter* species. HiCrome™ Enterobacter sakazakii Agar, Modified is recommended by ISO Committee for the isolation and identification of **C.sakazakii* (1). The chromogenic substrate is cleaved specifically (2) by **C.sakazakii* resulting in the formation of blue green colonies. Other organisms, which do not cleave this substrate, produce colorless to slightly violet coloured colonies.

Tryptone, soya peptone and yeast extract provide the essential growth nutrients along with nitrogenous and carbonaceous compounds, long chain amino acids and vitamins. Sodium chloride helps in maintaining

* : Formerly known as *Enterobacter sakazakii*

Quality Control

Appearance of Powder : Light yellow to pink coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Purple coloured (M1577) or light purple coloured (M1641), clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 5.16% w/v aqueous solution of M1577 at 25°C. pH : 7.3 ± 0.2 .
Reaction of 3.07% w/v aqueous solution of M1641 at 25°C. pH : 7.0 ± 0.2.

Cultural Response : M1577 Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	good-luxuriant	>=50%	yellow
Enterobacter aerogenes (13048) (00175*)	50-100	good-luxuriant	>=50%	green
**Cronobacter sakazakii* (12868)	50-100	good-luxuriant	>=50%	blue
Klebsiella pneumoniae (13883)	50-100	good-luxuriant	>=50%	green (mucoid)
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	—
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	—

(M1641) : Cultural characteristics observed after an incubation at 44±1°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	good-luxuriant	>=50%	colourless with blue centre
Enterobacter aerogenes (13048) (00175*)	50-100	good-luxuriant	>=50%	colourless with blue centre
**Cronobacter sakazakii* (12868)	50-100	good-luxuriant	>=50%	blue - green
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	—
Enterococcus faecalis (29212) (00087*)	>=103	inhibited	0%	—

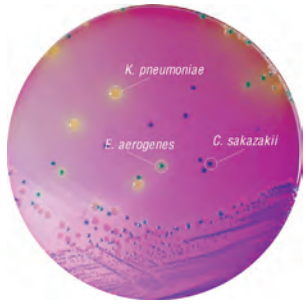
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. International Organization for Standardization Draft ISO/ TS 22964, 2006 (E).
2. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D. C.
3. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120,1983.



M1577 HiCrome™ Enterobacter sakazakii Agar

Salmonella Differential Agar / Modified (Twin pack) (RajHans Medium)

For identification and differentiation of *Salmonella* species from members of *Enterobacteriaceae*, especially *Proteus* species.

M1078 / M1082

Composition **	M1078	M1082
Ingredients	Grams/Litre	Grams/Litre
Part A :		
Peptone, special	8.00	8.00
Yeast extract	2.00	3.00
Sodium deoxycholate	1.00	1.00
Sodium chloride	—	5.00
B.C. indicator	2.00	2.00
Agar	12.00	12.00
Part B :		
Propylene glycol	10.00	10.00

Final pH (at 25°C) 7.3 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10 grams of fluid Part B in 1000 ml distilled water. Add 25 grams of Part A (M1078) or 31 grams of Part A (M1082). Mix well and heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45- 50°C. Mix well before and pour into sterile Petri plates.

Principle and Interpretation

Salmonella Differential Agar media are slight modification of original formulation of Rambach (3) used for differentiation of *Salmonella* species from *Proteus* species and other enteric bacteria. Production of acid from propylene glycol is a novel characteristic of *Salmonella* species and is utilized in these media. Many of the media such as SS Agar, XLD Agar recommended for the identification and differentiation of *Salmonella* species (1) are based on lactose fermentation and hydrogen sulphide production.

Peptone special and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acid, vitamins and other essential growth nutrients. Sodium deoxycholate inhibits gram-positive organisms rendering the medium selective for enteric microorganisms. The BC indicator turns pink in presence of acid produced from propylene glycol. Lactose fermenting ability is determined by using an indicator, which can detect the presence of enzyme β-galactosidase. Lactose fermenting (β-galactosidase producing) bacteria yield blue violet coloured colony (2). Salmonellae produce acid from propylene glycol and on combining with the BC indicator gives typical pink red colonies. Other enteric gram-negative bacteria form colourless colonies. *Salmonella* Typhimurium and *S* Enteritidis produce pink to red colonies. Specimen should be enriched in an appropriate selective enrichment broth. This enriched culture is then inoculated on Salmonella Differential Agar/ Salmonella Differential Agar, Modified and incubated at 35-37°C for 24-48 hours.

Quality Control

Appearance of Powder : Part A : Light yellow to light pink coloured, homogeneous, free flowing powder.
Part B : Colourless, viscous, solution.

Gelling : Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium : Light orange coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 2.5% w/v Part A of M1078 or 3.1% w/v of Part A of M1082 aqueous solution at 25°C. pH : 7.3 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24 - 48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	>=50%	pink-red
Salmonella Typhimurium (14028)	50-100	luxuriant	>=50%	pink-red
Salmonella Typhi (6539)	50-100	luxuriant	>=50%	colourless
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	blue-green
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=50%	blue-violet
Proteus mirabilis (25933)	50-100	luxuriant	>=50%	colourless
Shigella flexneri (12022)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

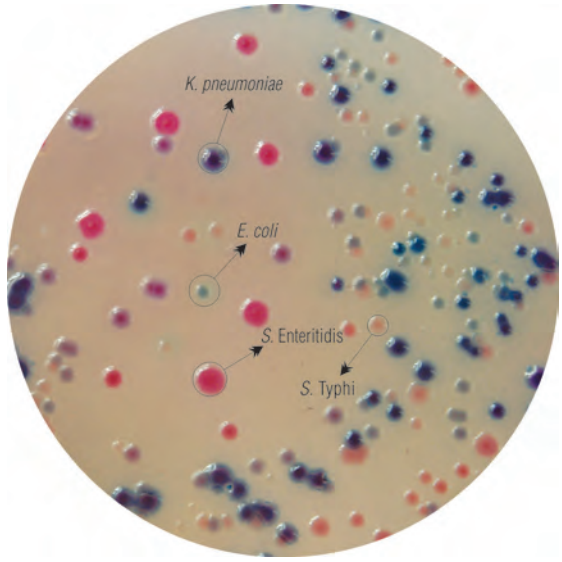
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Eaton A. D., Clesceri L. S., Rice E. W. and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
2. Greenwald R., Henderson R.W. and Yappaw S., 1991, J. Clin. Microbiol., 29:2354.
3. Rambach A., 1990, Appl Environ. Microbiol., 56:301.



M1082 – Salmonella Differential Agar Modified (Twin pack) (RajHans Medium)

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

Salmonella Differential Agar / Modified (Twin pack) (RajHans Medium)(M1078/M1082) is also available as Salmonella Differential HiVeg™ Agar / Modified (Twin pack) (RajHans Medium) (MV1078/MV1082) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

HiCrome™ RajHans Medium/Modified (Salmonella Agar/Modified)

Recommended for identification and differentiation of *Salmonella* species from among the members of *Enterobacteriaceae*, especially *Proteus* species.

M1633 / M1634

Composition **

	M1633	M1634
Ingredients	Grams/Litre	Grams/Litre
Tryptone	8.00	8.00
Yeast extract	5.00	5.00
Peptone	4.00	4.00
Sodium chloride	5.00	5.00
Sodium deoxycholate	1.00	1.00
Neutral red	0.02	0.02
Lactose	3.00	3.00
Chromogenic mixture	7.30	4.32
Agar	13.50	12.00

Final pH (at 25°C) 7.3 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.82 grams of M1633 and 42.34 grams of M1634 in 1000 ml distilled water. Mix well and heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45 - 50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ RajHans Medium/Modified is a modification of the original formulation of Rambach (2), used for differentiation of *Salmonella* species from *Proteus* species and other enteric bacteria. The original formulation is based on the novel characteristic of *Salmonella* species to produce acid from propylene glycol, which is detected by indicators present in the medium. These media are unique, because it is not based on acid production by propylene glycol. These media like many other media such as SS Agar, XLD Agar, recommended for the identification and differentiation of *Salmonella* species are based on lactose fermentation (1).

Tryptone, peptone and yeast extract supports the luxuriant growth of bacteria by providing carbonaceous and nitrogenous compounds, long chain amino acids, vitamin B complex and other essential nutrients. Sodium deoxycholate inhibits gram-positive organisms rendering the medium selective for enteric microorganisms. The chromogenic mixture incorporated in the medium yields pink to red colonies of *Salmonella*.

Quality Control

Appearance of Powder : Light yellow to beige coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.35% Agar gel of M1633 and 1.2% Agar gel of M1634.

Colour and Clarity of prepared medium : Light orange coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 4.68% w/v of M1633 and 4.23% w/v of M1634 aqueous solution at 25°C. pH:7.3 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24 - 48 hours.

Organisms (ATCC)	Inoculum	Growth (CFU)	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	light purple
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=50%	blue-violet
Proteus mirabilis (25933)	50-100	luxuriant	>=50%	colourless
Salmonella Typhi (6539)	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium (14028)	50-100	luxuriant	>=50%	pink-red
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	>=50%	pink-red
Shigella flexneri (12022)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

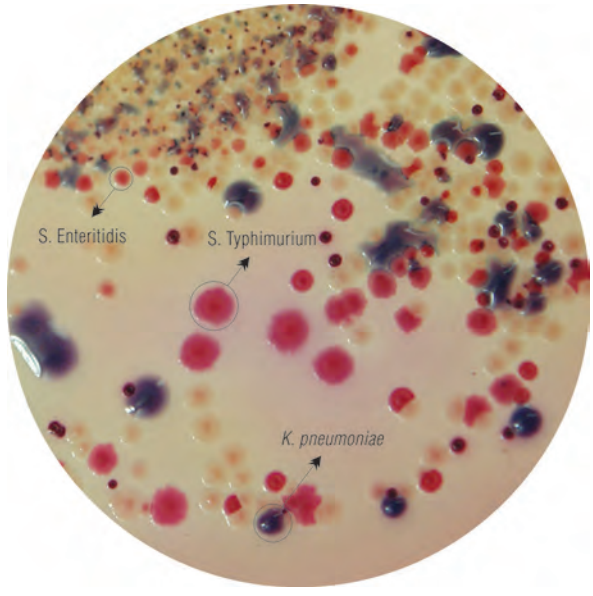
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Greenberg A.E., Trussel R.R., Clesceri L.S., (Eds.), (1985), Standard Methods for the Examination of water and waste water, 16th ed., APHA, Washington, D.C.
2. Rambach A., 1990, Environment. Microbiol, 56:301.



M1633 – HiCrome™ RajHans Medium (Salmonella Agar)

HiCrome™ Salmonella Agar /HiCrome™ Improved Salmonella Agar

Recommended for the simultaneous detection of *Salmonella* and *Escherichia coli* from food, water and clinical samples.

M1296 / M1466

Composition **

	M1296	M1466
Ingredients	Grams/Litre	Grams/Litre
Peptone	6.00	—
Peptone special	—	8.00
Yeast extract	2.50	2.00
Bile salts mixture	1.00	—
Sodium deoxycholate	—	1.00
Chromogenic mixture	5.40	3.25
Agar	13.00	12.00
Final pH (at 25°C)	7.7 ± 0.2	7.3 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.9 grams of M1296 or 26.25 grams of M1466 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Salmonella species have been isolated from humans and almost all animals throughout the world. They cause many types of infections from mild, self-limiting gastroenteritis to life threatening typhoid fever. *Salmonella Typhi* and *Salmonella* Paratyphi A & B cause gastroenteritis, bacteremia and enteric fever, *Salmonella* Choleraesuis causes gastroenteritis and enteric fever, especially in children. *Salmonella* Typhimurium is the most frequently isolated serotype of *Salmonella* (2).

HiCrome™ Salmonella Agar medium is a modification of the original formulation of Rambach (3) and is used for the differentiation of *Salmonella species* from other enteric bacteria. Rambach formulation differentiates *Salmonella* based on propylene glycol utilization and presence of a chromogenic indicator. However, HiCrome™ Salmonella Agar medium uses only a chromogenic mixture for identification and differentiation of *Salmonella species.*

Peptone, peptone special and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients.

Escherichia coli and *Salmonella* are easily distinguishable due to their colony characteristics. *Salmonella* forms light purple coloured colonies with a purple halo on (M1296) and pink to red colonies on (M1466). *E. coli* exhibits a characteristic blue colour, due to presence of the enzyme β-glucuronidase. Other organisms form colourless colonies. The characteristic light purple and blue colour is due to the chromogenic mixture (1). Bile salts mixture or sodium deoxycholate inhibits gram-positive organisms.

Quality Control

Appearance of powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.3% Agar gel of M1296 or 1.2% Agar gel of M1466.

Colour and Clarity of prepared medium : Light amber coloured (M1296) or reddish pink coloured (M1466), slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 2.79% w/v aqueous solution of M1296 at 25°C. pH:7.7 ± 0.2.
Reaction of 2.62% w/v aqueous solution of M1466 at 25°C. pH : 7.3 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony (M1296)	Colour of colony (M1466)
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	blue	blue to purple
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	>=50%	light purple with halo	pink to red
Salmonella Typhi (6539)	50-100	good-luxuriant	>=50%	light purple with halo	light pink
Salmonella Typhimurium (14028)	50-100	luxuriant	>=50%	light purple with halo	pink to red
Proteus vulgaris (13315)	50-100	good	40-50%	colourless	light brown
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-	-
Bacillus subtilis (6633)	>=10^3	inhibited	0%	-	-

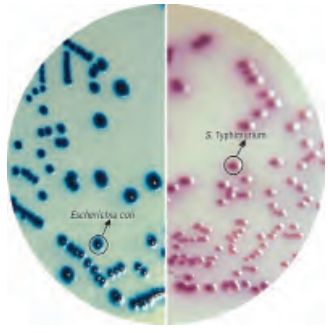
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

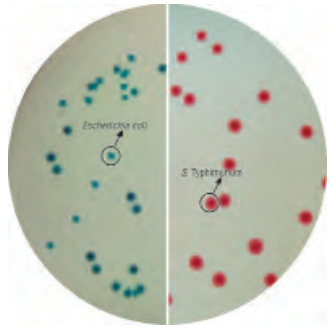
Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Greenwald R., Henderson R. W. and Yappan S., 1991, J. Clin. Microbiol., 29:2354.
2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. Rambach A., 1990, Appl. Environ. Microbiol., 56:301.



M1296-HiCrome™ Salmonella Agar



M1466-HiCrome™ Improved Salmonella Agar

HiCrome™ Selective Salmonella Agar Base

Recommended for the selective isolation of *Salmonella* species from food samples

M1842

Composition **

Ingredients	Grams/Litre
Heart Infusion powder	12.000
Yeast hydrolysate	5.000
Tryptose	5.000
Sodium cholate	3.000
Sodium taurocholate	5.000
Sodium deoxycholate	1.000
Chromogenic mixture	8.000
Agar	15.000

Final pH (at 25°C) 7.3 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.00 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Aseptically add the rehydrated contents of one vial of HiCrome™ Selective Salmonella Agar Supplement (FD274). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Salmonella species have been isolated from humans and almost all animals throughout the world. They cause many types of infections from mild, self-limiting gastroenteritis to life threatening typhoid fever. *Salmonella* Typhi and *Salmonella* Paratyphi A & B cause gastroenteritis, bacteremia and enteric fever, *Salmonella* Choleraesuis causes gastroenteritis and enteric fever, especially in children. *Salmonella* Typhimurium is the most frequently isolated serotype of *Salmonella*. *Salmonella* species are the major cause of food poisoning (1) Various chromogenic media are available for the differentiation of *Salmonella* species. The original media formulated by Rambach (2) differentiates *Salmonella* based on propylene glycol utilization and presence of a chromogenic indicator. However HiCrome™ Selective Salmonella Agar Base uses chromogenic mixture for identification and differentiation of *Salmonella* species. Sodium cholate, Sodium taurocholate and Sodium deoxycholate in the medium helps to restrict the growth of other organisms. Besides the selective supplement added to the medium inhibits competing microorganisms.

Heart Infusion powder, yeast hydrolysate and tryptose in the medium provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Due to the presence of chromogenic mix in the medium *Salmonella* are easily distinguishable and forms purple coloured colonies while some *Enterobacteriaceae* like *Klebsiella* and *Enterobacter* forms blue to dark blue coloured colonies. Conventional method employes the H_2S production property for *Salmonella* detection which is also exhibited by other non *Salmonella* species such as *Citrobacter, Proteus*, etc. Hence further biochemical confirmation is required for further identification.

This medium is specially employed for food samples where the sample is initially enriched in Salmonella Selective Enrichment Broth (M1843) and then isolated on HiCrome™ Selective Salmonella Agar Base. *Salmonella* species give purple coloured colonies due to the enzyme specificity.

Quality Control

Appearance of Powder : Light yellow to beige homogeneous free flowing powder

Gelling : Firm, comparable with 1.5 % Agar gel.

Colour and Clarity of prepared medium : Whitish cream coloured, opalescent gel forms in Petri plates

Reaction : Reaction of 5.4% w/v aqueous solution at 25°C. pH : 7.3 ± 0.2.

Cultural Response : Cultural characteristics observed with added HiCrome™ Selective Salmonella Agar Supplement (FD274), after an incubation at 35-37°C for 22-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Salmonella Typhimurium (14028)	50 -100	good-luxuriant	>=50 %	purple
Salmonella Enteritidis (13076) (00030*)	50 -100	good- luxuriant	>=50 %	purple
Klebsiella pneumoniae (13883)	50 -100	good	40 -50 %	blue
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0 %	-
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

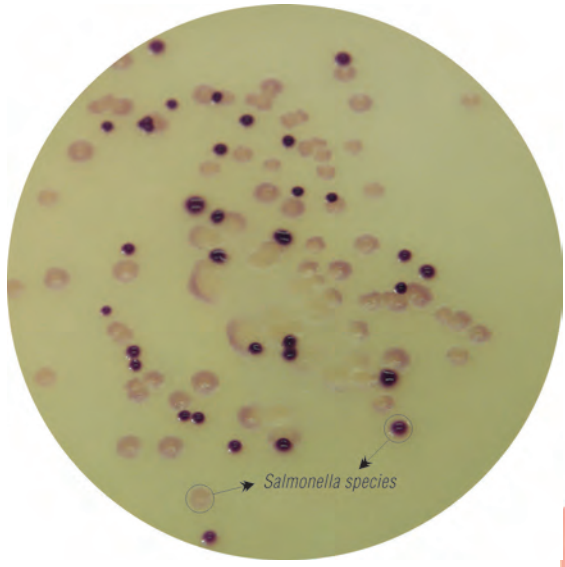
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder and prepared medium at 2-8°C in tightly closed container. Use before expiry period on the label

References

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Rambach A., 1990, Appl. Environ. Microbiol., 56:301.



M1842 – HiCrome™ Selective Salmonella Agar Base

HiCrome™ MM Agar

Recommended for identification and differentiation of *Salmonella* and non-salmonella like *Citrobacter* from food, water and clinical samples.

M1393

Composition **

Ingredients	Grams/Litre
Peptone	10.00
Meat extract B#	2.00
D-Cellobiose	3.00
Lactose	10.00
D-Mannitol	1.20
D-Trehalose	1.33
Chromogenic mixture	6.60
Agar	15.00

Final pH (at 25°C) 7.6 ± 0.2

** Formula adjusted, standardized to suit performance parameters

#Equivalent to Beef extract

Directions

Suspend 49.13 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ MM Agar was formulated by Miller and Mallison (1) for specific isolation and detection of Salmonellae. This medium is superior to XLT4 Agar in supporting growth of *Salmonella* due to the presence of appropriate proportion of four sugars. Most differential and selective media are formulated with one or more sugars and pH indicators respectively. The utilization of sugars by organisms results in pH-changes. This is used as a means of distinguishing *Salmonella* from competing bacteria on the basis of colony colour. *Salmonella* usually are unable to ferment the sugars (2) that support growth of competing bacteria. Thus other bacteria tend to overgrow Salmonellae, masking their presence. The inclusion of sugars like mannitol, cellobiose and trehalose stimulate the better initial growth of *Salmonella* cells. However, the low concentrations of these sugars do not interfere with the utilization of protein and H_2S production. Presence of lactose suppresses H_2S production by non-salmonellae like *Citrobacter freundii*. A chromogenic mixture, present in this medium helps to differentiate between lactose fermenters and nonfermenters. Lactose fermenters give bluish green coloured colonies, which would have been impossible to differentiate with an indicator based on pH change. Inclusion of tergitol 4 (included in chromogenic mixture) in the medium suppresses the presence of *Proteus* and *Providencia* colonies. Peptone and meat extract B# provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light amber coloured, slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 4.91% w/v aqueous solution at 25°C. pH:7.6 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	light blue
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	>=50%	black centered
Salmonella Typhimurium (14028)	50-100	luxuriant	>=50%	black centered
Citrobacter freundii (8090)	50-100	good-luxuriant	>=50%	colourless#
Pseudomonas aeruginosa (27853) (00025*)	50-100	good-luxuriant	>=50%	colourless
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	-

Key : # = may show bluish green colour on prolonged incubation

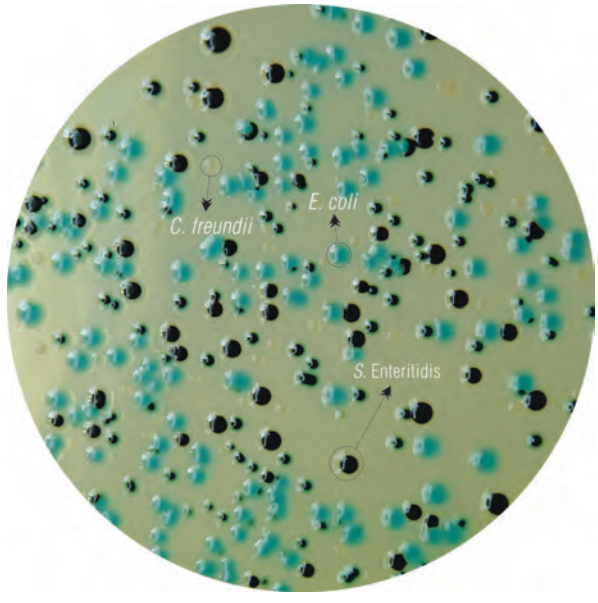
* = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Miller R.G. and Mallison E.T., 2000, J. Food Protection, 63(10), 1443-46.
2. Miller R.G., Tate C.R., Mallinson E.T. and Scherrer J.A., 1991, Pault Sa 70:2429-32.



M1393 – HiCrome™ MM Agar

HiCrome™ Veg Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

HiCrome™ MM Agar (M1393) is also available as HiCrome™ MM HiVeg ™ Agar (MV1393) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

HiCrome™ MM Agar Modified

Recommended for identification and differentiation of *Salmonella* and non-*Salmonella* like *Citrobacter* from clinical samples.

M1816

Composition **

Ingredients	Grams/Litre
Proteose peptone	6.00
Yeast extract	10.00
L-Lysine Hydrochloride	5.00
D-Cellobiose	10.00
Lactose	10.00
Sucrose	10.00
D-Xylose	3.75
Ferric Ammonium citrate	0.80
Sodium thiosulphate	6.80
Chromogenic mixture	0.20
Bromothymol blue	0.10
Agar	18.00

Final pH (at 25°C) 7.6 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.65 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ MM Agar was formulated by Miller and Mallison (1) for specific isolation and detection of Salmonellae. This medium is superior to XLT4 Agar in supporting growth of *Salmonella* due to the presence of appropriate proportion of four sugars. HiCrome™ MM Agar, Modified is a slight modification of HiCrome™ MM Agar and designed to differentiate *Enterobacteriaceae* especially *Salmonella* from *Proteus* and *Citrobacter* group. The utilization of sugars by organisms results in pH-changes. This is used as a means of distinguishing *Salmonella* from competing bacteria on the basis of colony colour.

Salmonella are gram negative rods in the family *Enterobacteriaceae* present in the stomach and intestinal tissues of human & animals and are found in their wastes. *Salmonella* usually are unable to ferment the sugars (2) that support growth of competing bacteria. Thus other bacteria tend to overgrow *Salmonellae*, masking their presence. Proteose peptone is a source of carbon, nitrogen and other essential amino acid and growth factor. Yeast extract provides vitamin especially Group B vitamins required for growth. To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. Bromothymol blue act as a pH indicator. The inclusion of sugars like lactose, sucrose, xylose and cellobiose provides source of fermentable carbohydrate which stimulate the better initial growth of *Salmonella* cells. Presence of lactose suppresses H_2S production by non-salmonellae like *Citrobacter freundii*. A chromogenic mixture, present in this medium helps to differentiate between lactose fermenters and nonfermenters. Lactose fermenters give bluish green coloured colonies, which would have been impossible to differentiate with an indicator based on pH change.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.8% Agar gel

Colour and Clarity of prepared medium : Bluish green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction : Reaction of 8.07 % w/v aqueous solution at 25°C. pH : 7.6 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18 - 24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Citrobacter freundii (8090)	50-100	good -luxuriant	>=50%	Yellow# coloured
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=50%	Bluish green
Salmonella Typhimurium (14028)	50-100	luxuriant	>=50%	black centered
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	>=50%	black centered with yellow zone
Salmonella Typhi (6539)	50-100	good -luxuriant	>=50%	Black centered
Proteus mirabilis (25933)	50-100	good -luxuriant	>=50%	Gray coloured
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=50%	Yellowish green, mucoid

key # : may show bluish green colour on prolonged incubation.

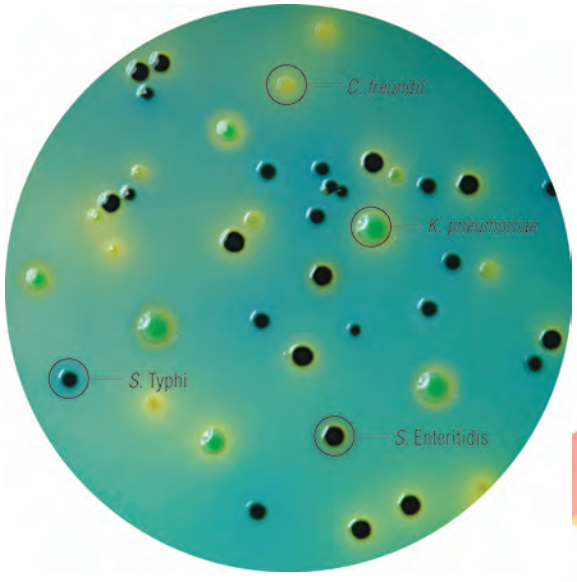
* = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Miller R.G. and Mallison E.T., 2000, J. Food Protection, 63(10), 1443-46.
2. Miller R.G., Tate C.R., Mallinson E.T. and Scherrer J.A., 1991, Pault Sa 70:2429-32.



HiCrome™ Klebsiella Selective Agar Base

M1573

Recommended for the isolation and detection of *Klebsiella species* from water and other sources.
This medium can also be used in membrane filtration procedure.

Composition **

Ingredients	Grams/Litre
Peptone, special	12.00
Yeast extract	7.00
Sodium chloride	5.00
Bile salts mixture	1.50
Sodium lauryl sulphate (SLS)	0.10
Chromogenic mixture	0.20
Agar	15.00

Final pH (at 25°C) 7.1 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.4 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of one vial of Klebsiella Selective Supplement (FD225). Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Klebsiella Selective Agar Base is recommended for isolation and enumeration of *Klebsiella species* based on chromogenic differentiation. *Klebsiella pneumoniae* strains are widely distributed in the environment and contribute to biochemical and geochemical process (1).

K. pneumoniae causes severe often fatal pneumonia. It also proves to be the source of lung infections that generally occur in patients with debilitating conditions such as alcoholism, diabetes mellitus, and chronic obstructive pulmonary disease (2). The chromogenic substrate incorporated in the media is cleaved specifically by *Klebsiella* species. *K. pneumoniae*, the causative agent of pneumonia, produces a purple-magenta coloured colony thereby aiding in the easy detection of the organisms. Most of the frequently encountered gram-negative faecal contaminants are inhibited on this media using a selective supplement.

Peptone special and yeast extract provide nitrogeneous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients required for the growth of the organism. Sodium chloride maintains the osmotic equilibrium of the medium. Bile salts mixture and sodium lauryl sulphate (SLS) inhibits most of the accompanying flora. Addition of the selective supplement further increases the selectivity of the medium.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 4.08% w/v aqueous solution at 25°C. pH:7.1 ± 0.2.

Cultural Response : Cultural characteristics observed with added Klebsiella Selective Supplement (FD225) after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=50%	purple-magenta (mucoid)
Enterobacter aerogenes (13048) (00175*)	>=10^3	inhibited	0%	-
Escherichia coli (25922) (00013*)	>=10^3	inhibited	0%	-
Serratia marcescens (8100)	>=10^3	inhibited	0%	-
Salmonella Typhi (6539)	>=10^3	inhibited	0%	-

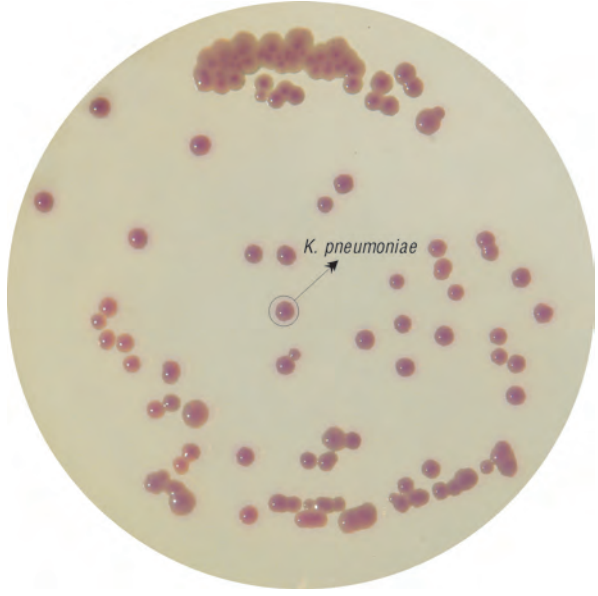
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Krieg, N. R., and J. G. Holt, (Eds.), 1984, Bergey's Manual of Systematic Bacteriology, Vol. 1, p. 408 - 516. The Williams and Wilkins Co., Baltimore, Md.
2. Wyngaarden J. B., Smith L. H., (Eds.), Cecil Text book of Medicine, 16th Ed, pp 1430 -1432, Philadelphia, W. B. Saunders, 1982.



M1573 – HiCrome™ Klebsiella Selective Agar Base

HiCrome™ ESBL Agar Base

Recommended for selective isolation of Extended-Spectrum β-lactamase-producing *Enterobacteriaceae*.

M1829

Composition **

Ingredients	Grams/Litre
Peptone mix	12.000
Chromogenic mixture	4.000
Sodium chloride	5.000
Buffer mix	4.000
Agar	15.000

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add rehydrated contents of two vials of HiCrome™ ESBL Agar Supplement (FD278). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Extended-spectrum β-lactamase (ESBL)-producing organisms are an increasing challenge for healthcare practitioners fighting healthcare-associated infections (HAIs). *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca* are the most common ESBL-producing pathogens (1). ESBL-producing organisms are generally resistant to many classes of antibiotics, including aminoglycosides and fluoroquinolones; ESBL-producing organisms are able to attack newer cephems and monobactams as well as narrow-spectrum cephalosporins and anti gram-negative penicillins (1). They are associated with increased mortality and are difficult to detect and treat. The widespread use of extended-spectrum, third-generation cephalosporins, introduced in the 1980s to treat antibiotic-resistant bacteria, is believed to be a major contributor to the emergence of ESBL-producing organisms.

HiCrome™ ESBL Agar Base is chromogenic screening medium for the selective isolation of ESBL producing organisms. It contains peptone mix which serves as the carbon and nitrogen sources, long chain amino acids, vitamins and other growth nutrients. Chromogenic mixture is used to differentiate the ESBL producing organisms on the basis of colour. HiCrome™ ESBL Agar Supplement (FD278) helps in inhibition of other contaminating organisms. ESBL producing *E.coli* grow as pink to purple colonies. ESBL producing members of the KESC group produce bluish green colonies; *Proteus, Morganella* and *Providencia* do not utilize any chromogen resulting in colourless to light brown colonies. This medium can be inoculated with liquid suspension equivalent to 0.5 McFarland turbidity, prepared from rectal screening swabs, faecal samples or from isolated colony. Isolated colonies should not be directly plated on to this medium, because the high level inoculum may cause false positive results. Further confirmation using biochemical identification tests is recommended.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Yellow coloured opalescent gel forms in Petri plates.

Reaction : Reaction of 4.0% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2.

Cultural Response : Cultural characteristics observed with added HiCrome™ ESBL Agar Supplement (FD278) after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli (NCTC 13351)	50-100	luxuriant	>=50%	pink to purple
Klebsiella pneumoniae (700603)	50-100	luxuriant	>=50%	bluish green
Enterobacter cloacae (23355)	>=10^3	inhibited	0%	—
Citrobacter freundii (8090)	>=10^3	inhibited	0%	—
Candida albicans (10231)	>=10^3	inhibited	0%	—

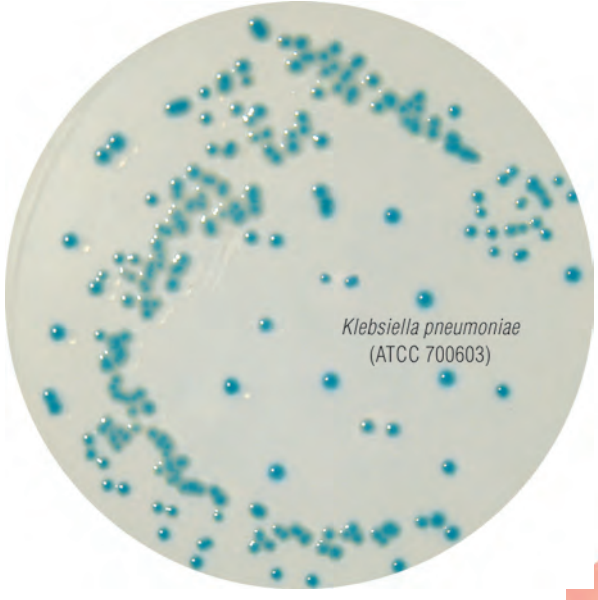
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Journal of Clinical Microbiology, February 2007, Page 501-505, Vol. 45, No. 2 pp 1430 -1432, Philadelphia, W. B. Saunders, 1982.



M1829 – HiCrome™ ESBL Agar Base

HiCrome™ KPC Agar Base

Recommended for the detection of gram negative bacteria with a reduced susceptibility to a carbapenem agents.

M1831

Composition **

Ingredients	Grams/Litre
Peptone	15.000
Chromogenic mixture	3.000
Agar	15.000

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16.50 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of HiCrome™ KPC Agar Supplement (FD279). Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ KPC Agar Base is a chromogenic medium designed for the detection and differentiation of KPC producing gram negative bacterial species without selective pre-enrichment. Carbapenems are the last line of defense against invasive or serious infections and are used to treat these life threatening infections that are caused by gram negative, drug resistant pathogens (2). Production of carbapenemase enzyme results in resistance to penicillins, cephalosporins (i.e. cefepime, ceftriaxone), carbapenems (i.e. meropenem, ertapenem) and aztreonam there by making these pathogens multi drug resistant.

Most carbapenemase producing bacteria are included in the family *Enterobacteriaceae* and are thus termed as carbapenem resistant *Enterobacteriaceae* (CRE). Besides the *Enterobacteriaceae* family, rare strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have also found to produce carbapenemase (1, 2, 3).

Peptone provides nitrogenous and carbonaceous compounds long chain amino acids and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections. Selective supplement have been added to inhibit the growth of yeast, gram positive organisms and gram negative organisms that do not produce carbapenemase.

This medium is intended to be used as a screening medium. Isolates should be tested further for carbapenem susceptibility following CLSI guidelines. Indole test may be perform for the confirmation of carbapenem resistant *E. coli* because some rare strains of *C. freundii* may produce small pink to magenta coloured colonies similar to *E. coli.* Carbapenem resistant strains of *Klebsiella, Enterobacter* and *Serratia* species produce bluish green colonies. *Acinetobacter* and *Salmonella* species produce smooth, colourless colonies. *Pseudomonas* species produce colourless to light yellowish green, translucent colonies with wrinkled edges. Further biochemical tests may be needed for complete identification.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 3.3% w/v aqueous solution at 25°C. pH : 7.0 ± 0.2.

Cultural Response : Cultural characteristics observed with added HiCrome™ KPC Agar Supplement (FD279) after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	—
Klebsiella pneumoniae (BAA 1705)	50-100	luxuriant	>=50%	bluish green
Klebsiella pneumoniae (13883)	>=10^3	inhibited	0%	—
Candida albicans (10231)	>=10^3	inhibited	0%	—
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	—

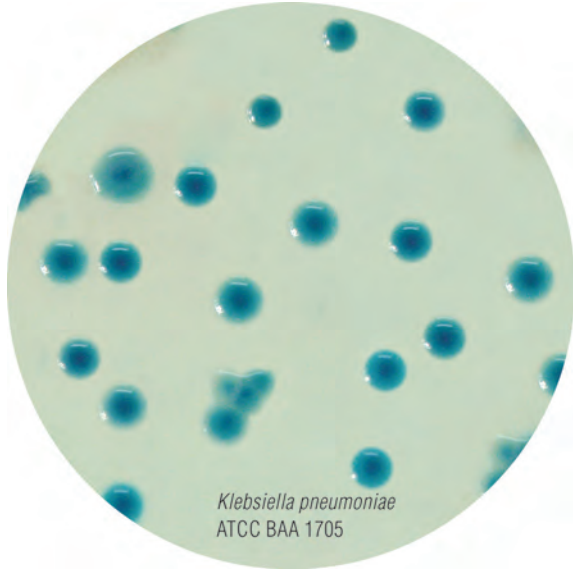
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Hindiyeth, M., et. al. 2008, J. Clin. Microbiol.; Vol. 46, p.2879 -2883
2. Pillai D.R. et.al. 2009. Emerg. Infect. Dis; Vol. 15, P.827-829
3. Samra, Z., 2008, J. Clin. Microbiol; Vol. 146, P.3110-3111.



M1831 – HiCrome™ KPC Agar Base

HiCrome™ Vibrio Agar

Recommended for the isolation and selective chromogenic differentiation of *Vibrio* species from seafood.

M1682

Composition **

Ingredients	Grams/Litre
Peptone	10.00
Sodium chloride	25.00
Sodium thiosulphate	5.00
Sodium citrate	6.00
Sodium cholate	1.00
Chromgenic mixture	5.50
Agar	15.00

Final pH (at 25°C) 8.5 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 67.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Vibrio's have played a significant role in human history. Outbreaks of cholera, caused by *Vibrio cholerae*, can be traced back in time to early recorded descriptions of enteric infections. The *Vibrios* have also received the attention of marine microbiologists who observed that the readily cultured bacterial population in near-shore waters and those associated with fish and shell fish were predominantly *Vibrio* species (4). *Vibrio species* are mainly responsible for causing cholera and food poisoning in humans. *Vibrio cholerae* causes cholerae due to the intake of contaminated food such as raw oysters. *Vibrio parahaemolyticus* is a major cause of food borne infections, causing food poisoning (1). Since *Vibrio* species naturally occur in sea-water, worth special mention is their need for sodium chloride, although some species can grow with minimum sodium chloride concentration (4). The widely used media for *Vibrio* isolation are TCBS Agar and Alkaline Peptone Water (2). However accompanying sucrose-fermenting bacteria pose a problem in the identification of *Vibrio species* on TCBS Agar. On HiCrome™ Vibrio Agar, the colour development by *Vibrio species* is not affected by the presence of colonies of other bacteria. This is because, the amount of colour developed depends on the reaction of the bacterial beta-galactosidase with the substrate contained in the media (3).

Peptone provides carbonaceous and nitrogeneous compounds, long chain amino acids and essential nutrients to the organisms. High concentration of sodium chloride in addition to maintaining the osmotic equilibrium also has an inhibitory action on the accompanying microflora. Sodium thiosulphate, sodium citrate and sodium cholate are used in the formulation because they can inhibit the growth of gram positive and some gram negative bacteria, but not members of *Enterobacteriaceae*. The proprietary chromogenic mixture incorporated in the medium helps in the chromogenic differentiation of *Vibrio cholerae* and *Vibrio parahaemolyticus*. The high (alkaline) pH of the medium helps in selective isolation of *Vibrio species*.

Quality Control

Appearance of Powder : Light yellow to light tan coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light yellow coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 6.75% w/v aqueous solution at 25°C. pH:8.5 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Vibrio cholerae (15748)	50-100	good-luxuriant	>=50%	purple
Vibrio parahaemolyticus (17802)	50-100	good-luxuriant	>=50%	bluish-green
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	
Escherichia coli (25922) (00013*)	>=10^3	inhibited	0%	

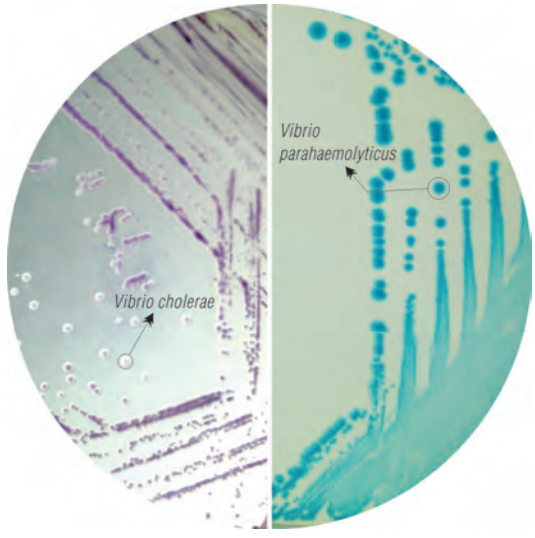
Key * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Alcamo. E.I, 2001. Fundamentals of Microbiology, 6th ed, Jones and Bartlett Publishers, Inc. pg 254, 244.
2. Clesceri, Greenberg and Eaton (ed.). 1998. Standard Method for the examination of Water and Waste water, 20th ed. American Public Health Association, Washington, D. C.
3. Kudo. H. Y et al, 2001. Improved Method for Detection of Vibrio parahaemolyticus in Seafood. ASM. Vol 67, No. 12, pg 5819-5823.
4. Thompson et al (ed.). 2006. The Biology of Vibrios, ASM Press, chapter1, pg 3.



M1682– HiCrome™ Vibrio Agar

HiCrome™ UTI Agar, Modified / HiCrome™ UTI Selective Agar

M1418/ M1505

A chromogenic differential medium for identification, differentiation and confirmation of enteric bacteria from specimens such as urine which may contain large number of *Proteus* species as well potentially pathogenic gram - postive organisms.

Composition **

	M1418	M1505
Ingredients	Grams/Litre	Grams/Litre
Peptone	18.00	18.00
Tryptone	4.00	4.00
Meat extract B#	6.00	6.00
Chromogenic mixture	12.44	12.44
Bile salts	-	1.50
Agar	15.00	15.00

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

#Equivalent to Beef extract

Directions

Suspend 56.94 grams of M1505 or 55.44 grams of M1418 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ UTI Agar, Modified/ HiCrome™ UTI Selective Agar is formulated on the basis of work carried out by Pezzlo (4), Wilkie et al (6), Friedman et al (1), Murray et al (3), Soriano and Ponte (5) and Merlino et al (2). These media are the modifications of HiCrome™ UTI Agar (M1353), which can be used in place of MacConkey Agar for isolation, and confirmation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates.

Enzymes produced by *Enterococcus species*, *Escherichia coli* and coliforms cleave the chromogenic substrates incorporated in the medium. Presence of rich source of phenylalanine and tryptophan from peptone and tryptone provides an indication of tryptophan deaminase activity, revealed with TDA Reagent (R036) indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species, which appear brown. One chromogenic substrate is cleaved by β-glucosidase possessed by enterococci resulting in formation of blue colonies. *E. coli* produce purple to magenta colonies due to the enzyme β-D-galactosidase which cleaves the other chromogenic substrate. Further confirmation of *E. coli* can be done by performing indole test using DMACA Reagent (R035). Also, some strains of *Enterobacter cloacae* lacking β-glucosidase show pink colonies indistinguishable from *E. coli*. The DMACA reagent for indole test (should be performed on filter paper) distinguishes between *E. coli* and *Enterobacter*, and TDA reagent between *Proteus mirabilis* and other species. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrates. Peptone, Meat extract B and Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. HiCrome™ UTI Selective Agar is made selective by the addition of bile salts, which selectively inhibits gram-positive bacteria.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 5.54% w/v of M1418 or 5.69% w/v of M1505 aqueous solution at 25°C. pH:7.2 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth «	Recovery «	Growth ««	Recovery ««	Colour of colony	TDA #	DMACA ##
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=70%	luxuriant	>=50%	purple- magenta	-	+
Proteus mirabilis (12453)	50-100	luxuriant	>=70%	luxuriant	>=50%	light brown	+	-
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=70%	luxuriant	>=50%	blue to purple, mucoid	-	-
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	>=70%	luxuriant	>=50%	colourless greenish pigment may be observed	-	-
Enterococcus faecalis (29212) (00087*)	50-100	luxuriant	>=70%	fair	20-30%	blue -blue green(small)	-	-
Staphylococcus aureus (25923)	50-100	luxuriant	>=70%	inhibited	0%	golden yellow*	-	-

Key : TDA + : Tryptophan deaminase present , TDA - : Tryptophan deaminase absent

DMACA + : Indole positive, DMACA - : Indole negative,

«: on HiCrome UTI Agar, Modified «« : on HiCrome UTI Selective Agar

: Add 1-2 drops of TDA reagent directly on suspected colony. Brown colouration-positive.

: Transfer suspected colony on filter paper, dipped in DMACA reagent. Bluish purple colouration- positive.

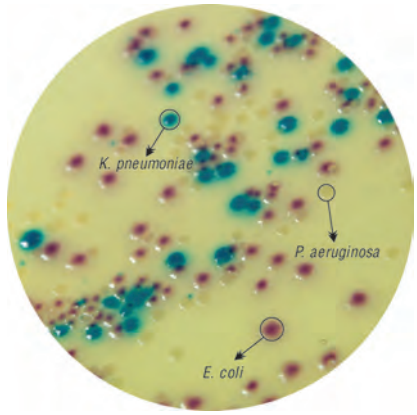
* = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Friedman M. P. et al, 1991, J. Clin. Microbiol., 29:2385-2389.
2. Merlino et al, 1995, Abstr. Austr. Microbiol., 16(4):17-3.
3. Murray P. R., Traynor P. and Hopson D., 1992, J. Clin. Microbiol., 30:1600-1601.
4. Pezzlo M., 1998, Clin. Microbiol. Rev., 1:268-280.
5. Soriano F. and Ponte C., 1992, J. Clin. Microbiol., 30:3033-3034.
6. Wilkie M. E., Almond M. K. and Marsh F. P., 1992, British Medical Journal, 305:1137-1141.



M1418 – HiCrome™ UTI Agar, Modified

HiCrome™ UTI Agar

M1353 / M1353R

A differential medium recommended for presumptive identification and confirmation of microorganisms mainly causing urinary tract infections. Can also be used for testing water, food, environmental and other clinical samples.

Composition **

	M1353	M1353R
Ingredients	Grams/Litre	Grams/Litre
Peptone, special	15.00	-
Peptone	-	15.00
Chromogenic mixture	2.45	26.80
Agar	15.00	15.00

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.45 grams of M1353 or 56.8 grams of M1353R in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli*, but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella species*, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome UTI Agar is formulated on basis of work carried out by Pezzlo (5) Wilkie et al (7), Friedman et al (2), Murray et al (4), Soriano and Ponte (6) and Merlino et al (3). These media are recommended for the detection of urinary tract pathogens where HiCrome™ UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus species*, *E. coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus species*, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by β-glucosidase possessed by enterococci resulting in formation of blue colonies. *E. coli* produce pink-purple colonies due to the enzyme β-D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E. coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus*, *Morganella* and *Providencia species* appear brown because of tryptophan deaminase activity. Peptone or peptone special provides nitrogenous, carbonaceous compounds long chain amino acids and other essential growth nutrients.

This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

Quality Control

Appearance of Powder : Cream to yellow (M1353) or white to cream (M1353R) coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity : Light amber coloured, clear to slightly opalescent of prepared medium gel forms in Petri plates of M1353 or white coloured opaque gel forms with precipitate in Petri plates of M1353R.

Reaction : Reaction of 3.24% w/v of M1353 or 5.68% w/v of M1353R aqueous solution at 25°C. pH : 6.8 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=70%	pink-purple
Proteus mirabilis (12453)	50-100	luxuriant	>=70%	light brown
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=70%	blue to purple, mucoid
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	>=70%	colourless, greenish pigment may be observed
Staphylococcus aureus (25923)	50-100	luxuriant	>=70%	golden yellow
Enterococcus faecalis (29212) (00087*)	50-100	luxuriant	>=70%	blue, small

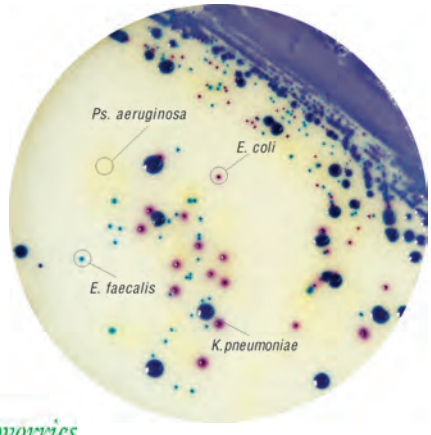
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
2. Friedman M. P. et al, 1991, J. Clin. Microbiol., 29:2385-2389.
3. Merlino et al, 1995, Abstr. Austr. Microbiol. 16(4):17-3.
4. Murray P., Traynor P. Hopson D., 1992, J. Clin. Microbiol., 30:1600-1601.
5. Pezzlo M., 1998, Clin. Microbiol. Rev., 1:268-280
6. Soriano F., Ponte C., 1992, J. Clin. Microbiol., 30:3033-3034.
7. Wilkie M. E., Almond M. K., Marsh F. P., 1992, British Medical Journal 305:1137-1141.



M1353R HiCrome™ UTI Agar

HiCrome™ Universal Differential Medium

HiCrome™ Universal Differential Medium is a differential medium recommended for presumptive identification of microorganisms from clinical and non-clinical specimens.

M1600

Composition **

Ingredients	Grams/Litre
Peptone	15.00
Tryptone	4.00
Chromogenic mixture	2.50
Agar	13.50

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.00 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Universal Differential Medium is a modification of the medium formulated on basis of the work carried out by Pezzlo (4), Wilkie et al (6), Friedman et al (1), Murray et al (3), Soriano and Ponte (5) and Merlino et al (2). HiCrome™ Universal Differential Medium is recommended for the presumptive identification of microorganisms from clinical and non-clinical specimens where the medium has broader application as a general nutrient agar for isolation of various microorganisms. This medium helps in the identification of some gram-positive bacteria and gram-negative bacteria on the basis of different colony colours exhibited by them. These colours are formed due to the reactions of genus or species specific enzymes with the two chromogenic substrates incorporated in the medium. *Enterococcus species, Escherichia coli* and coliforms produce enzymes which specifically cleave these chromogenic substrates to give characteristically distinctive colony colours. Peptone in the medium serve as sources of amino acids like phenylalanine and tryptophan which aids in indicating tryptophan deaminase activity, thereby facilitating the identification of *Proteus species*, *Morganella* species and *Providencia* species. One of the chromogenic substrate is cleaved by β-glucosidase enzyme possessed by Enterococci resulting in the formation of bluish green colonies. *Escherichia coli* possesses the enzyme β-galactosidase which specifically cleaves the other chromogenic substrate resulting in the formation of purple coloured colonies. *Escherichia coli* can be differentiated and confirmed from other similar coloured colonies, by performing the indole test.

Coliforms cleave both the chromogenic substrates forming blue to purple coloured colonies. Colonies of *Proteus, Morganella* and *Providencia* species appear brown due to tryptophan deaminase activity. Peptone and Tryptone provide nitrogenous and carbonaceous compounds, essential growth nutrients and also serve as a source of amino acids.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.35% Agar gel.

Colour and Clarity : Light amber coloured, clear to slightly of prepared medium opalescent gel forms in Petri plates

Reaction : Reaction of 3.5% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis (29212) (00087*)	50-100	luxuriant	>=70%	blue, small
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=70%	purple
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=70%	blue - green, mucoid
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	>=70%	colourless (greenish pigment may be observed)
Proteus mirabilis (12453)	50-100	luxuriant	>=70%	light brown
Staphylococcus aureus (25923)	50-100	luxuriant	>=70%	golden yellow
Salm*onella* Typhi (6539)	50-100	luxuriant	>=70%	colourless
Salmonella Typhimurium (14028)	50-100	luxuriant	>=70%	colourless

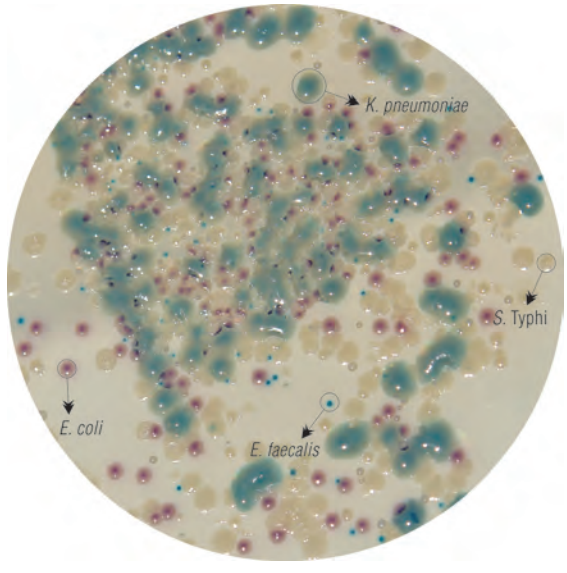
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Friedman M.P. et al (1991), Journal of Clinical Microbiology, 29:2385-2389.
2. Merlino et al (1995) Abstr. Austr. Microbiol. 16(4):17-3.
3. Murray P., Traynor P. Hopson D., (1992), Journal of Clinical Microbiology 30:1600-1601.
4. Pezzlo M (1998), Clinical Microbiology Reviews 1:268-280.
5. Soriano F., Ponte C., (1992), Journal of Clinical Microbiology 30:3033-3034.
6. Wilkie M.E.,Almond M.K.,Marsh F.P.(1992),British Medical Journal 305:1137-1141.



M1600 – HiCrome™ Universal Differential Medium

HiCrome™ Mueller Hinton Agar

HiCrome™ Mueller Hinton Agar is used for differentiation of organisms based on chromogenic differentiation and determination of susceptibility of microorganisms to antimicrobial agents.

M2010

Composition **

Ingredients	Grams/Litre
Casein acid hydrolysate	20.00
Chromogenic mixture	1.50
Agar	17.00

Final pH (at 25°C) 7.3±0.1

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.50 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

The Mueller Hinton formulation was originally developed for the cultivation of pathogenic *Neisseria species* (1). Other mediawere subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria species,* but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (2).

Casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT). Chromogenic mixture incorporated helps in colour differentiation. One of the chromogenic substrate is cleaved by β-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink to purple colonies due to the enzyme β-D-galactosidase that cleaves the other chromogenic substrate. *Staphylococcus aureus* produces colourless colonies. *Pseudomonas aeruginosa* produces greenish pigmentation. *Klebsiella* and *Enterobacter species* produces metallic blue colured colonies. Colonies of *Proteus, Morganella* and *Providencia species* appear brown .This medium can be employed in screening urinary tract pathogens wherein organisms can be differentiated based on colour and simultaneously the antibiotic sensitivity can be determined.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.7% agar gel.

Colour and Clarity : Light amber coloured clear to slightly opalescent gel froms in Petri plates

Reaction : Reaction of 3.85% w/v aqueous solution at 25°C. pH : 7.3±0.1

Cultural Response : Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	>=70%	pink-purple
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	>=70%	greenish pigment may be observed
Staphylococcus aureus (25923)	50-100	luxuriant	>=70%	colourless-golden yellow
Enterococcus faecalis (29212) (00087*)	50-100	luxuriant	>=70%	blue
Klebsiella pneumoniae (13883)	50-100	luxuriant	>=70%	metallic blue

Key : * = corresponding WDCM Numbers

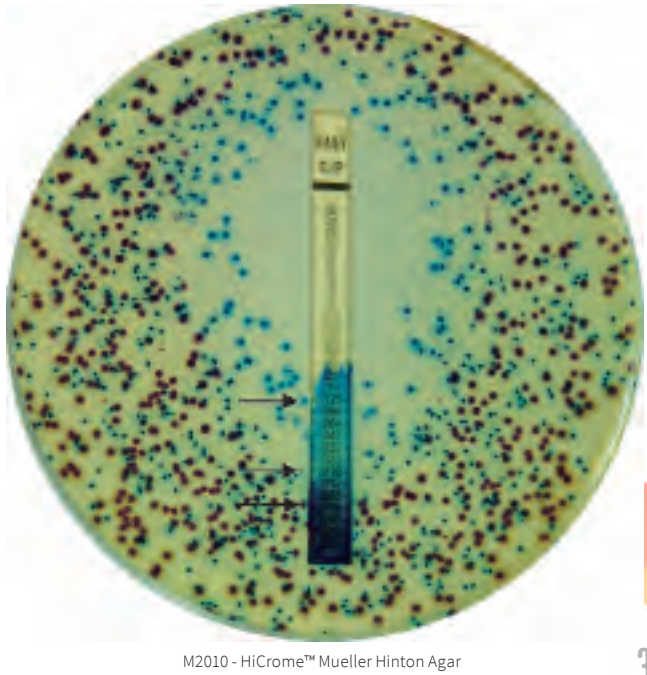
Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
2. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.

Mixture of *Klebsiella, S. faecalis* and *E. coli*



HiCrome™ Rapid ECC Broth

HiCrome™ Rapid ECC Broth is recommended for rapid detection of Escherichia coli and other Enterobacterioaceae from water samples.

M2011

Composition **

Ingredients	Grams/Litre
Peptone special	24.00
Sodium chloride	5.00
Disodium hydrogen phosphate	1.00
Sodium thiosulphate	5.00
Ferric citrate	1.00
Lactose	5.00
Phenol red	0.018
Selective mix	1.50
Chromogenic substrate	3.83

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.35 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle and Interpretation

HiCrome™ Rapid ECC Broth is designed for detection and confirmation of Escherichia coli and other coliforms from water samples. The major microbial water contaminants are coliforms include Escherichia coli, Klebsiella pneumoniae, Salmonella, Citrobacter, Vibrio, and Pseudomonas (1). This test was designed for the rapid detection and differentiation of these organisms.

Peptone special provides nitrogen and carbon source, long chain amino acids, vitamins and other essential growth nutrients. Phosphates buffer the medium. Lactose is the fermentable carbohydrate and phenol red is the indicator. Lactose fermenting organisms gives yellow colour to the medium while lactose non-fermentors gives pink to red colour. The chromogenic substrate is used to detect the presence of ß-D-glucuronidase produced by E.coli thus imparting blue colour to the medium. However since E.coli also ferments lactose, the presence of E.coli is indicated by bluish gren to green colour. The The detection of H_2S production is enhanced by the presence of specific H_2S detectors. The medium turns black in case of H_2S producers such as Salmonella, Citrobacter etc are present. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium chloride helps to maintain the osmotic balance. Selective mix present in the medium suppresses the growth of gram positive microorganisms. Recovery of these pathogens is faster and reliable.

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

Colour and Clarity : Red coloured clear solution in tubes

Reaction : Reaction of 4.83% w/v aqueous solution at 25°C. pH : 7.4±0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 12-18 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Colour change in medium
Escherichia coli ATCC 25922	50-100	luxuriant	green
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	yellow
Citrobacter freundii ATCC 8090	50-100	luxuriant	black
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	black
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	

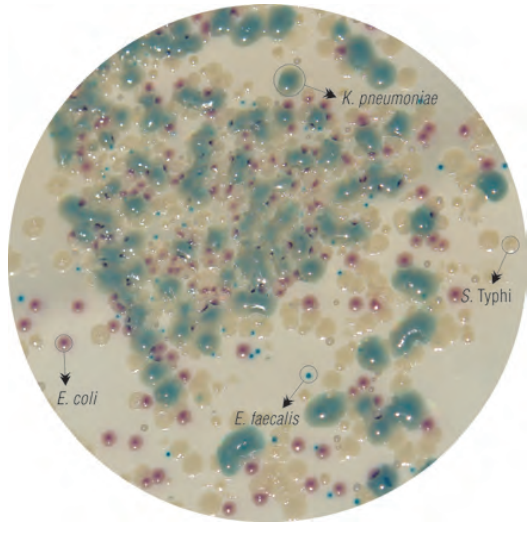
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder and prepared medium at 2-8°C in tightly closed container. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

References

1. Methods for Examination of Waters and Associated Materials, Environment Agency, 1998, Standing Committee of Analysts.
2. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
3. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
4. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.



M1600 – HiCrome™ Universal Differential Medium

UTI Causing Organisms

HiCrome™ Yersinia Agar Base

HiCrome™ Mueller Hinton Agar is used for detection and isolation of pathogenic Yersinia enterocolitica from clinical specimens and food samples.

M2025

Composition **

Ingredients	Grams/Litre
Peptone mix	24.24
Selective mix	7.74
Chromogenic mixture	10.45
Growth factor	3.00
Agar	12.50

Final pH (at 25°C) 7.4±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 57.93 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C and aseptically add reconstituted contents of 1 vial of Yersinia Selective Supplement (FD034). Mix well before pouring into sterile Petri plates.

Principle and Interpretation

Yersinia enterocolitica is widely distributed in lakes and reservoirs. Epizootic outbreaks of diarrhea, lymphadenopathy, pneumonia and spontaneous abortions occur in various animals. It is the most common species of Yersinia recovered from clinical specimens. Y.enterocolitica is biochemically more active at room temperature than at 37°C. Yersinia Selective Agar Base with added Yersinia Selective Supplement is used to isolate Y.enterocolitica from clinical and food samples. Yersinia Selective Agar Base is recommended for selective isolation of Yersinia (1,2) with modification of chromogenic identification .

Peptone mix and growth factor provides nitrogen and carbon source, long chain amino acids, vitamins and other essential growth nutrients. The medium is selective due to the presence of selective mix, which inhibit gram-positive and a number of gram-negative bacteria. Addition of antibiotic supplement makes it highly selective for Yersinia. thus imparting additional selectivity. One of the chromogen is split by Yersinia species and results in purple coloured colonies. Other organisms are either inhibited or results in colourless colonies.

For the isolation of Y. enterocolitica by direct plating and pour plating, inoculate the specimen directly onto the medium. Incubate at 22-32°C for 24-48 hours or suspend the sample (food, faeces, etc.) in sterile Phosphate Buffer Saline and incubate for upto 21 days (4) at 4°C.

Quality Control

Appearance of Powder : Light yellow to greenish yellow homogeneous free flowing powder.

Gelling : Firm, comparable with 1.25% Agar gel.

Colour and Clarity : Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

Cultural Response : Cultural characteristics observed with added Yesinia Selective Supplement (FD034) after an incubation at 22-32°C for 24-48 hours.

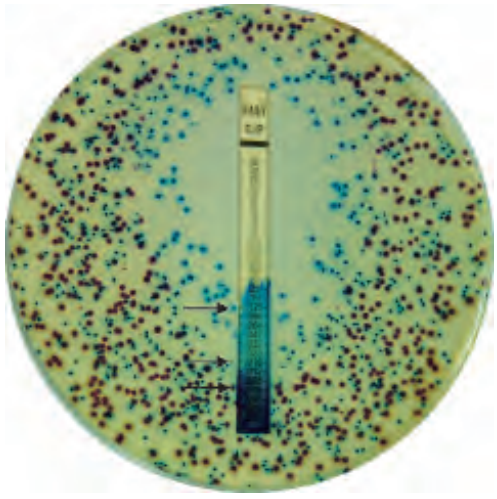
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli O157:H7 (NCTC 12900)	>=10^3	inhibited	green	
Salmonella Typhimurium ATCC 14028	>=10^3	inhibited	yellow	
Listeria monocytogenes ATCC 19112	>=10^3	inhibited	black	
Campylobacter jejuni ATCC 29428	>=10^3	inhibited	black	
Yersinia enterocolitica ATCC 27729	50-100	good - luxuriant	>=50%	Purple
Escherichia coli ATCC 25922	>=10^3	inhibited		
Enterococcus faecalis ATCC 29212	>=10^3	inhibited		

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Schiemann D. A., 1979, Can. J. Microbiol., 25: 1298.
2. Schiemann D. A., 1980, Can. J. Microbiol., 26: 1232.
3. International Organization for Standardization (ISO), 1994, Draft ISO/DIS 10273.
4. Weissfeild and Sonnenwirth, 1982, J. Clin. Microbiol. 15 :508.



M2010 - HiCrome™ Mueller Hinton Agar
Mixture of *Klebsiella, S. faecalis* and *E. coli*

HiCrome™ Clostridial Agar Base

HiCrome™ Rapid ECC Broth is recommended for selective isolation and presumptive identification of Clostridium species

M2026

Composition **

Ingredients	Grams/Litre
Tryptone	15.00
Yeast extract	10.00
Dextrose	1.00
Sodium chloride	5.00
Sodium thioglycollate	0.50
Chromogenic mixture	3.31
Agar	13.00

Final pH (at 25°C) 7.1 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 47.81 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of one vial of Perfringens Supplement II (FD012). Mix well and pour into sterile Petri plates.

Principle and Interpretation

One of the major species of anaerobic bacteria to cause disease in humans is Clostridium . Clostridium species cause tetanus and gas gangrene that ultimately leads to tissue damage. Another Clostridium species produces the lethal botulinum toxin, the causative agent of botulism (1). Clostridial Agar formulated by Vera is recommended for the selective isolation of pathogenic Clostridia form mixed flora (2). HiCrome is the modification for chromogenic differentiation.

Tryptone and yeast extract provide the essential nutrients, mainly the nitrogen compounds. Dextrose serves as the carbon or fermentable carbohydrate source. Sodium thioglycollate is the reducing agents that help to create low oxidation-reduction potential enabling the growth of Clostridia.Also the media is well supplemented to support luxuriant growth of Clostridium species.The selective supplements inhibits other enteric bacteria.

The ideal method of inoculation of Clostridial Agar is direct inoculation of sterile, cooled medium with the specimen (in tubes). Alternatively agar plates of the medium can also be inoculated by streaking.

Quality Control

Appearance of Powder : Cream to beige homogeneous free flowing powder.

Gelling : Firm, comparable with 1.3% Agar gel

Colour and Clarity : Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction : Reaction of 4.78% w/v aqueous solution at 25°C. pH : 7.1±0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24-48hours(under anaerobic condition).

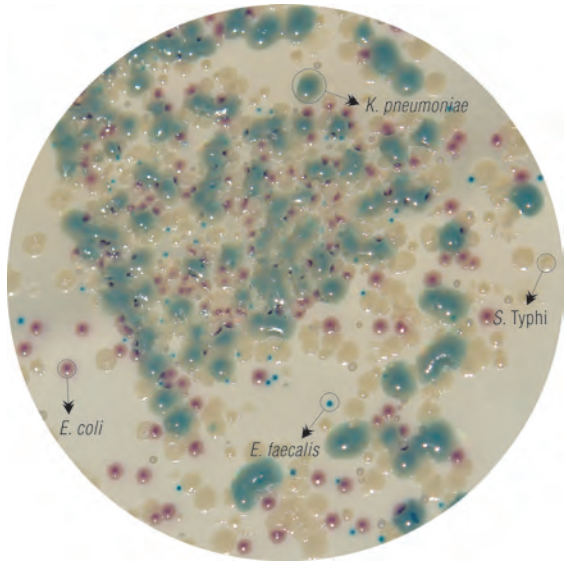
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium perfringens NCTC 8237	50-100	luxuriant	>=50%	Pale yellow-ish green
Clostridium perfringens ATCC 13124	50-100	luxuriant	>=50%	Pale yellow-ish green
Clostridium sporogenes ATCC 11437	50-100	luxuriant	>=50%	Pale green-blu-ish green
Clostridium sporogenes ATCC 19404	50-100	luxuriant	>=50%	Pale green-blu-ish green
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf-life

Store dehydrated powder and the prepared medium at 2-8°C in tightly closed container. Use before expiry date on the label.

References

1. Alcamo E. I., 2001, Fundamentals of Microbiology, 6th Ed., Jones and Bartlett Publishers.
2. Vera, 1962, Presented Pa. Soc. Med. Tech., York, Pa.



M1600 – HiCrome™ Universal Differential Medium

HiCrome™ ECC Selective Agar Base, Modified

HiCrome™ Mueller Hinton Agar is recommended for detection of Escherichia coli and coliforms in water and food samples.

M2056

Composition **

Ingredients	Grams/Litre
Peptone	10.00
Sodium dihydrogen phosphate	2.20
Disodium hydrogen phosphate	2.70
Sodium chloride	5.00
Sodium pyruvate	1.00
L-Tryptophan	1.00
Sorbitol	1.00
Potassium nitrate	1.00
Sodium lauryl sulphate	0.200
Chromogenic mixture	0.200
Agar	15.00

Final pH (at 25°C) 7.0±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.30 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure(121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ ECC Selective Agar, Modified is a selective medium recommended for the simultaneous detection of Escherichia coli and total coliforms in water and food samples (1,2). The chromogenic mixture contains two chromogenic substrates. The enzyme ß-D-galactosidase produced by coliforms cleaves one of the chromogen to form salmon to red coloured colonies (3). The enzyme ß-D-lucuronidase produced by E. coli, cleaves X-glucuronide, the other chromogen (4). Colonies of E.coli give dark blue to violet coloured colonies due to cleavage of both the chromogens. Addition of LTryptophan improves the indole reaction, thereby increasing the detection reliability. Peptone provide nitrogenous and carbonaceous compounds, long chain amino acids and and other essential growth nutrients for the organisms. Sodium pyruvate serves as a growth factor and sorbitol is the fermentable carbohydrate Phosphates buffer the medium. The media formulation helps even sublethally injured coliforms to recover and grow rapidly. Sodium lauryl sulphate inhibits grampositive bacteria.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Gelling : Firm, comparable with 1.5 % Agar gel.

Colour and Clarity : Light yellow coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 3.93% w/v aqueous solution at 25°C. pH : 7.00±0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	50-100	good - luxuriant	>=50%	dark blue
Enterobacter aerogenes ATCC 13048 (00175*)	50-100	luxuriant	>=50%	pink
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	>=50%	pink
Citrobacter freundii ATCC 8090	50-100	luxuriant	>=50%	pink
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0%	
Key : (*) corresponding WDCM numbers				

Storage and Shelf-life

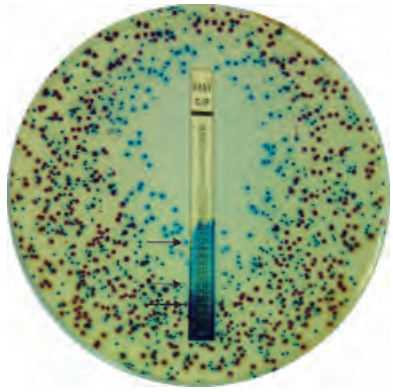
Store below 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

References

1. Frampton E.W., Restaino L. and Blaszko N., 1988, J.Food Prof., 51:402.
2. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
3. LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur > 102:267.
4. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
8. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
9. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
10. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.

Mixture of *Klebsiella, S. faecalis* and *E. coli*



M2010 - HiCrome™ Mueller Hinton Agar

HiCrome™ Cronobacter Isolation Agar(CCI Agar)

M2062I

HiCrome™ Rapid ECC Broth is recommended for the isolation and identification of Cronobacter sakazakii from food products. The composition and performance of this media are as per specifications laid down in in ISO /TS 22964: 2017

Composition **

Ingredients	Grams/Litre
Tryptone#	7.00
Yeast extract	3.00
Sodium chloride	5.00
Sodium deoxycholate	0.25
5-Bromo-4-chloro-3-indolyl α–D-glucopyranoside	1.50
Ammonium iron(III) citrate	1.00
Sodium thiosulfate	1.00
Agar	15.00

Final pH (at 25°C) 7.3 ± 0.2

** Formula adjusted, standardized to suit performance parameters

- Equivalent to Tryptic digest of casein

Directions

Suspend 32.4 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Enterobacter species are widely distributed in nature occurring in fresh water, soil, sewage, plants, vegetables, animal and human feaces. *Cronobacter sakazakii has been closely associated with neonatal meningitis and sepsis (1). HiCrome™ Cronobacter isolation Agar is recommended by ISO Committee for the isolation and identification of *C.sakazakii from food samples.

(2). The chromogenic substrate (5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside) is cleaved specifically (3) by *C.sakazakii resulting in the formation of blue green colonies. Other organisms, which do not cleave this substrate, produce colourless coloured colonies.

Tryptone and yeast extract provides nitroge nous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Sodium deoxycholate inhibits the accompanying gram-positive flora.

Key: *: Formerly known as Enterobacter sakazakii.

Quality Control

Appearance of Powder : Cream to yellow to pink homogeneous free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity : Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction : Reaction of 3.24% w/v aqueous solution at 25°C. pH : 7.3±0.2

Cultural Response : Cultural characteristics observed after an incubation at 41.5±1°C for 24±2 hours.

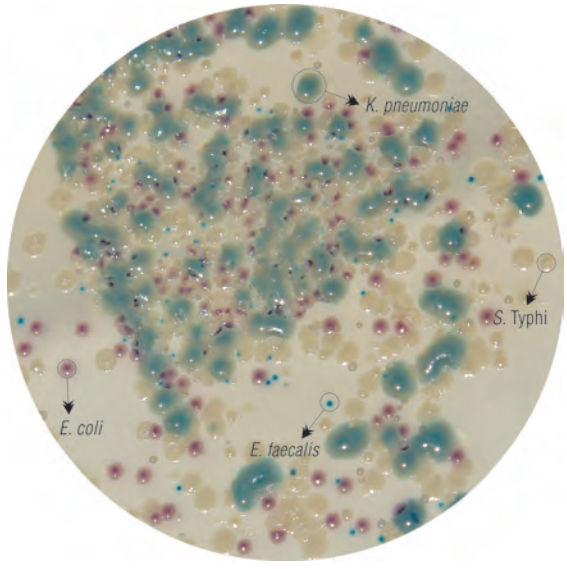
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good-luxuriant	>=50%	blue-green
Cronobacter muyt-jensii ATCC 51329 (00213*)	50-100	good-luxuriant	>=50%	blue-green
Enterobacter cloacae ATCC 13047 (00083*)	50-100	good-luxuriant	>=50%	colourless without green or blue green colour
Staphylococcus aureus ATCC 25923 (00034*)	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 6538 (00032*)	>=10^3	inhibited	0%	
Key: *: Corresponding WDCM numbers				

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983.
2. International Organization for Standardization. Microbiology of the food chain-Horizontal method for the detection of Cronobacter spp. Draft ISO/ TS 22964, 20176 (E).



M1600 – HiCrome™ Universal Differential Medium

HiCrome™ m-Coliconfirm Broth

HiCrome™ m-Coliconfirm Broth is recommended for detection of E.coli and other total coliforms in water samples by membrane filtration.

M2064

Composition **

Ingredients	Grams/Litre
Tryptone	8.00
Yeast extract	0.50
Lactose	0.60
Sodium chloride	3.00
Dipotassium hydrogen phosphate	1.75
Potassium dihydrogen phosphate	1.25
Sodium pyruvate	1.00
Octyphenol ethoxylate	0.50
Magnesium sulphate	0.30
Sodium azide	0.02
L-Methionine	0.10
Methylene blue	0.016
Cyclohexylammonium salt	0.20
Chromogenic mixture	0.20

Final pH (at 25°C) 7.0±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17.43 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Aseptically add the rehydrated contents of ECC Selective Supplement (FD344) and 7ml of TTC Solution, 1% (FD057). Mix well and aseptically add desired quantity (2 to 5 ml) of broth on sterile absorbent cotton pad or sterile filter paper for saturation. The nutrient pad should be used within 24 hours of saturation.

Principle and Interpretation

This is a selective medium recommended for the simultaneous detection of Escherichia coli and total coliforms in water (1). The water sample is filtered through membranes and then placed on pad saturated with medium and incubated at 35 ±5°C for 24 hours in sealed Petri plates. Tryptone provides nitrogeneous anddcarbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Yeast extract serves as a source of vitamins. Lactose is the fermentable carbohydrate. The phosphates in the medium buffers the medium. Sodium chloride maintains the osmotic balance. The enzyme beta-glucuronidase produced by E.coli utilizes the chromogenic substrate to produce blue-purple coloured colonies. Coliforms other than Escherichia coli turn red as they reduce TTC (2,3,5-triphenyl tetrazolium chloride). Thus, the resulting colour distinction allows simple interpretation of test without further confirmation. Methylene blue and ECC selective supplement containing imparts selectivity to the medium. Non-coliforms usually give white coloured colonies.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations

Overgrowth of non-coliform organisms may interfere with the total coliform organisms.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Colour and Clarity : Cream, clear to slightly opalescent solution,may have slight precipitate.

Reaction : Reaction of 1.74% w/v aqueous solution at 25°C. pH : 7.0±0.2

Cultural Response : Cultural characteristics observed after an incubation at 34.5-35.5°C for 24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Colour of colony on membrane
Citrobacter freundii ATCC 8090	50-100	luxuriant	red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	blue
Escherichia coli ATCC 35218	50-100	luxuriant	blue
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	-
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	red
Staphylococcus aureus ATCC 25923 (00034*)	>=10^3	inhibited	-
Staphylococcus aureus ATCC 6538 (00032*)	>=10^3	inhibited	-
Key : (*) Corresponding WDCM numbers			

HiCrome™ m-Coliconfirm Broth

HiCrome™ m-Coliconfirm Broth is recommended for detection of E.coli and other total coliforms in water samples by membrane filtration.

M2064

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

References

1 Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

2. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.

3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

HiCrome™ Lactobacillus Selective Agar Base

M2065

HiCrome™ Rapid ECC Broth is recommended for isolation and differentiation between various species of Lactobacillus from a mixed culture by chromogenic method

Composition **

Ingredients	Grams/Litre
Peptone	10.00
HM Extract #	1.00
M-Protein powder ##	5.00
D-Mannitol	10.00
Sodium chloride	10.00
Chromogenic mixture	3.20
Phenol red	0.025
Agar	15.00

Final pH (at 25°C) 7.1 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Equivalent to Meat Extract

Equivalent to Milk Protein

Directions

Suspend 54.22 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Ciprofloxacin Supplement (FD345). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Lactobacillus is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria. They are a major part of the lactic acid bacteria group. As more LABs have been developed and sold in mixed forms as probiotics, it is necessary to develop a method for counting each LAB in a mixture(1)

The medium contains peptone and HM extract, which provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by phenol red. M-protein aids in detecting casein hydrolysis activity. The chromogenic mixture present in the medium is cleaved by the enzyme beta-glucosidase resulting in greenish blue to blue coloured colonies.

For selective isolation of Lactobacillus, Ciprofloxacin supplement is added (FD345) which inhibits the accompanying bacteria.

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity : Red coloured, clear to slightly opalescent gel forms in Petri plates

Reaction : Reaction of 5.42% w/v aqueous solution at 25°C. pH : 7.1±0.2

Cultural Response : Cultural characteristics observed with addition of Ciprofloxacin supplement (FD345) after an incubation at 25-30°C for 24-48 hours (with 5% CO_2).

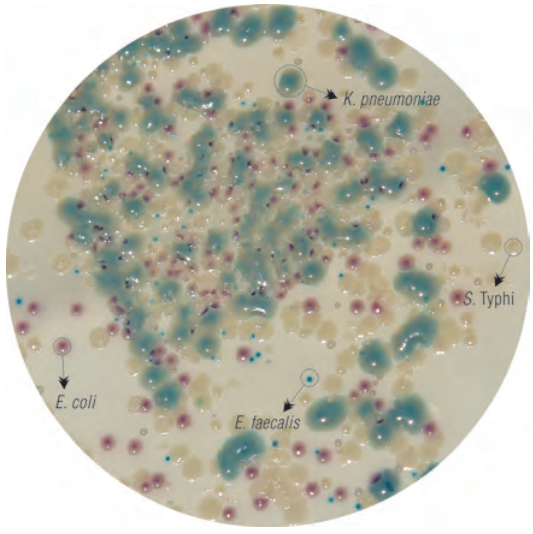
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Lactobacillus acidophilus ATCC 4356 (WDCM 00098)	50-100	good - luxuriant	>=50%	Pale pink - pink
Lactobacillus rhamnosus ATCC 9595	50-100	good	>=50%	Light green
Lactobacillus fermentum ATCC 9338	50-100	good - luxuriant	>=50%	Yellow
Lactobacillus plantarum ATCC 8014	50-100	good - luxuriant	>=50%	Light green-green colonies w/ hazy back-ground
Lactococcus lactis sub-sp. lactis ATCC 19435	50-100	good - luxuriant	>=50%	Light green-green colonies w/ hazy back-ground
Bacillus spizizenii ATCC 6633 (WDCM 00003)	>=10^3	inhibited	0%	
Bacillus cereus ATCC 10876	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 6538 (WDCM 00032)	>=10^3	inhibited	0%	

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. De Man, J.C., Rogosa, M. and Sharpe, E.M. (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23, 30–35.



M1600 – HiCrome™ Universal Differential Medium

HiCrome™ Enterococci Agar / Broth

Recommended for the identification and differentiation of Enterococci from water samples.

M1414 / M1376

Composition **

	M1414	M1376
Ingredients	Grams/Litre	Grams/Litre
Peptone, special	10.00	10.00
Sodium chloride	5.00	5.00
Sodium azide	0.30	0.30
Chromogenic substrate	0.06	0.040
Polysorbate 80	2.00	2.00
Disodium hydrogen phosphate	1.25	1.25
Agar	15.00	–

Final pH (at 25°C) 7.5 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.61 gm of M1414 and 18.59 grams (single strength) or 37.18 grams (double strength) of M1376 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour/dispense into sterile Petri plates / tubes.

Principle and Interpretation

HiCrome™ Enterococci media are formulated on the basis of the work carried out by Althous et al (1), Amoras (2), Litsky et al (3), and Manafi and Sommer (4) and Snyder and Lichstein (5). These media are recommended for the rapid detection of Enterococci from water samples. The presence of *Enterococcus* group, which is a subgroup of the faecal streptococci, serves as a valuable bacterial indicator for determining the extent of faecal contamination (1, 6) and it is more specific than the detection of coliforms, which may originate from non-faecal sources. The enzyme β-glucosidase produced by Enterococci cleaves the chromogenic substrate, resulting in a bluish green colour.

The medium contains peptone special, which provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Sodium chloride maintains the osmotic balance of the medium. Sodium azide inhibits the accompanying microflora, especially gram-negative organisms. Polysorbate 80 acts as a source of fatty acids.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel of M1414.

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates / clear solution in tubes.

Reaction : Reaction of 3.36% w/v of M1414 and 1.86% w/v of M1376 aqueous solution at 25°C. pH:7.5 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery on M1414	Colour of colony (M1414)/ Medium (M1376)
Enterococcus faecalis (29212) (00087*)	50-100	good	40-50%	blue green
Staphylococcus aureus (25923)	50-100	good	40-50%	colourless
Escherichia coli (25922) (00013*)	50-100	none to poor	<=10%	-
Pseudomonas aeroginosa (27853) (00025*)	50-100	none to poor	<=10%	-

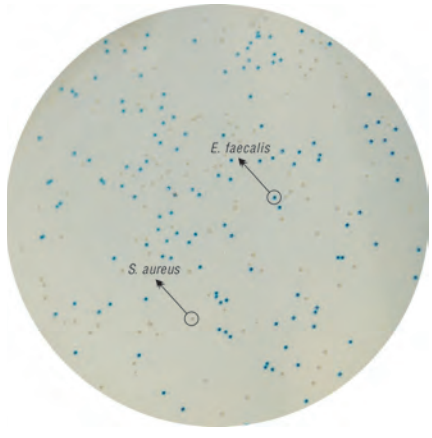
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Althous, H., Dott, W., Havemeister, G, Muller, H.E, a. Sacre',C., 1982, Zbl. Bakt. Hyg. I. Abt. Orig. A. 252:154-165.
2. Amoras I, 1995, Poster präsentation congress of Spanish Society of Microbiology, Madrid.
3. Litsky, W., Mallmann, W.L., a Fifield, C.W. 1953, Amer. J. Pbl. Hlth. 43:873-879.
4. Manafi M., a. Sommer R, 1993, Wat. Sci. Tech. 27:271-274.
5. Snyder M.L., and Lichstein, H.C. 1940, J. Infect. Dis. 67. 113-115.
6. Standard Methods for the Examination of Water and Wastewater, 20th Edition, Edited by L.S.Clesceri, A.E., Greenberg and A.D. Eaton, Published by APHA, AWWA and WEF (1998).



M1414 – HiCrome™ Enterococci Agar

HiCrome™ Enterococcus faecium Agar Base

Recommended for the identification of *Enterococcus faecium* from water, faeces and sewage samples.

M1580

Composition **

Ingredients	Grams/Litre
Peptone, special	23.00
Corn starch	1.00
Sodium chloride	5.00
Chromogenic substrate	0.10
Arabinose	10.00
Phenol red	0.10
Agar	15.00

Final pH (at 25°C) 7.8 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.1 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add the rehydrated contents of 1 vial of Enterococcus faecium Selective Supplement (FD226). Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Enterococcus faecium Agar Base is recommended for the chromogenic detection of *Enterococcus faecium* from urine, faeces, soil, food, water, plants and animals. *E. faecium* is commonly found in the gastrointestinal tracts of humans (3). The resistance exhibited by *Enterococcus* species to various antimicrobials has led them to being a major cause of human infections including nosocomial infections (1). *E. faecalis* causes 80-90% of infection while *E. faecium* causes the majority of the remainder (3). The use of selective media for the isolation of Enterococci has been previously reviewed, including those containing chromogenic substrates (4) and media containing cephalexin-aztreonam supplements. *Enterococcus* species possess the enzyme glucosidase, which specifically cleaves the chromogenic substrate to produce blue coloured colonies. *E. faecium* ferment arabinose; and cleaves the chromogenic substrate present in the media to produce green coloured colonies along with yellow colouration to the medium. *E. faecalis* does not ferment arabinose and therefore retains the blue colour.

Peptone special serves as a source of carbon, nitrogen and essential growth nutrients. Corn starch neutralizes the toxic metabolites while sodium chloride maintains the osmotic equilibrium. Phenol red serves as a pH indicator with arabinose being the fermentable carbohydrate.

Quality Control

Appearance of Powder : Light yellow to pinkish beige coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Red coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 5.42% w/v aqueous solution at 25°C. pH:7.8 ± 0.2 .

Cultural Response : Cultural characteristics observed (with added Enterococcus faecium Selective Supplement (FD226)) after an incubation at 35-37°C for 24-48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecium (19434)	50-100	luxuriant	>=50%	green
Enterococcus faecalis (29212) (00087*)	50-100	luxuriant	>=50%	blue
Enterococcus hirae (10541)	50-100	luxuriant	>=50%	blue
Escherichia coli (25922) (00013*)	>=10^3	inhibited	0%	-
Pseudomonas aeruginosa (27853) (00025*)	>=10^3	inhibited	0%	-
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

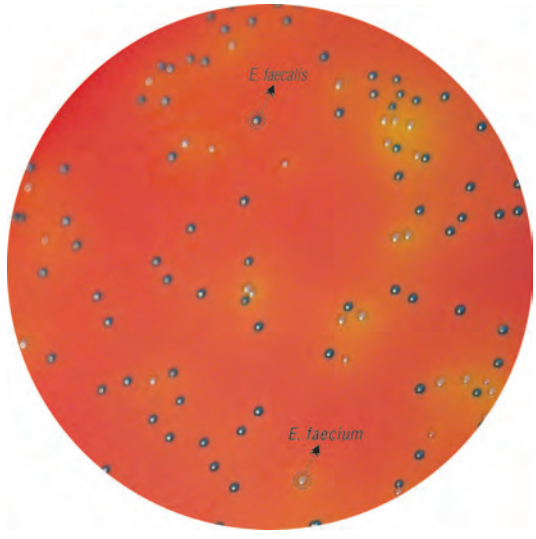
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Chenoweth C., Schaberg D., The Epidemiology of Enterococci, Eur. J. Clin. Micorbiol. Infect. Dis., 9:80-89, 1990.
2. Moellering R. C., 1992, Clin. Infect. Dis. 14: 1173.
3. Skinner F. A. and Quesnel L. B., (Ed.), 1978, Streptococci. Academic Press, Inc. (London) Ltd., London, United Kingdom, p. 245 261
4. Willinger B. and Manafi M., 1995, Lett. Appl. Microbiol., 20: 300-302.



M1580 – HiCrome™ Enterococcus faecium Agar Base

HiCrome™ Strep B Selective Agar Base

HiCrome™ Strep B Selective Agar Base is recommended for selective isolation of Group B streptococci.

M1840

Composition **

Ingredients	Grams/Litre
Protein hydrolysate	17.50
Buffers	2.50
Chromogenic mixture	2.54
Selective agents	0.11
Agar	15.00

Final pH (at 25°C) 7.3 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.65 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add the rehydrated contents of one vial of Hicrome Strep B Selective Supplement (FD273). Mix well and pour in sterile Petri plates.

Principle and Interpretation

Group B Streptococcus infection is a leading illness causing death in newborns. Group B streptococci can also cause serious diseases in pregnant women, the elderly, and adults with other illnesses. GBS normally reside in the vagina of women and rectum of men and women (1). In newborns, group B strep is the most common cause of sepsis (infection of the bloodstream) and meningitis (infection of the lining and fluid surrounding the brain) and a common cause of pneumonia. In adults, group B strep can rarely lead to serious bloodstream infections, urinary tract infections, skin infections, and pneumonia, especially in people with weak immune systems. Heavy colonization of the maternal genital tract is associated with colonization of infants and risk of neonatal disease (2).

The sample collection is usually done by collection of vaginal and rectal swab between 35 and 37weeks of pregnancy. The swab is then processed on HiCrome™ Strep B Selective Agar Base. For the conventional methods optimum recovery is however achieved by selective enrichment into Todd Hewitt broth with colistin and nalidixic acid and then subculture on Blood Agar (3,4).

Protein hydrolysate provides essential nutrients for the growth of Streptococci. Buffers present provides buffering to the medium. Selective agents in the medium inhibits accompanying flora. One of the substrate in the chromogenic mixture is cleaved by beta glucosidase possesed by Group B Streptococci resulting in blue coloured colonies.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Yellow coloured opaque gel forms in Petri plates

Reaction : Reaction of 3.77% w/v aqueous solution at 25°C. pH : 7.3 ± 0.2.

Cultural Response : Cultural characteristics observed with added HiCrome™ Strep B Selective Supplement (FD273), after an incubation at 35-37°C for 18 - 24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli (25922) (00013*)	>=10^3	inhibited	0%	—
Neisseria meningitidis (13090)	>=10^3	inhibited	0%	—
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	—
Streptococcus agalactiae (13813)	50-100	luxuriant	>=50%	blue

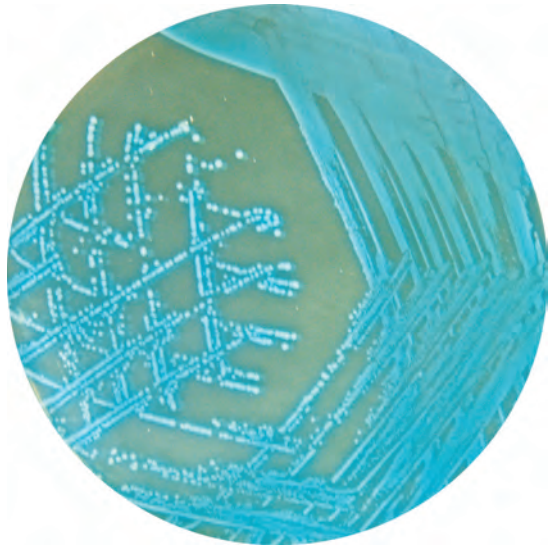
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Anthony BF, Okada DM, Hobel CJ. Epidemiology of group B Streptoccoccus: longitudinal observations during pegnancy. J.Infect Dis 1978; 137:524-30.
2. Murray P.R. , Baron J.H., Manual of Clinical Microbiology Murray P. R., Baron J. H. Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. NHS Processing swabs for Group B Streptococcal carriage Issue no.2.1,2006.
4. Prevention of perinatal group B Streptococcal disease: a public health perspective. Centres for Disease control and Prevention. MMWR Recomm Rep 1996; 51:1-22.



Streptococcus agalactiae ATCC 13813
M1840 HiCrome™ Strep B Selective Agar Base

HiCrome Strep B Selective Agar Base, Modified

Recommended for selective isolation of Group B streptococci.

M1966

Composition **

Ingredients	Grams/Litre
Peptone special	10.000
Yeast extract	4.300
Chromogenic mixture	7.500
Phenol red	0.025
Agar	15.000

Final pH (at 25°C)7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.83 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add the rehydrated contents of one vial of Hicrome Strep B Selective Supplement (FD273). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Group B *Streptococcus* is a leading infection causing illness and death in newborns. Group B streptococci can also cause serious diseases in pregnant women, the elderly and adults with other illnesses. GBS normally reside in the vagina of women and rectum of men and women (1). In newborns, group B strep is the most common cause of sepsis (infection of the bloodstream)and meningitis (infection of the lining and fluid surrounding the brain) and a common cause of pneumonia. In adults, group B strep can rarely lead to serious bloodstream infections, urinary tract infections, skin infections, and pneumonia, especially in people with weak immune systems. Heavy colonization of the maternal genital tract is associated with colonization of infants and risk of neonatal disease (2).

The sample collection is usually done by collection of vaginal and rectal swab between 35 and 37weeks of pregnancy. The swab is then processed on HiCrome Strep B Selective Agar Base. For the conventional methods optimum recovery is however achieved by selective enrichment into Todd Hewitt broth with colistin and nalidixic acid and then subculture on Blood Agar(3,4).

Peptone special and yeast extract provides nitrogeneous and carbonaceous compounds, long chain amino acids, vitamins and essential nutrients for the growth of Streptococci. One of the chromogenic substrate is utilized by Group B Streptococci resulting in purple coloured colonies, while the other Streptococci either give blue or bluish green coloured colonies with yellow background. Phenol red is the indicator dye.

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 3.68 w/v aqueous solution at 25°C. pH : 7.4±0.2

Cultural Response : Cultural characteristics observed with added HiCrome Strep B Selective Supplement (FD273), after an incubation at 35-37°C for 24 - 48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	
Streptococcus agalactiae ATCC 13813	50-100	luxuriant	>=50%	purple
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	bluish green
Enterococcus faecium ATCC 19434	50-100	luxuriant	>=50%	green w/yellow background

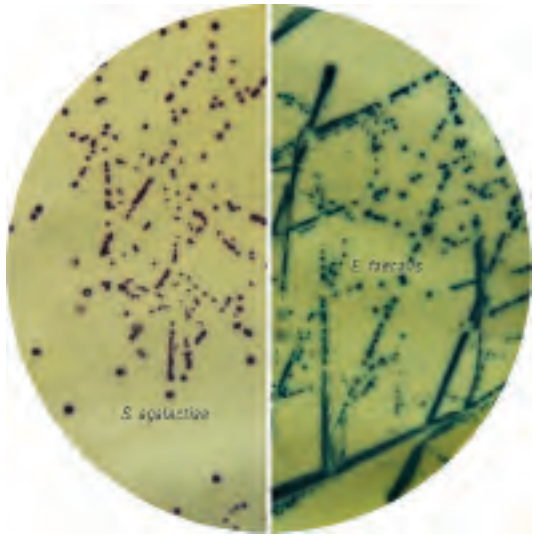
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Anthony BF, Okada DM, Hobel CJ. Epidemiology of group B Streptoccoccus: longitudinal observations during pegnancy. J.Infect Dis 1978; 137:524-30.
2. Murray P.R. , Baron J.H., Manual of Clinical Microbiology Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. Prevention of perinatal group B Streptococcal disease: a public health perspective . Centres for Disease control and Prevention. MMWR Recomm Rep 1996; 51:1-22
4. NHS Processing swabs for Group B Streptococcal carriage Issue no.2.1,2006



M1966 HiCrome Strep B Selective Agar Base, Modified

HiCrome™ VRE Agar Base

HiCrome™ VRE Agar Base is recommended for identification of Vancomycin Resistant Enterococci from clinical specimens

M1830

Composition **

Ingredients	Grams/Litre
Peptone special	25.00
Chromogenic mixture	0.45
Sodium chloride	5.00
Buffering agent	1.25
Salt mixture	4.25
Agar	15.00

Final pH (at 25°C) 6.5 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.95 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add the rehydrated contents of two vials of HiCrome™ VRE Agar Supplement (FD277). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Enterococci are the common habitants of the normal flora residing in the intestines of mammals (1). Vancomycin Resistant Enterococci are the group of Enterococci that have developed resistance towards many antibiotics particularly vancomycin. Enterococcal infections that result in human disease can be fatal, particularly those caused by strains of vancomycin-resistant enterococci (VRE) (2). Early detection of VRE is important to prevent the emergence of vancomycin resistance in *Enterococcus faecalis*.

VRE can be transmitted from person to person, especially in a hospital or chronic-care facility. Microscopic amounts of fecal material from an infected or colonized patient can contaminate the hospital environment and be a reason for the spread of infection. There are many traditional media for the detection of VRE which includes Vancomycin Resistant Enterococci Broth Base/ Agar or Bile Esculin Agar supplemented with vancomycin.

Peptone special in the medium supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other necessary nutrients required for the growth of microorganisms. Sodium chloride maintains the osmotic balance. Buffering agents provides buffering to the medium. *Enterococcus faecalis* cleaves the chromogenic substrate in the medium to produce blue coloured colonies, which are clearly visible against the opaque background. The supplement added to the medium allows the selective isolation of Vancomycin Resistant Enterococci. This medium can be inoculated directly from screening swab, isolated colony prepared as a liquid suspension approximately equivalent to 0.5 McFarland turbidity.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Off white coloured opaque gel forms in Petri plates.

Reaction : Reaction of 5.1% w/v aqueous solution at 25°C. pH : 6.5 ± 0.2

Cultural Response : Cultural characteristics observed with added HiCrome™ VRE Agar Supplement (FD277) after an incubation at 35-37°C for 24-48 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis (VRE) (51299)	50-100	luxuriant	>=50%	bluish green
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	—
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	—

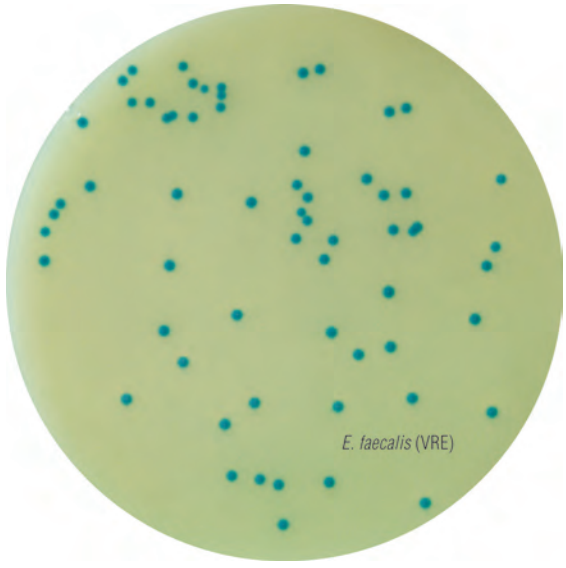
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Mara D., Horan NJ: The Handbook of water, wastewater and microbiology, Amsterdam,
The Netherlands, Academic Press; 2003.
2. Mascini EM, Bonten MJ: Vancomycin- resistant enterococci: consequences for therapy and infection control. Clin Microbiol Infect.2005,11 (Suppl.4) :43-56



M1830 – HiCrome™ VRE Agar Base

HiCrome™ VRE Agar Base, Modified

M1925

HiCrome™ VRE Agar Base, Modified is recommended for selective isolation and differentiation of Vancomycin Resistant *Enterococcus faecalis* and *Enterococcus faecium* from clinical specimens.

Composition **

Ingredients	Grams/Litre
Peptone special	20.00
Chromogenic mixture	3.60
Sodium chloride	5.00
Arabinose	10.000
Phenol red	0.100
Agar	15.000

Final pH (at 25°C) 7.80 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 53.70 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add the rehydrated contents of two vials of HiCrome™ VRE Agar Supplement (FD277). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Enterococci are the common habitants of the normal flora residing in the intestines of mammals (1). Vancomycin Resistant Enterococci are the group of Enterococci that have developed resistance towards many antibiotics particularly vancomycin. Enterococcal infections that result in human disease can be fatal, particularly those caused by strains of vancomycin-resistant enterococci (VRE) (2). Early detection of VRE is important to prevent the emergence of vancomycin resistant in *Enterococcus faecalis*.

VRE can be transmitted from person to person, especially in a hospital or chronic-care facility. Microscopic amounts of fecal material from an infected or colonized patient can contaminate the hospital environment and be a reason for the spread of infection. There are many traditional media for the detection of VRE which includes Vancomycin Resistant Enterococci Broth Base/ Agar or Bile Esculin Agar supplemented with vancomycin.

Peptone special in the medium supplies the necessary nutrients and vitamins required for the growth of microorganisms. Sodium chloride maintains the osmotic balance. Phenol red is the pH indicator and arabinose is the fermentable carbohydrate *Enterococcus* species possess the enzyme -glucosidase which cleaves the chromogenic substrate in the medium to produce blue coloured colonies. *Enterococcus faecium* ferments arabinose and cleaves the substrate thereby producing green colonies with yellow background. *Enterococcus faecalis* does not ferment arabinose thereby producing blue colonies due to cleavage of chromogenic substrate. The supplement added to the medium allows the selective isolation of Vancomycin Resistant Enterococci. This medium can be inoculated directly from screening swab, isolated colony prepared as a liquid suspension approximately equivalent to 0.5 McFarland turbidity.

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Red coloured opaque gel forms in Petri plates

Reaction : Reaction of 5.37% w/v aqueous solution at 25°C. pH : 7.80 ± 0.2

Cultural Response : Cultural characteristics observed with added HiCrome™ VRE Agar Supplement (FD277) after an incubation at 35-37°C for 24-48 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Enterococcus faecalis (VRE) (51299)	50-100	luxuriant	>=50%	blue
Enterococcus faecium (VRE) (700221)	50-100	luxuriant	>=50%	green w/yellow background
Enterococcus faecalis (29212) (00087*)	>=10^3	inhibited	0%	-
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

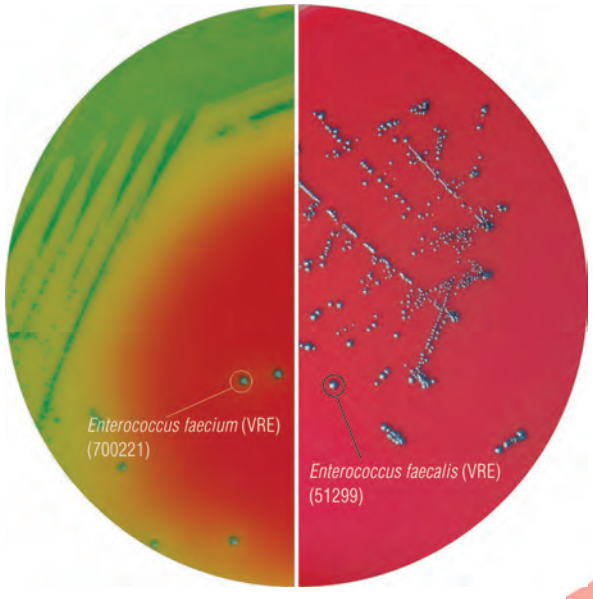
Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C Use before expiry date on the label.

References

1. Mara D., Horan NJ: The Handbook of water, wastewater and microbiology, Amsterdam, The Netherlands, Academic Press; 2003.
2. Mascini EM, Bonten MJ: Vancomycin- resistant enterococci: consequences for therapy and infection control. Clin MicrobiolInfect.2005,11 (Suppl.4) :43-56



M1925 – HiCrome™ VRE Agar Base, Modified

Vancomycin Resistant Enterococci

HiCrome™ Listeria Agar Base / Modified

A selective and differential agar medium recommended for rapid and direct identification of *Listeria species*.

M1417F/ M1417

Composition **

Ingredients	M1417 Grams/Litre	M1417F Grams/Litre
Peptone, special	23.00	-
Peptone	-	30.00
Sodium chloride	5.00	-
Yeast extract	1.00	1.00
Meat extract	5.00	5.00
Lithium chloride	5.00	9.00
Rhamnose	10.00	-
D-xylose	-	10.00
Phenol red	0.12	0.12
Chromogenic mixture	5.13	5.13
Agar	13.00	13.00
Final pH (at 25°C)	7.3 ± 0.2	7.3 ± 0.1

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.62 grams of M1417 or 36.63 grams of M1417F in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Add rehydrated contents of 1 vial of HiCrome™ Listeria Selective Supplement (FD181) aseptically. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Listeria Agar Base Modified medium is a modification of a medium first developed by Notermans et al. (3) and Mengaud et al. (2) for the detection of *Listeria species* from food stuffs. HiCrome™ Listeria Agar Base Modified allows growth of *Listeria species* and gives a presumptive identification of *L. monocytogenes* within 24-48 hours after pre-enrichment. HiCrome™ Listeria Agar Base Modified (M1417) is based on rhamnose fermentation while HiCrome™ Listeria Agar Base (M1417F) is based on xylose fermentation. HiCrome™ Listeria Agar Base (M1417F) is in accordance with FDA BAM (1) where D-Xylose is the fermentable carbohydrate. This medium is based on the specific chromogenic detection of β-glucosidase activity and also sugar fermentation. *Listeria species* hydrolyse the purified chromogenic substrate in the medium forming bluish green coloured colonies. Since β-glucosidase activity is specific for *Listeria species*, other organisms cannot utilize the chromogenic substrate and therefore form colourless colonies. Differentiation between *Listeria species* is based on the property of rhamnose or xylose fermentation. The colonies of *L. monocytogenes* and *L. innocua* appear bluish green with a yellow halo (rhamnose positive) while the colonies of *L. ivanovii* appear blue without a yellow halo (rhamnose negative) in M1417. In case of M1417F, the colonies of *L. ivanovii* appear bluish green with yellow halo (xylose positive) while *L. monocytogenes* and *L. innocua* appear bluish green without a yellow halo (Xylose negative).

Peptone special, peptone, yeast extract and meat extract provide nitrogenous substances, carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Rhamnose or xylose are the fermentable carbohydrates with phenol red as an indicator. Sodium chloride maintains the osmotic equilibrium.

Quality Control

Appearance of powder : Light yellow to pink coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium : Red coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 6.72% w/v aqueous solution of M1417 at 25°C. pH : 7.3 ± 0.2.

Reaction of 7.32% w/v aqueous solution of M1417F at 25°C. pH : 7.3 ± 0.1.

Cultural Response : Cultural characteristics observed with added Hicrome™ Listeria Selective Supplement (FD181) after incubation at 35-37°C for 24-48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	(M1417) Rhamnose fermentation	(M1417F) Xylose fermentation
Listeria monocytogenes (19118)	50-100	luxuriant	>=50%	bluish-green	+ (yellow halo)	-
Listeria ivanovii (19119)	50-100	luxuriant	>=50%	bluish-green	-	+ (yellow halo/ background)
Listeria innocua (33090)	50-100	luxuriant	>=50%	bluish-green	+ (yellow background)	-
Escherichia coli (25922) (00013*)	>=10^3	inhibited	0%	-	-	-
Bacillus subtilis (6633)	>=10^3	inhibited	0%	-	-	-
Pseudomonas aeruginosa (27853) (00025*)	>=10^3	inhibited	0%	-	-	-
Candida albicans (10231)	>=10^3	inhibited	0%	-	-	-

Key : + = positive reaction, - = negative reaction.

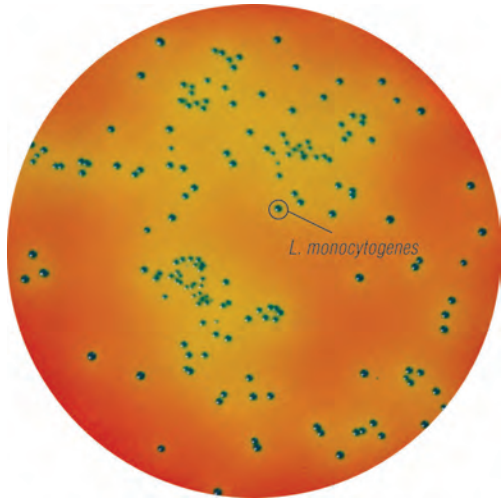
* = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. FDA U.S., Bacteriological Analytical Manual 8 ed. Gaithersburg, MD, AOAC international, 1998.
2. Mengaud J., Braun-Breton C. and Cossart P., (1991), Molecular Microbiology, (2): 367-372.
3. Notermans S.H. and Dufrenne J., (1991), Applied and Environmental Microbiology, 57(09):2666-70.



L. Mono Differential Agar Base

Recommended for the selective and differential isolation of *Listeria monocytogenes* based on PIPLC activity.

M1540

Composition **

Ingredients	Grams/Litre
Meat peptone	18.00
Casein enzymic hydrolysate	6.00
Yeast extract	10.00
Sodium pyruvate	2.00
Glucose	2.00
Magnesium glycerophosphate	1.00
Magnesium sulphate	0.50
Sodium chloride	5.00
Lithium chloride	10.00
Disodium hydrogen phosphate anhydrous	2.50
Chromogenic substrate	0.05
Agar	15.00

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.02 grams in 460 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of L .mono Selective Supplement I (FD212) and L .mono Selective Supplement II (FD213). For enrichment add sterile content of L. mono Enrichment Supplement I (FD214). Mix well and pour into sterile Petri plates.

Principle and Interpretation

L. mono Differential Agar Base is based on the formulation of Ottoviani and Agosti (2, 3) for the selective and differential isolation of *Listeria monocytogenes* from food and animal feeds which is adopted by ISO Committee (1).

Meat peptone, casein enzymic hydrolysate, yeast extract and sodium pyruvate provide essential growth nutrients and nitrogenous substances, carbonaceous compounds, long chain amino acids and vitamin B complex. Glucose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplements (FD212 and FD213) inhibit accompanying microflora and allow the growth of *Listeria species. Listeria* species hydrolyse the chromogenic substrate which produces greenish-blue coloured colonies. Differentiation of *Listeria monocytogenes* from other *Listeria species* is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies.

Quality Control

Appearance of Powder : Cream to yellow coloured, homogenous free flowing powder.
Gelling : Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium : Light amber coloured, opalescent gel forms in Petri plates.
Reaction : Reaction of 7.2% w/v aqueous solution at 25°C.
pH : 7.2 ± 0.2
Cultural Response : Cultural characteristics observed withadded L. mono Selective Supplement I(FD212), L. mono Selective Supplement II (FD213) and L. mono Enrichment Supplement I (FD214) after an incubation at 35-37°C for 24-48 hours.

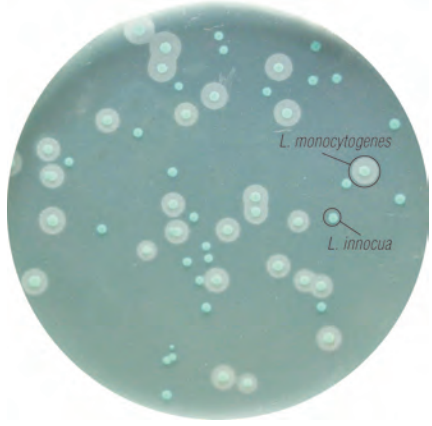
Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of the Colony	PIPLC activity*
Candida albicans (10231)	≥10^3	inhibited	0%	-	-
Enterococcus faecalis (29212)	≥10^3	inhibited	0%	-	-
Escherichia coli (25922)	≥10^3	inhibited	0%	-	-
Listeria innocua (33090)	50-100	luxuriant	≥50%	greeish-blue	-
Listeria grayi (19120)	50-100	luxuriant	≥50%	greeish-blue	-
Listeria ivanovii (19119)	50-100	luxuriant	≥50%	greeish-blue	+
Listeria monocytogenes (19112)	50-100	luxuriant	≥50%	greeish-blue	+
Listeria seeligeri (35967)	50-100	luxuriant	≥50%	greeish-blue	-
Listeria welshimeri (43549)	50-100	luxuriant	≥50%	greeish-blue	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Draft Amendment ISO 11290-2:1996/DAM 1.
2. Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.
3. Ottaviani F., Ottaviani M., and Agosti M. (1997 b), Quimper Froid Symposium Proceedings p. 6, A.D.R.I.A. Quimper, France, 16-18 June 1997.



M1540 L. Mono Differential Agar Base

HiCrome™ L. mono Rapid Differential Agar Base

M1924

Recommended for the rapid identification and differentiation of *Listeria monocytogenes* from other *Listeria species* based on rhamnose fermentation and PIPLC activity.

Composition **

Ingredients	Grams/Litre
Peptone special	23.000
Tryptone	10.000
Soya peptone	2.000
Sodium chloride	4.000
Lithium chloride	5.000
Chromogenic mixture	1.160
Rhamnose	10.000
Phenol red	0.120
Agar	15.000

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.14 grams in 470 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of L. mono Enrichment Supplement I (FD214) and sterile rehydrated contents of 1 vial of HiCrome™ Listeria Selective Supplement (FD181). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Listeria monocytogenes is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles. The pathogenicity of *Listeria ivanovii* for humans is uncertain (5). Since *L. monocytogenes* and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). This medium is based on the specific chromogenic detection of β-glucosidase activity, rhamnose fermentation and PIPLC activity. *Listeria* species hydrolyse the purified chromogenic substrate in the medium giving blue coloured colonies. Since β-glucosidase activity is specific for *Listeria* species, other organisms cannot utilize the chromogenic substrate and therefore give white colonies. Differentiation between *Listeria* species is based on the property of rhamnose fermentation and PIPLC activity. The colonies of *L. monocytogenes* appear bluish green with a yellow halo (rhamnose positive) while the colonies of *L.ivanovii* appear bluish green without a yellow halo (Rhamnose negative) (1, 2). The differentiation of *L.mono* and *L.innocua* is based on PIPLC (phosphatidylinositol-specific phospholipase C) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies. *L.ivanovii* also demonstrates PIPLC activity however since it does not ferment rhamnose it can be easily distinguished from *L. monocytogenes* (3, 4).

Peptone special, tryptone and soya peptone provide nitrogenous compounds, carbon, long chain amino acids vitamin B complex and other essential growth nutrients. Rhamnose is the fermentable carbohydrate with phenol red as an indicator. Sodium chloride maintains the osmotic equilibrium. The added lithium chloride and HiCrome™ Listeria Selective Supplement (FD181) inhibit growth of most gram- positive bacteria, gram-negative bacteria, yeasts and moulds. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies demonstrating PIPLC activity.

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Red coloured, opalescent gel forms in Petri plates

Reaction : Reaction of 7.03% w/v aqueous solution at 25°C. pH : 7.4±0.2.

Cultural Response : Cultural characteristics observed w/added HiCrome™ Listeria Selective Supplement (FD181) and L.mono Enrichment supplement I(FD214), after an incubation at 35-37°C for 24-48 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	Rhamnose fermentation	PIPLC Activity
Bacillus subtilis (6633)	>=10^3	inhibited	0%			
Candida albicans (10231)	>=10^3	inhibited	0%			
Escherichia coli (25922)	>=10^3	inhibited	0%			
Listeria innocua (33090)	50-100	luxuriant	>=50%	bluish green (yellow	positive reaction,	negative reaction
Listeria ivanovii (19119)	50-100	luxuriant	>=50%	bluish green	negative reaction	*positive
Listeria monocytogenes (19118)	50-100	luxuriant	>=50%	bluish green (yellow back-ground)	positive reaction,	*positive,
Pseudomonas aeruginosa (27853)	>=10^3	inhibited	0%			

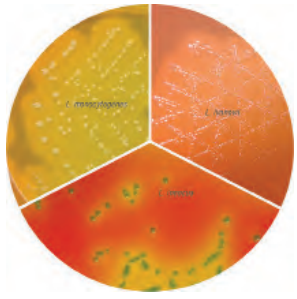
* : opaque halo around the colony exhibiting phosphatidyl inositol specific phospholipase activity.

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Mengaud J., Braun-Breton C. and Cossart P., (1991), Molecular Microbiology, 5(2): 367-372.P
2. Notermans S.H. and Dufrenne J., (1991), Applied and Environmental Microbiology, 57(09): 2666-70.
3. Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.
4. Ottaviani F., Ottaviani M., and Agosti M. (1997 b), Quimper Froid Symposium Proceedings p. 6, A.D.R.I.A. Quimper, France, 16-18 June 1997.
5. Schlech WF, Lavigne PM, Bortolussi RA, et al.(January 1983). "Epidemic listeriosis-evidence for transmission by food". N. Engl. J.Med. 308(4): 203–6. doi:10.1056.



M1924 – HiCrome™ L. mono Rapid Differential Agar Base

HIMEDIA®

HiCrome L. mono Differential Agar Base

M2009

This media is recommended for the selective and differential isolation, enumeration and identification of *Listeria monocytogenes* and *Listeria species* based on PCPLC activity.

Composition **

Ingredients	Grams/Litre
Peptone	15.000
Tryptone	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Maltose	4.000
Magnesium glycerophosphate	1.000
Magnesium sulphate	0.500
Sodium chloride	5.000
Lithium chloride	5.000
Disodium hydrogen phosphate anhydrous	2.500
Chromogenic substrate	2.200
Agar	14.000

Final pH (at 25°C) 7.2±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.60 grams in 480 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of Lecithin solution (FD332) and sterile rehydrated contents of Modified L.mono Selective Supplement (FD333). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Listeria monocytogenes is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles (2). The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L.monocytogenes* and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). HiCrome L.mono Differential Agar Base is based on for the selective and differential isolation of *Listeria species* on the basis of utilization of chromogenic substrate and lecithinase activity [Phosphotidylcholine phospholipase C (PCPLC)] (3). PI-PLC and PC-PLC, the major virulence factors, are only produced by pathogenic *L. monocytogenes* and *Listeria ivanovii* (1)

Peptone, tryptone, yeast extract and sodium pyruvate provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and essential growth nutrients . Maltose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplement (FD333) inhibit accompanying microflora and allow the growth of *Listeria species*. *Listeria species* hydrolyse the chromogenic substrate and produces green coloured colonies. Lecithin solution (FD332) helps in detecting PCPLC activity. Differentiation of *Listeria species* is based on phosphatidylcholine phospholipase C (PCPLC) activity. *Lmonocytogene*s and *L.ivanovii* exhibits PCPLC activity which is seen as opaque halo around the colony.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder
Gelling : Firm, comparable with 1.4% Agar gel
Colour and Clarity of prepared medium : Light amber coloured, opalescent gel forms in Petri plates.
Reaction : Reaction of 6.72% w/v aqueous solution at 25°C. pH : 7.2±0.2
pH : 7.00-7.40
Cultural Response : Cultural characteristics observed with added sterile Modified L.mono Selective Supplement (FD333) and Lecithin solution (FD332) after an incubation at 35 - 37°C for 24 - 48 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	PIPLC Activity*
Enterococcus faecalis (29212)	>=10^3	inhibited	0%	-	-
Listeria innocua (33090)	>=10^3	luxuriant	>=50%	greeish-blue	negative
Listeria ivanovii (19119)	50-100	luxuriant	>=50%	greeish-blue	positive#
Listeria monocytogenes	50-100	luxuriant	>=50%	greeish-blue	positive#

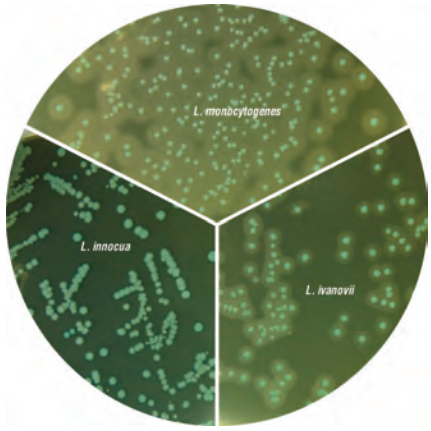
: opaque halo around the colony exhibiting phophatidylcholine phospholipase acivity

Storage and Shelf-life

Store dehydrated powder and the prepared medium at 2-8°C in tightly closed container. Use before expiry date on the label.

References

1. Mengaud J, Braun-Breton C, Cossart P 1991. Identification of phosphatidylinositol-specific phospholipase C activity in Listeria monocytogenes : a novel type of virulence factor. Mol. Microbiol. 5:367–372. doi:10.1111/j.1365-2958.1991
2. Painter J, Slutsker L. 2007. Listeriosis in humans, p 85–109. In Ryser ET, Marth EH (ed), Listeria, listeriosis, and food safety. Marcel Dekker, New York, NY.
3. Sang-Hyun Park, Pahn-Shick Chang, Sangryeol Ryu and Dong-Hyun Kang. Development of a Novel Selective and Differential Medium for the Isolation of Listeria monocytogenes. Applied and Environmental Microbiology 2014.



M2009 - HiCrome L. mono Differential Agar Base

HiCrome™ Aureus Agar Base

HiCrome™ Aureus Agar Base is recommended for isolation and identification of *Staphylococci* from environmental samples.

M1468

Composition **

Ingredients	Grams/Litre
Casein enzymic hydrolysate	12.000
Gelatin peptone	3.000
Meat extract B#	6.000
Yeast extract	5.000
Sodium pyruvate	10.000
Lithium chloride	5.000
Chromogenic mixture	2.100
Agar	20.000

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

#Equivalent to Beef extract

Directions

Suspend 63.1 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 50 ml concentrated Egg Yolk Tellurite Emulsion (FD046). Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Aureus Agar Base is recommended for isolation and enumeration of coagulase positive *Staphylococcus aureus* from environment samples. Coagulase positive *S. aureus* gives brown black colonies with clear zone around the colony whereas *S. epidermidis* gives slightly brownish colonies. Other organisms give either colourless colonies or bluish coloured colonies due to the presence of chromogen. *Listeria monocytogenes* colonies are bluish in colour whereas *Bacillus, E. coli* and *Micrococcus* give colourless colonies.

Casein enzymic hydrolysate, gelatin peptone, meat extract B and yeast extract provide nitrogenous substances carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Sodium pyruvate protects injured cells, helps recovery and enhances growth of *Staphylococcus*. Lithium chloride and potassium tellurite inhibit most of the contaminating microflora except *Staphylococcus aureus* (1). Due to addition of egg yolk, proteolytic bacteria produce a clear zone around colony (1).

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 2.0 % Agar gel.

Colour and Clarity of prepared medium : Yellow coloured opaque gel forms in Petri plates.

Reaction : Reaction of 6.31% w/v aqueous solution at 25°C. pH:7.0±0.2

Cultural Response : Cultural characteristics observed with added Egg Yolk Tellurite Emulsion (FD046) after an incubation at 35-37°C for 24-48 hours.

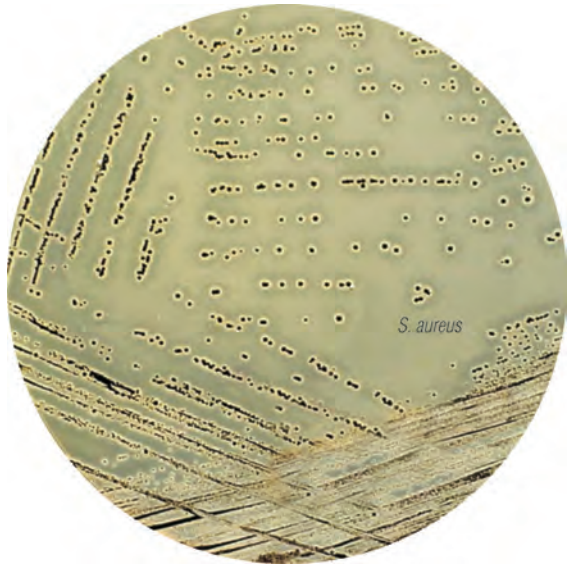
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase activity
Bacillus subtilis (6633)	50-100	none to poor	<=10%	colourless	Negative reaction
Escherichia coli (25922)	50-100	none to poor	<=10%	colourless	Negative reaction
Listeria monocytogenes (19112)	50-100	fair - good	30-40%	bluish	Negative reaction
Micrococcus luteus (10240)	50-100	none to poor	<=10%	colourless	Negative reaction
Staphylococcus aureus (25923)	50-100	good-lux-uriant	>=50%	brown-black halo or clear zone around the colony	Positive reaction
Staphylococcus epidermidis (12228)	50-100	none to poor	<=10%	yellow-slight brownish	Negative reaction

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Baird Parker, Ac (1962) J appl. Bact., 25:12.



M1468 – HiCrome™ Aureus Agar Base

HiCrome™ Staph Agar Base, Modified

HiCrome™ Staph Agar Base, Modified is a selective medium recommended for the isolation and enumeration of *Staphylococcus aureus.*

M1837

Composition **

Ingredients	Grams/Litre
Peptone special	23.000
Sodium pyruvate	4.000
Sodium chloride	40.000
Lithium chloride	5.000
Chromogenic mixture	5.300
Agar	15.000

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.15 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Polymyxin B Selective supplement (FD003). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Staphylococci are widespread in nature, though they are mainly found living on the skin, skin glands and mucous membranes of mammals and birds. Humans and animals are the primary source of this organism. Because of its widespread nature it is easily transferred to food and a cause of food poisoning if not handled properly.(1)

The coagulase positive species *S.aureus* is well documented as a human opportunistic pathogen. *Staphylococcus species* are a major cause of food poisoning and produces a wide variety of enterotoxins, thus causing various types of disease symptoms. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (2).

This medium is a selective chromogenic medium recommended for the isolation and enumeration of coagulase positive staphylococci in foods within 24 hours. This medium has an advantage over the traditional media which requires 48 hours. Peptone special in the medium supplies the essential nitrogeneous and carbonanceous compounds required for the growth. The chromogenic mixture incorporated in the medium is specifically cleaved by *Staphylococcus aureus* to give bluish green coloured colonies which are clearly visible against the opaque background. Sodium pyruvate enhances the growth of *Staphylococcus species.* Sodium chloride in the medium helps to maintain the osmotic equilibrium of the medium. High concentration of sodium chloride also helps in inhibiting the accompanying microflora. Lithium chloride inhibits most of the contaminating microflora. Addition of Polymyxin B Sulphate (FD003) helps to restrict growth of gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa.*

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Off white coloured opaque gel forms in Petri plates

Reaction : Reaction of 9.23 % w/v aqueous solution at 25°C. pH : 7.2±0.2.

Cultural Response : Cultural characteristics observed with added Polymyxin B Selective Supplement (FD003) after an incubation at 35-37°C for 24 hours.

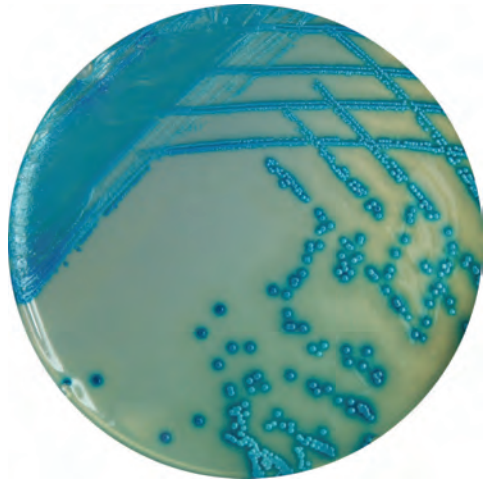
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Staphylococcus aureus (25923)	50 -100	luxuriant	>=50 %	blue colonies
Staphylococcus aureus (6538)	50 -100	luxuriant	>=50 %	blue colonies
Staphylococcus saprophyticus (15305)	50 -100	luxuriant	>=50 %	blue colonies
Bacillus cereus (10876)	50 -100	none- poor	<=10 %	-
Staphylococcus epidermidis (12228)	50 -100	none- poor	<=10 %	
Enterococcus faecalis (29212)	50 -100	none- poor	<=10 %	-
Escherichia coli (25922)	>=10^3	inhibited	0 %	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Victor , Lachica F, Weiss KF, Deibel RH (1969) Appl Microbiol 18 126-27



M1837 – HiCrome™ Staph Agar Base, Modified

HiCrome™ Staph Selective Agar

HiCrome™ Staph Selective Agar is a selective medium recommended for the isolation and enumeration of *Staphylococcus aureus*.

M1931

Composition **

Ingredients	Grams/Litre
Peptone special	25.000
Sodium chloride	50.000
Chromogenic mixture	3.200
Selective mixture	2.800
D-Mannitol	10.000
Phenol red	0.025
Agar	12.000

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 103.03 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Staphylococci are widespread in nature, though they are mainly found living on the skin, skin glands and mucous membranes of mammals and birds. Humans and animals are the primary source of this organism. Because of its widespread nature it is easily transferred to food and a cause of food poisoning if not handled properly.(1)

The coagulase positive species *S.aureus* is well documented as a human opportunistic pathogen. *Staphylococcus species* are a major cause of food poisoning and produces a wide variety of enterotoxins, thus causing various types of disease symptoms. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (2).

This medium is a selective chromogenic medium recommended for the isolation and enumeration of coagulase positive staphylococci in foods within 24 hours. Peptone special in the medium supplies the essential nitrogeneous compounds required for the growth. Phenol red is pH indicator. Mannitol in the medium is fermented by *Staphylococcus aureus* and the chromogenic mixture incorporated in the medium is specifically cleaved by *Staphylococcus aureus* to give greenish coloured colonies which are easily distinguishable. *Staphylococcus epidermidis* does not ferment mannitol hence blue coloured colonies are observed. Sodium chloride in the medium helps to maintain the osmotic equilibrium of the medium. High concentration of sodium chloride also helps in inhibiting the accompanying microflora.

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder
Gelling : Firm, comparable with 1.2% Agar gel
Colour and Clarity of prepared medium : Red coloured clear to slightly opalescent gel forms in Petri plates.
Reaction : Reaction of 10.30 % w/v aqueous solution at 25°C. pH : 7.4±0.2.
Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

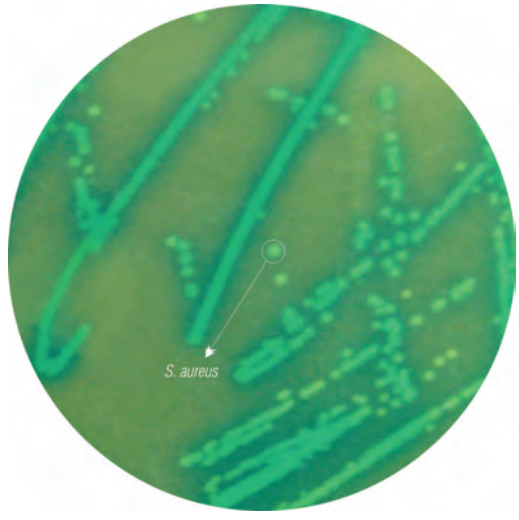
Organisms (ATCC)	Inoculum CFU)	Growth	Recovery	Colour of colony
Staphylococcus aureus (25923)	50 -100	luxuriant	>=50 %	green colonies
Staphylococcus aureus (6538)	50 -100	luxuriant	>=50 %	green colonies
Bacillus cereus (10876)	>=10^3	inhibited	0%	-
Staphylococcus epidermidis (12228)	50 -100	good-poor	40-50 %	blue colonies
Enterococcus faecalis (29212)	>=10^3	inhibited	0 %	-
Escherichia coli (25922)	>=10^3	inhibited	0 %	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Victor , Lachica F, Weiss KF , Deibel RH (1969) Appl Microbiol 18 126-27.



M1931 – HiCrome™ Staph Selective Agar

HiCrome™ MeReSa Agar Base

M1674

For the isolation and selective identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) from clinical isolates.

Composition **

Ingredients	Grams/Litre
Casein enzymic hydrolysate	13.00
Yeast extract	2.50
Meat extract B#	2.50
Sodium pyruvate	5.00
Sodium chloride	40.00
Chromogenic mixture	5.30
Agar	15.00

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

#Equivalent to Beef extract

Directions

Suspend 41.65 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of MeReSa Selective Supplement (FD229) and Cefoxitin supplement (FD259) for selectivity. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Staphylococcus aureus is an invasive pathogen that can cause disease in almost any tissue or organ in the human body, primarily in compromised individuals (3). Staphylococcal infections were earlier treated using Penicillin. But over the year's resistance to this drug developed. Methicillin was the next drug of choice. While methicillin is very effective in treating most *Staphylococcus* infections some strains have developed resistance to methicillin and can no longer be killed by this antibiotic. These resistant bacteria are called Methicillin Resistant *Staphylococcus aureus* (MRSA) (4). Patients with breaks in their skin due to wounds, indwelling catheters or burns are those with certain risk of developing MRSA infection (2). Spread of MRSA infections can be controlled to a great extent by maintaining personal hygiene after interaction with an MRSA infected person (4).

CLSI recommends the usage of cefoxitin instead of oxacillin for determination of resistance against Methicillin for *S. aureus* (1). To increase the sensitivity for the detection of heterogeneously resistant MRSA strains, cefoxitin is used which selectively inhibits the susceptible strains.

Casein enzymic hydroylsate, meat extract B and yeast extract provide the essential nutrients along with carbonaceous, nitrogenous and Vitamin B complex nutrients. The proprietary chromogenic mixture incorporated in the medium is specifically cleaved by *Staphylococcus aureus* to form bluish green coloured colonies. Sodium pyruvate enhances the growth of *Staphylococcus species.* Sodium chloride in the medium helps to maintain the osmotic equilibrium of the medium. High concentration of sodium chloride also helps in inhibiting the accompanying microflora. The medium is made selective for MRSA by the addition of MeReSa Selective Supplement (FD229) and Cefoxitin Supplement (FD259).

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light yellow coloured, opaque gel forms in Petri plates.

Reaction : Reaction of 8.33% w/v aqueous solution at 25°C. pH:7.0 ± 0.2.

Cultural Response : Cultural characteristics observed with added MeReSa Selective Supplement (FD229) and Cefoxitin Supplement (FD259) after an incubation at 30-35°C for 18-48 hours.

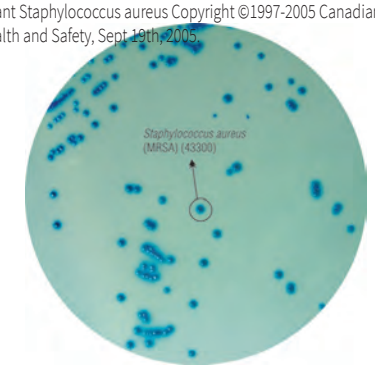
Organisms (ATCC)	Inoculum CFU)	Growth w/FD229 & FD259	Recovery w/FD229 & FD259	Colour of colony
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-
Staphylococcus aureus (MRSA) (43300)	50-100	luxuriant	>=50%	bluish - green
Staphylococcus epidermidis (12228)	>=10^3	inhibited	0%	-
Escherichia coli (25922)	>=10^3	inhibited	0%	-
Enterococcus faecalis (29212)	>=10^3	inhibited	0%	-
Staphylococcus aureus (6538)	>=10^3	inhibited	0%	-
Staphylococcus xylosus (29971)	>=10^3	inhibited	0%	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. CLSI 2010. Performance Standard for antimicrobial disk susceptibility testing, Twentieth Informational Supplem.
2. Dr. Alan Johnson, methicillin resistant staphylococcus aureus (MRSA) infection. The Support group for MSRA sufferers and Dependents, Aug 1st, 2005.ent.
3. DWorkin M et. al 2006. The Prokaryotes (a Handbook on the Biology of Bacteria) 3rd ed, Vol. 2, page 345.
4. Methicillin Resistant Staphylococcus aureus Copyright ©1997-2005 Canadian Centre for Occupational Health and Safety, Sept 19th, 2005.



M1674 – HiCrome™ MeReSa Agar Base

Methicillin Resistant Staphylococcus

Hicrome™ MRSA Agar Base, Modified

HiCrome™ MRSA Agar Base,Modified is recommended for the differentiation and identification of MRSA and MRSE *Staphylococcus species.*

M1953

Composition **

Ingredients	Grams/Litre
Peptone	23.000
Sodium chloride	10.000
Sodium pyruvate	5.000
Chromogenic substrate	0.770
Inhibitor mixture	7.000
Agar	15.000

Final pH (at 25°C) 7.2±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.38 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of MeReSa Selective Supplement (FD229) and Cefoxitin supplement (FD259), both in combination for more selectivity as desired. Mix well and pour into sterile Petri plates.

Principle and Interpretation

MRSA is a resistant variation of the common bacterium *Staphylococcus aureus* and MRSE is a resistant variation of the common bacterium *Staphylococcus epidermidis. Staphylococcus aureus* is an invasive pathogen that can cause disease in almost any tissue or organ in the human body, primarily in compromised individuals (1). Staphylococcal infections were earlier treated using Penicillin. But over the years resistance to this drug developed. Methicillin was the next drug of choice. While methicillin is very effective in treating most *Staphylococcus* infections some strains have developed resistance to methicillin and can no longer be killed by this antibiotic. These resistant bacteria are called Methicillin Resistant *Staphylococcus aureus* (MRSA) (2).Patients with breaks in their skin due to wounds, indwelling catheters or burns are those with certain risk of developing MRSA infection (3). Spread of MRSA infections can be controlled to a great extent by maintaining personal hygiene after interaction with an MRSA infected person (2).

Peptone provide the essential nutrients along with carbonaceous, nitrogenous and Vitamin B complex nutrients. The chromogenic mixture incorporated in the medium is specifically cleaved by *Staphylococcus aureus* to give green coloured colonies whereas Methicillin Resistant *Staphylococcus epidermidis* gives blue coloured colonies. This medium helps in identification and differentiation of MRSA and MRSE. Sodium pyruvate enhances the growth of *Staphylococcus species.* Sodium chloride in the medium helps to maintain the osmotic equilibrium of the medium. High concentration of sodium chloride also helps in inhibiting the accompanying microflora. Inhibitor mixture imparts selectivity to the medium. Cefoxitin is recommended to use for selective isolation of MRSA. The medium is made selective for MRSA by the addition of MeReSa Selective Supplement (FD229) and Cefoxitin supplement (FD259) in combination.

Quality Control

Appearance of Powder : Cream to beige homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Light purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction at : Reaction of 6.08% w/v aqueous solution 25°C. pH : 7.2±0.2.

Cultural Response : Cultural characteristics observed with added MeReSa Selective Supplement (FD229) and Cefoxitin Supplement (FD259) after an incubation at 30-35°C for 18-48 hours.

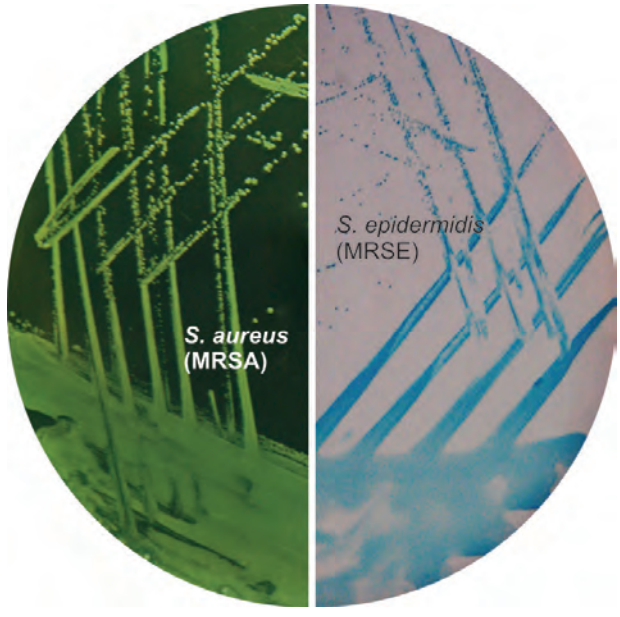
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922)	>=10^3	inhibited	0%	-
Enterococcus faecalis (29212)	>=10^3	inhibited	0%	-
Staphylococcus aureus, MRSA (43300)	50-100	luxuriant	>=50%	green
Staphylococcus epidermidis, MRSE	50-100	luxuriant	>=50%	blue
Staphylococcus xylosus (29971)	>=10^3	inhibited	0%	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. DWorkin M et. al 2006. The Prokaryotes (a Handbook on the Biology of Bacteria) 3rd ed, Vol. 2, page 345.
2. Methicillin Resistant Staphylococcus aureus Copyright ã 1997-2005 Canadian Centre for



M1953 – Hicrome™ MRSA Agar Base,Modified

HiCrome Rapid MRSA Agar Base

It is recommended for rapid isolation and identification of Methicillin Resistant *Staphylococcus aureus* (MRSA).

M1974

Composition **

Ingredients	Grams/Litre
Special peptone	20.000
Casein peptone	20.000
Sodium chloride	8.500
Carbohydrate	14.000
Phenol red	0.025
Chromogenic mix	6.500
Amino-Vitamin mix	1.200
Agar	15.000

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 85.23 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of MRSA Selective Supplement (FD319). Mix well and pour into sterile Petri plates. DO NOT AUTOCLAVE.

Principle and Interpretation

MRSA is a resistant variation of the common bacterium *Staphylococcus aureus* . It is an invasive pathogen that can cause disease in almost any tissue or organ in the human body, primarily in compromised individuals (1). Staphylococcal infections were earlier treated using Penicillin. But over the years resistance to this drug developed. Methicillin was the nextdrug of choice. While methicillin is very effective in treating most *Staphylococcus* infections some strains have developed resistance to methicillin and can no longer be killed by this antibiotic. These resistant bacteria are called Methicillin Resistant *Staphylococcus aureus* (MRSA) (2).Patients with breaks in their skin due to wound, indwelling catheters or burns are thosewith certain risk of developing MRSA infection (3).

Special peptone, Casein peptone and amino-vitamin mix provides essential nutrients for growth. Carbohydrate is the source of carbon and energy. Phenol red is the pH indicator. The chromogenic mixture incorporated in the medium is specifically cleaved by *Staphylococcus aureus* (MRSA) to give greenish yellow coloured colonies. Sodium chloride in the medium helps to maintain the osmotic equilibrium of the medium. High concentration of sodium chloride also helps in inhibiting the accompanying microflora. Agar acts as solidifying agent.

Quality Control

Appearance of Powder	:	Cream to beige homogeneous free flowing powder
Gelling	:	Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium	:	Red coloured,clear to slightly opalescent gel forms in Petri plates.
Reaction	:	Reaction of 8.52% w/v aqueous solution at 25°C. pH:7.4 ± 0.2.
Culture Response	:	Cultural characteristics observed with added MRSA Selective Supplement (FD319) after an incubation at 30-35°C for 18-24hours

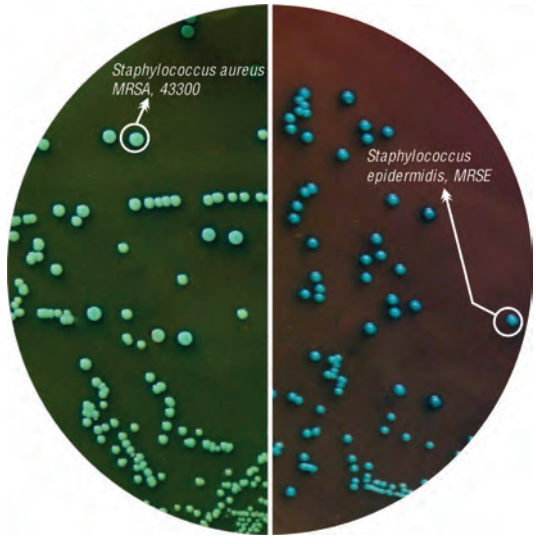
Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Staphylococcus aureus, MRSA ATCC 43300	50-100	luxuriant	>=50%	greenish yellow (Note: Green colour may develop after 48 hours)
Staphylococcus epidermidis, blue MRSE		50-100	luxuriant	>=50%
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	-
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0%	-
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	-
Candida albicans ATCC 10231	>=10^3	inhibited	0%	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. DWorkin M et. al 2006. The Prokaryotes (a Handbook on the Biology of Bacteria) 3rd ed, Vol. 2, page 345.
2. Methicillin Resistant Staphylococcus aureus Copyright ã 1997-2005 Canadian Centre for Occupational Health and Safety,Sept 19th, 2005.
3. Dr. Alan Johnson, methicillin resistant staphylococcus aureus (MRSA) infection. The Support group for MSRA sufferersand Dependents, Aug 1st , 2005.



M1974 HiCrome Rapid MRSA Agar Base

HiCrome™ Bacillus Agar

M1651

For isolation and differentiation between various species of *Bacillus* from a mixed culture by chromogenic method.

Composition **

Ingredients	Grams/Litre
Peptone	10.00
Meat extract	1.00
D-Mannitol	10.00
Sodium chloride	10.00
Chromogenic mixture	3.20
Phenol red	0.025
Agar	15.00

Final pH (at 25°C) 7.1 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 49.22 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Bacillus Selective Supplement (FD324) if desired. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Majority of *Bacillus species* apparently have little or no pathogenic potential and are rarely associated with disease in humans or lower animals. The principal exception to this are *Bacillus anthracis*, the agent of anthrax, and *Bacillus cereus*. However a number of other species, particularly those of the *B. subtilis* group, have been implicated in food poisoning and other human and animal infections (4). *Bacillus cereus* causes food poisoning due to consumption of contaminated rice (2, 1, 5) other starchy foods such as potato, pasta and cheese have also been implicated, eye infections and a wide range of other clinical conditions like abscess formation, meningitis, septicemia and wound infection.

HiCrome™ Bacillus Agar is based on the formulation of MYP Agar formulated by Mossel et al (2) used for enumeration of *Bacillus cereus* and *Bacillus thuringiensis* when present in large number in certain foodstuffs. The medium contains peptone and meat extract, which provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other growth nutrients. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by phenol red. Mannitol fermenting organisms like *B. megaterium* yield yellow coloured colonies. The chromogenic mixture present in the medium is cleaved by the enzyme β-glucosidase found in *B. cereus* resulting in the formation of blue colonies. *B. thuringiensis* also grows as blue/green colonies on this medium as *B. cereus* and *B. thuringiensis* are biochemically identical. If selective isolation of *B. cereus* or *B. thuringiensis* is required aseptically add Bacillus Selective Supplement (FD324).

Quality Control

Appearance of Powder	:	Light yellow to pink coloured, homogeneous, free flowing powder.
Gelling	:	Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium	:	Red coloured, clear to slightly opalescent gel forms in Petri plates.
Reaction	:	Reaction of 4.92% w/v aqueous solution at 25°C. pH : 7.1 ± 0.2.

Organisms (ATCC)	Inoculum (CFU)	Growth *	Recovery *	Growth **	Recovery **	Colour of colony
Bacillus subtilis (6633)	50-100	fair	20-30%	inhibited		0% yellowish green to green
Bacillus cereus (10876) with	50-100	good-luxuriant	>=50%	good-luxuriant	>=50%	light blue,# large, flat blue centre
Bacillus thuringiensis (10792)	50-100	good-luxuriant	>=50%	good-luxuriant	>=50%	light blue, large, flat with irregular margins
Bacillus megaterium (14581)	50-100	good-luxuriant	>=50%	inhibited		0% yellow, mucoid colonies
Bacillus coagulans (7050)	50-100	good-luxuriant	>=50%	inhibited		0% pink, small, raised colonies
Bacillus pumilis (14884)	50-100	good-luxuriant	>=50%	poor	10-20%	light green to green colonies
Staphylococcus aureus colonies (25923)	50-100	luxuriant	>=50%	inhibited		0% yellow
Enterococcus faecalis to (29212)	50-100	luxuriant	>=50%	inhibited		0% light green to green

Key : * : Growth without addition of FD324 ** : Growth with addition of FD324

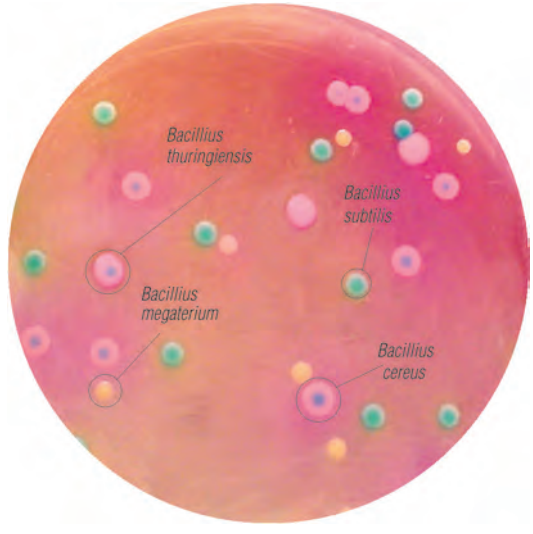
: Colony surrounded by pink halo

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Bouza E., Grant S., Jordan C. et al, 1979, Arch. Ophthamol., 97:488.
2. Mortimer P. R. and McCann G., 1974, Lancet, 1043.
3. Mossel D. A. A., Koopman M. J. and Jongerium E., 1967, Appl. Microbiol.,15:650.
4. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed. American Society for Microbiology, Washington, D.C.
5. Wohlgemuth K., Kirkbride C. A., Bicknell E. J. and Ellis R. P., 1972 Am. Vet. Met,



M1651 –HiCrome™ Bacillus Agar

HiCrome™ Single Streak Rapid Differentiation Series HiCynth Free from BSE/TSE/GMO

HiCrome™ Bacillus Agar (M1651) is also available as HiCrome™ Bacillus HiCynth™ Agar (MCD1651)wherein all animal/vegetable based nutrients are substituted with chemically defined nutrients.

HIMEDIA®

M-CP Agar Base

M-CP Agar Base with selective supplement is recommended by the Directive of the Council of the European Union 98/83/EC for isolation and enumeration of *Clostridium perfringens* from water sample using membrane filtration technique.

M1354

Composition **

Ingredients	Grams/Litre
Tryptose	30.00
Yeast extract	20.00
Sucrose	5.00
L-Cysteine hydrochloride	1.00
Magnesium sulphate, $7H_2O$	0.10
Bromo cresol purple	0.04
Ferric chloride, $6H_2O$	0.09
Indoxyl-β-D-glucoside	0.06
Agar	15.00

Final pH (at 25°C) 7.6 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.60 grams (the equivalent weight of dehydrated powder per 485 ml) in 485 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add the rehydrated contents of 1 vial of M-CP Selective Supplement I (FD153) and 1 vial of M-CP Selective Supplement II (FD154) or rehydrated contents of 1 vial of M-CP Selective Supplement II Modified (FD154A). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Clostridial species are one of the major causes of food poisoning/ gastro-intestinal illnesses. They are gram-positive, spore-forming rods that occur naturally in the soil (2). Among the family are: *Clostridium botulinum* which produces one of the most potent toxins in existence; *Clostridium tetani*, causative agent of tetanus; and *Clostridium perfringens* commonly found in wound infections and diarrhoea cases. The use of toxins to damage the host is a method deployed by many bacterial pathogens. The major virulence factor of *C. perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributes to food poisoning and other gastrointestinal illnesses (2). Several solid media have been devised for quantitation of *C. perfringens*. The selectivity of the media is achieved by incorporation of one or more antibiotics that inhibit certain anaerobes or facultative anaerobes. M-CP Agar Base is prepared as per the formula of Armon and Payment (1). It is also recommended by the Directive of the Council of the European Union 98/83/EC (3) for isolation and enumeration of *Clostridium perfringens* from water sample using membrane filtration technique.

Tryptose, yeast extract provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients compounds while sucrose is the fermentable carbohydrate. Bromocresol purple serves as a pH indicator. Indoxyl-β-D-glucoside is a chromogenic substrate for β-D-glucosidase or cellobiase and phenolphthalein diphosphate for the detection of acid phosphatase. The addition of D-cycloserine and polymyxin B (FD153) makes the medium inhibitory to accompanying non-clostridial microflora and thus allows analysis of both clostridial vegetative cells and spores. Further selectivity is provided by incubation under anaerobic conditions. Yellow (cellobiase-negative) colonies becoming old rose to pink-red upon exposure to ammonia fumes for 30 seconds are considered to be presumptive *C. perfringens*. Colour differentiation on M-CP Agar Base is sometimes difficult, so typical colonies (yellow turning into pink) as well as a typical colonies (green or those that remain yellow upon exposure to ammonia fumes) are picked for confirmation. Presumptive *C. perfringens* can be confirmed by sulphite reduction, gram-positive, sporulating rods, non-motile, reduction of nitrate, gelatine liquefaction, lactose fermentation and other biochemical tests (4).

Quality Control

Appearance of Powder : Light yellow to light green coloured, homogeneous, free flowing powder.
Gelling : Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium : Purple coloured, clear to slightly opalescent gel forms in Petri plates.
Reaction : Reaction of 7.12% w/v aqueous solution at 25°C. pH:7.6 ± 0.2.
Culture Response : Cultural characteristics observed after an incubation at 44°C for 24-48 hours with added contents of 1 vial of M-CP Selective pplement I (FD153) and 1 vial of M-CP Selective Supplement II (FD154) or rehydrated contents of 1 vial of M-CP Selective Supplement II Modified (FD154A) under anaerobic conditions.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of Colonies	
Clostridium perfringens (12924)		50-100	good	yellow*
Staphylococcus aureus (25923)		>=10^3	inhibited	-
Bacillus subtilis (6633)	>=10^3	inhibited	-	
Salmonella Typhi (6539)	>=10^3	inhibited	-	

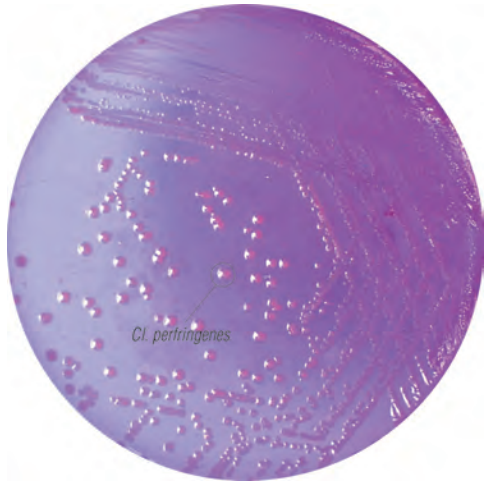
Key: *: colonies becomes old rose to light pink-red upon exposure to ammonia fumes for 30 seconds.

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Armon R. and Payment P., 1988, Can. J. Microbiol., 34:78-79.



M1354 M-CP Agar Base

Clostridium perfringens

HIMEDIA®

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

M-CP Agar Base (M1354) is also available as M-CP HiVeg™ Agar Base (MV1354) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

HiCrome™ Nickels and Leesment Medium

For the enumeration of citrate-fermenting lactic acid bacteria from milk, milk products and mesophilic starter cultures.

M1712

Composition **

Ingredients	Grams/Litre
Casein enzymic hydrolysate	18.00
Yeast extract	4.50
Gelatine	2.25
Glucose	4.50
Lactose	4.50
Sodium chloride	3.60
Trisodium citrate dihydrate	1.80
Calcium lactate pentahydrate	8.00
Tricalcium dicitrate tetrahydrate	6.65
Carboxymethyl cellulose (CMC)	0.40
Chromogenic substrate (X-gal)	0.20
Agar	15.000

Final pH (at 25°C) 6.65 ± 0.05

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 66.0 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add rehydrated contents of 2 vials of HiCrome™ Nickels and Leesment Selective Supplement (FD245). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Lactic acid bacteria are widespread in nature and are best known for their activities in major food such as dairy, meat and vegetable products (1). Testing for lactic acid bacteria in dairy products may be useful for various reasons like evaluating lactic starter cultures; determining the cause of acid defects in milk products, controlling the quality of cured cheese, cultured milks and uncultured products containing added cultures (2). HiCrome™ Nickels and Leesment Medium is a modification of Modified Nickels and Leesment Medium formulated as per APHA (1) and is used for the enumeration of citrate-fermenting lactic acid bacteria using colony count technique at 25°C. Casein enzymic hydrolysate and yeast extract serve as carbon and nitrogen sources, long chain amino acids, vitamin B complex and other essential growth nutrients. Lactose and glucose are the carbohydrate source in the medium.

X-gal differentiates between *Lactococcus lactis* subsp. *lactis* and *Leuconostoc* species. *Lactococcus lactis* subsp. *lactis biovar* diacetylactis colonies are white with a clear zone. *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* colonies are white without a clear zone. *Leuconostoc* species are blue, with or without a clear zone. HiCrome™ Nickels and Leesment Medium with the addition of HiCrome™ Nickels and Leesment Supplement (FD245) can be used for enumeration of *Leuconostoc* (1). Vancomycin acts as a supplement for the selective isolation of *Leuconostoc* from a mix flora of lactic acid bacteria. Sodium chloride maintains osmotic equilibrium and various salts provide essential ions.

Quality Control

Appearance of Powder : Cream to light yellow coloured homogeneous, free flowing powder.
Gelling : Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium : White coloured opaque gel forms in Petri plates containing white precipitate.
Reaction : Reaction of 6.6% w/v aqueous solution at 25°C. pH : 6.65 ± 0.05.
Cultural Response : Cultural characteristics observed after incubation at 25-30°C for 48-72 hours.

Organisms (ATCC)	Growth	Growth*	Colour of colony
Lactococcus lactis subsp *lactis biovar diacetylactis*	good-luxuriant	inhibited	white with a clear zone
Lactococcus lactis subsp *lactis (19435)*	good-luxuriant	inhibited	white without a clear zone
Lactococcus lactis subsp *cremoris* (19257)	good-luxuriant	inhibited	white without a clear zone
Leuconostoc mesenteroides (9135)	good-luxuriant	good-luxuriant	blue without clear zone

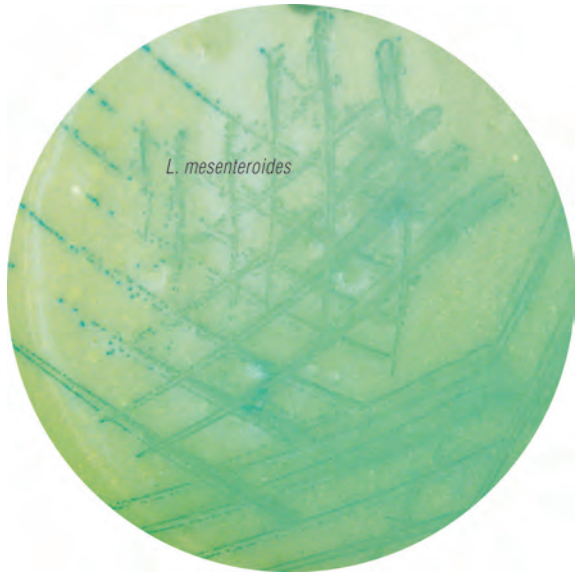
Key : * = with the addition of HiCrome Nickels and Leesment Selective Supplement (FD245)

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Downes F.P. and I to K., (Eds) 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA Washington D.C.
2. Marshall R.T., 1992, Standard Methods for the Examination of Dairy products, 16th Ed,



M1712 HiCrome™ Nickels and Leesment Medium

HiCrome™ Bifidobacterium Agar

Recommended for the differentiation of *Bifidobacterium* and *Lactobacillus* species.

M1960

Composition **

Ingredients	Grams/Litre
Peptone special	23.000
Sodium chloride	5.000
Milk Protein	5.000
Chromogenic mixture	10.480
Agar	16.000

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 59.48 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

The genus *Bifidobacterium* is the third most numerous bacterial populations found in the human intestine after *Bacteroides* and *Eubacterium*. It is an anaerobic bacteria that makes up the gut microbial flora. It resides in the colon and have health benefits for their hosts. Bifidobacteria are also associated with lower incidences of allergies (1, 2). Bifidobacterium Agar isused for the cultivation and maintenance of *Bifidobacterium* species (3).

Peptone special provides nitrogeneous and carbanaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride maintains osmotic balance. Milk protein aids in detecting casein hydrolysis activity which is exhibited by *Bifidobacterium breve*. A halo zone is observed around the colony in case of casein hydrolysis. The indicator system in the chromogenic mixture helps in distinguishing between *Lactobacillus* and *Bifidobacterium* species. *Lactobacillus* species usually produce green colonies with opaque background. *Bifidobacterium infantis* produces dark blue to bluish green colonies. Agar serves as an solidifying agent.

Quality Control

Appearance of Powder	:	Cream to yellow homogeneous free flowing powder
Gelling	:	Firm, comparable with 1.6% Agar gel
Colour and Clarity of prepared medium	:	Reddish orange coloured clear to slightly opalescent gel forms in Petri plates
Reaction	:	Reaction of 5.95% w/v aqueous solution at 25°C. pH :7.2±0.2
Cultural Response	:	Cultural characteristics observed after an incubation at 35-37°C for 48 hours in an anaerobic conditions.

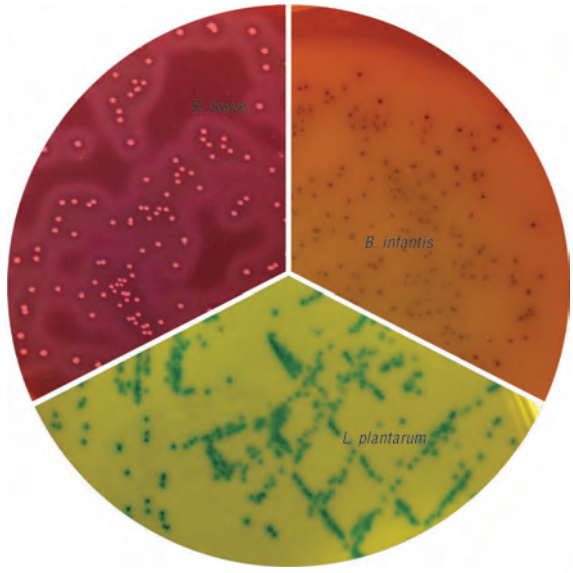
Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of the colony
Bifidobacterium infantis - bluish (25962)	50-100	good-luxuriant		>=50% Dark blue green
Bifidobacterium breve with (15698)	50-100	good-luxuriant		>=50% Red-pink halo zone
Lactobacillus plantarum colonies w/ (8014)	50-100	good-luxuriant		>=50% Green hazy background
Lactobacillus fermentum without (9338)	50-100	good-luxuriant		>=50% Pink halo zone

Storage and Shelf-life

Store dehydrated medium in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Björkstén B., Sepp E., Julge K., Voor T., and Mikelsaar M., 2001, J. Allergy Clin. Microbiol., Volume 108, Issue 4, 516-520.
2. Guarner F., and Malagelada J. R., 2003, The Lancet, Vol. 361, Issue 9356, 8 February 2003, 512-519.



M1960 - HiCrome™ Bifidobacterium Agar

HiCrome™ Acinetobacter Agar Base

Recommended for selective isolation of *Acinetobacter* species from environmental and clinical samples.

M1938

Composition **

Ingredients	Grams/Litre
Peptone special	9.00
Sodium chloride	5.00
Selective mix	0.50
Chromogenic mixture	1.35
Agar	15.00

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.85 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and add the rehydrated contents of two vials of MDR Selective Supplement (FD271). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Acinetobacter species are gram negative ubiquitous bacteria that have been isolated from patients with nosocomial infection, environment, soil, and water. *Acinetobacter* is mostly found in every type of infections (3). There is an alarming situation as *Acinetobacter baumannii* is found to be resistant to most commonly used antibiotics which includes beta-lactams and aminoglycosides (2,3). Immunocompromised patients requiring mechanical respirations are at more risk of infection by *Acinetobacter species*.(1)

Peptone special provides nitrogeneous and carbonaceous compounds, long chain amino acids and vitamins to the organisms. Sodium chloride maintains the osmotic balance. Selective mix inhibits gram positive organisms. The chromogenic mixture in the medium allows the differentiation of *Acinetobacter species* from other organisms.

Quality Control

Appearance of Powder : Light yellow to yellow homogeneous free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Yellow coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 3.09% w/v aqueous solution at 25°C. pH:7.0 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

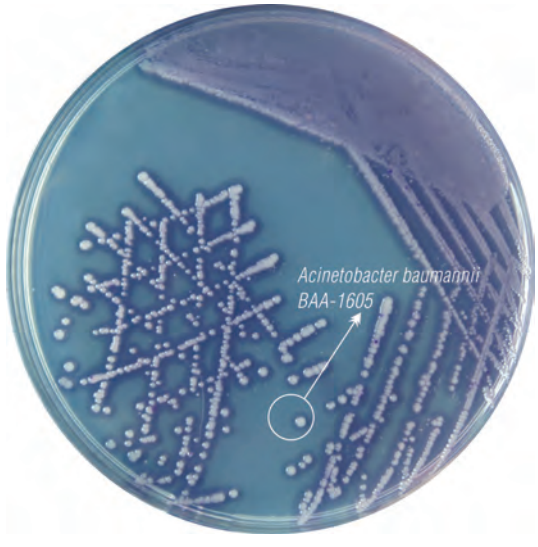
Organism (ATCC)	Inoculum (CFU)	Growth	Recovery with FD271	Colour of colony
Acinetobacter baumannii (BAA-1605)	50-100	luxuriant	>=50%	Light purple with halo
Acinetobacter baumannii (BAA-747)	>=10^3	inhibited	0%	-
Acinetobacter baumannii (19606)	>=10^3	inhibited	0%	-
Acinetobacter lwofii (15309)	>=10^3	inhibited	0%	-
Acinetobacter haemolyticus (19002)	>=10^3	inhibited	0%	-
Escherichia coli (25922)	>=10^3	inhibited	0%	-
Enterococcus faecalis (29212)	>=10^3	inhibited	0%	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Bergogne- Berezin, E., m. L. Joly-Guillou, and J.F. Vieu. 1987. Epidemiology of nosocomial infections due to Acinetobacter calcoaceticus . J. Hosp. Infect. 10:105-113
2. Montefour, K., et.al.2008. Acinetobacter baumanni : An EmergingMultidrug Resistant pathogen in critical care Nurse; 28:15-25
3. Valentine, S.C., et.al. 2008 Phenotypic and molecular characterization of Acinetobacter baumanni. Clinical isolates from nosocomial outbreaks in Los Angeles



M1938 – HiCrome™ Acinetobacter Agar Base

HiCrome™ OGYE Agar Base

For isolation and enumeration of yeasts and moulds from milk and milk products by chromogenic method.

M1467

Composition **

Ingredients	Grams/Litre
Yeast extract	4.00
Dextrose	20.00
Chromogenic mixture	1.10
Agar	12.00

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 18.55 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add reconstituted contents of one vial of Oxytetra Selective Supplement (FD032). Mix well and pour into sterile Petri plates.

Principle and Interpretation

OGYE Agar Media were originally formulated by Mossel et al (1, 2) for the isolation and enumeration of yeasts and moulds from foodstuffs. Mossel et al (3) further added Oxytetracycline as a selective agent and found that the use of Oxytetracycline in a medium with a neutral pH gives increased counts of yeasts and moulds as compared to media having a low pH to suppress bacterial growth. HiCrome™ OGYE Agar is a selective and differential medium, which facilitates rapid isolation of yeasts and moulds from milk and milk products.

Yeast extract provides essential growth nutrients. Dextrose acts as carbon and energy source. Although the low pH helps to reduce the bacterial flora, Oxytetracycline makes the medium, more selective by inhibiting the growth of lactobacilli encountered in milk and milk-products at low pH. Incorporation of chromogenic compounds into the growth medium helps in identification of yeasts and moulds isolates directly on primary isolation. **Aspergillus brasiliensis* appear as light blue coloured colonies with black spores due to presence of chromogenic mixture, *C.albicans* shows green coloured colonies and *Saccharomyces cerevisiae* forms colourless colonies.

Quality Control

Appearance of Powder	:	Cream to yellow coloured homogeneous, free flowing powder.
Gelling	:	Firm, comparable with 1.2% Agar gel.
Colour and Clarity of prepared medium	:	Light amber coloured, clear to slightly opalescent gel forms in Petri plates.
Reaction	:	Reaction of 3.71% w/v aqueous solution at 25°C. pH:7.0 ± 0.2.
Cultural Response added ment (FD032)	:	Cultural characteristics observed with Oxytetra Selective Supple- after anincubation at 25-30°C for 2-3 days.

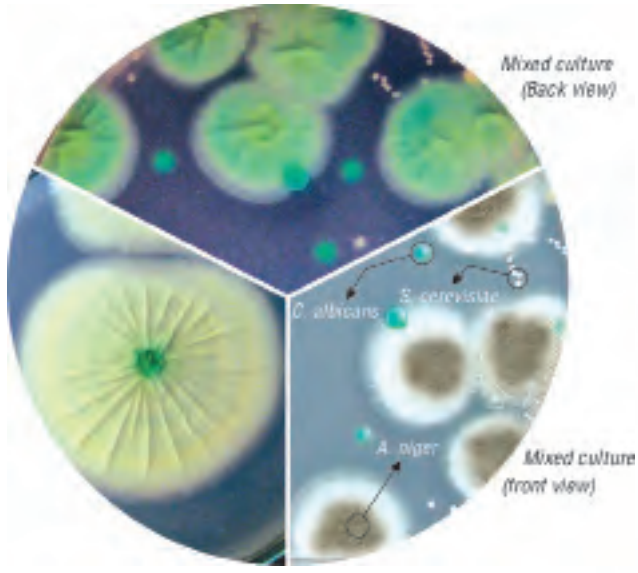
Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
**Aspergillus brasiliensis* (16404) with spores		50-100	luxuriant	— light blue black
Candida albicans (10231)	50-100	luxuriant	>=50%	green
Escherichia coli (25922)	>=10^3	inhibited	0%	—
Saccharomyces cerevisiae (9763) colourless		50-100	luxuriant	>=50%

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Mossel D.A.A. et al, 1970, J. Appl. Bact., 33:454.



M1467 – HiCrome™ OGYE Agar Base

*Formerly known as Aspergillus niger

HiCrome™ Candida Differential Agar / Base, Modified

Recommended for rapid isolation and identification of *Candida species* from mixed cultures.

M1297A / M1456A

Composition **	M1297A	M1456A
Ingredients	**Grams/Litre**	**Grams/Litre**
Peptone	—	5.00
Peptone special	15.00	—
Yeast extract	4.00	3.00
Malt extract	—	3.00
Dipotassium hydrogen phosphate	1.00	—
Glucose	—	10.00
Chromogenic mixture	7.22	3.00
Chloramphenicol	0.50	0.05
Agar	15.00	18.00
Final pH (at 25°C)	6.3 ± 0.2	7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.72 grams of M1297A in 1000 ml distilled water and 21.02 grams of M1456A in 500 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of HiCrome™ Candida Selective Supplement (FD192) to M1456A. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Perry and Miller (1) reported that *Candida albicans* produces an enzyme β-N-acetyl- galactosaminidase and according to Rousselle et al (2) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of *C. albicans* isolates directly on primary isolation. HiCrome™ Candida Differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida species* namely *C. albicans, C. krusei, C. tropicalis* and *C. glabrata* on the basis of colouration and colony morphology. On this medium, results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts in Mycology and Clinical Microbiology Laboratory.

Peptone, peptone special, malt extract and yeast extract provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Phosphate buffers the medium well. Chloramphenicol suppresses the accompaning bacterial flora. *C. albicans* appear as light green coloured smooth colonies, *C. tropicalis* appear as blue to purple coloured raised colonies. *C. glabrata* colonies appear as cream to white smooth colonies, while *C. krusei* appear as purple fuzzy colonies. *C. glabrata, C. kefyr, C. parapsilosis* colonies appear as cream to white, beige/yellow due to natural pigmentation and some alkaline phosphatase activity. The use of HiCrome Selective Supplement (FD192) in M1456A imparts additional selectivity to the medium.

Quality Control

Appearance of Powder : Cream to beige coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel of M1297A or 1.8% Agar gel of M1456A.

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction of : Reaction of 4.27% w/v aqueous solution M1297A at 25°C. pH:6.3 ± 0.2

of Reaction of 4.20% w/v aqueous solution M1456A at 25°C. pH:7.2 ± 0.2

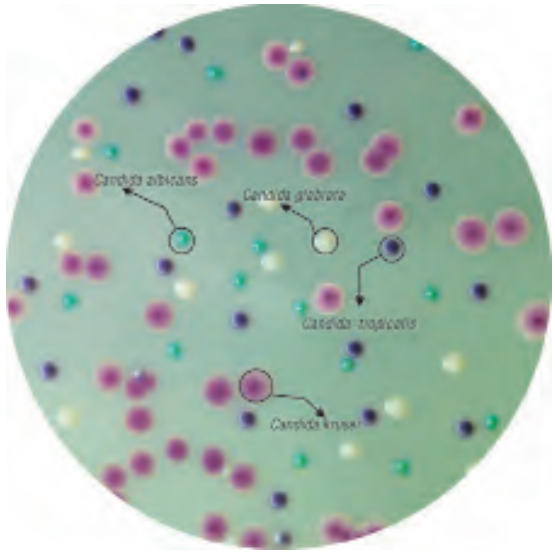
Cultural Response an hours on addition tive Supplement : Cultural characteristics observed after incubation at 30°C for 40-48 of HiCrome™ Candida Selec-

(FD192) in M1456A.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of the colony
Candida albicans (10231) light green		50-100	good-luxuriant	>=50%
Candida tropicalis (750) blue to purple		50-100	good-luxuriant	>=50%
Candida krusei (24408) purple, fuzzy		50-100	good-luxuriant	>=50%
Candida glabrata (15126) cream to white	50-100	good-luxuriant		>=50%
Candida parapsilosis (22019) cream to white have mauve	50-100	good-luxuriant		>=50% (may center)
Escherichia coli (25922)	>=10^3	inhibited	0%	—
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	—

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.



M1297A HiCrome™ Candida Differential Agar

HiCrome™ Candida Differential Agar Base

M1297AR

HiCrome™ Candida Differential Agar Base is selective and differential medium for rapid isolation and identification of *Candida* species from mixed cultures.

Composition **

Ingredients	Grams/Litre
Peptone	4.000
Chromogenic mixture	13.600
Agar	13.600

Final pH (at 25°C) 6.0±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.6 grams in 500 ml distilled water. Add the rehydrated contents of one vial of HiCrome™ Candida Differential Selective Supplement (FD283R). Heat to boiling with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Perry and Miller (1) reported that *Candida albicans* produces an enzyme β-N-acetyl- galactosaminidase and according to Rousselle et al (2) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of *C. albicans* isolates directly on primary isolation. HiCrome™ Candida Differential Agar Base incorporates two chromogenes X-NAG which detects the activity of hexosaminidase and BCIP which detects phosphatase activity.

HiCrome™ Candida Differential Agar Base is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida species* namely *C.albicans, C.krusei, C.tropicalis* and *C.glabrata* on the basis of colouration and colony morphology. On this medium results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts in Mycology and Clinical Microbiology Laboratory.

Peptone provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Chloramphenicol from the supplement suppresses the accompanying bacterial flora. *C.albicans* appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to metallic blue coloured raised colonies. *C.glabrata, C.kefyr, C.parapsilosis* colonies appear as cream to white, beige/yellow due to natural pigmentation and some alkaline phosphatase activity, while *C.krusei* appear as pink-purple, fuzzy, dry colonies.

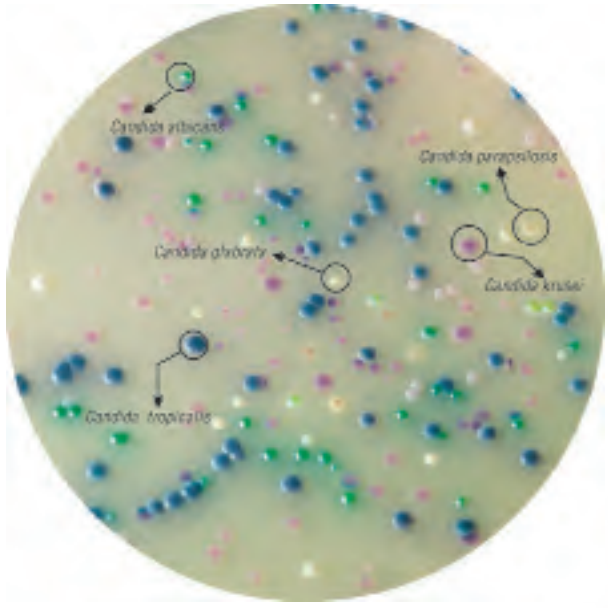
Quality Control

Appearance of Powder	:	Cream to beige homogeneous free flowing powder
Gelling	:	Firm, comparable with 1.36% Agar gel
Colour and Clarity of prepared medium	:	Light amber coloured, opaque gel forms in Petri plates
Reaction	:	Reaction of 3.12% w/v aqueous solution at 25°C. pH : 6.0±0.2
Cultural Response	:	Cultural characteristics observed with HiCrome™ Candida Differential Selective Supplement (FD283R) after an incubation at 20-25°C for 40-48 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans (10231)	50-100	good-luxuriant		>=50% light green
Candida krusei (24408)	50-100	good-luxuriant		>=50% Purple, fuzzy
Candida tropicalis (750)	50-100	good-luxuriant		>=50% Blue to purple
Candida kefyr (66028)	50-100	good-luxuriant		>=50% Cream to white
Candida parapsilosis (22019)	50-100	good-luxuriant		>=50% Cream to white
Candida glabrata (15126)	50-100	good-luxuriant		>=50% Cream to white
Escherichia coli (25922)	>=10^3	inhibited	0%	
Escherichia coli (8739)	>=10^3	inhibited	0%	

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared



M1297AR – HiCrome™ Candida Differential Agar

For Identification of Malassezia

HiCrome Malassezia Agar (Twin Pack)

For isolation, cultivation and identification of *Malassezia furfur*

M1985

Composition **

Ingredients	Grams/Litre
Part A -	
Peptone special	30.000
Chromogenic mixture	1.400
Agar	15.000
Part B -	
Tween 40	10.000
Glycerol mono-oleate	5.000
Fatty acids	10.000

Final pH (at 25°C) 5.80±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25ml of fluid Part B in 1000 ml distilled/purified water. Add 46.4 grams of Part A. Mix well and heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates .

Principle and Interpretation

Malassezia is a genus of fungi, naturally found on the skin surfaces of many animals, including humans. Media based on malt extract is appreciated by many microbiologists due to their richness and nutrient balance especially for the cultivation of fastidious microorganisms. With acidic pH, they are used for the isolation, cultivation and maintenance of yeast and moulds.

M. furfur is a lipophilic yeast, therefore in vitro growth must be stimulated by natural oils or other fatty substances. Peptone special provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Low pH favours fungal growth and inhibits contaminating bacteria from test samples (1).Tween 40, Glycerol monooleate and fatty acids enhances the growth of *Malessezia* species as it is a lipophilic yeast Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.

Quality Control

Appearance of Powder : Part A : Cream to yellow homogeneous free flowing powder Part B: Colourless to pale yellow viscous solution

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Yellow coloured, opalescent gel with scum forms in Petri plates

Reaction : Reaction of 4.64% w/v aqueous solution of Part A and 2.5% v/v of Part B at 25°C. pH : 5.80±0.2

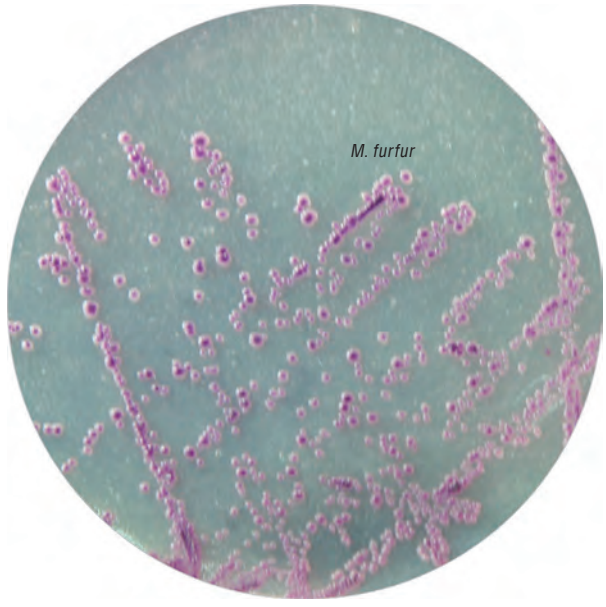
Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 48-72 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Malassezia furfur (14521)	50-100	good-luxuriant		>=50% mauve, small
Candida albicans (10231)	50-100	good-luxuriant		>=50% pale green to green
Candida glabrata (15126)	50-100	good-luxuriant		>=50% colourles
Candida krusei (24408)	50-100	good-luxuriant		>=50% purple
Candida tropicalis (750)	50-100	good-luxuriant		>=50% metallic blue

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

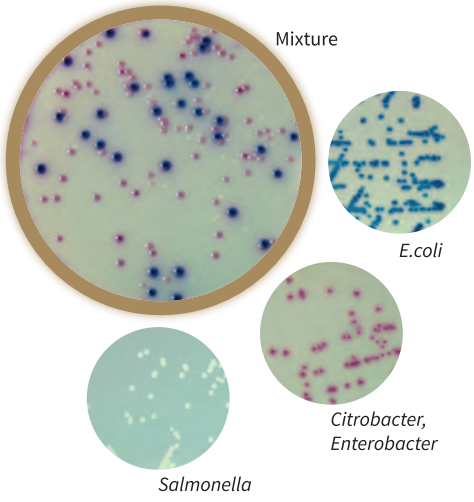


M1985 HiCrome Malassezia Agar

2015 INNOVATION IN MICROBIOLOGY MEDIA

Rapid & Reliable Method of Detection

M1991I - HiCrome™ Chromogenic Coliform Agar

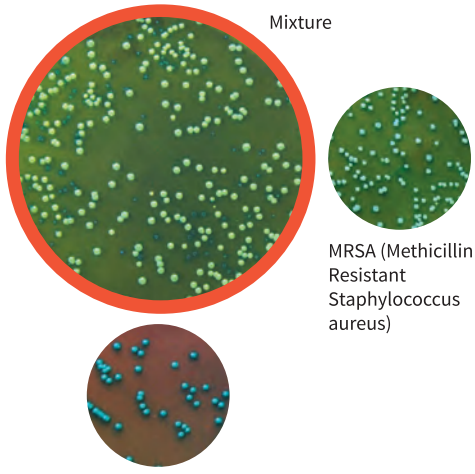


HiCrome™ CHROMOGENIC COLIFORM AGAR (M1991I)

- » Recommended by ISO 9308 for water testing
- » Recommended for simultaneous detection of E.coli and coliforms in water testing
- » Distinct colours allows easy differentiation
  - E.coli : dark blue to violet
  - Other Enterobacteriaceae : pink-pink-red or colourless
  - Gram positive bacteria : inhibited

Also available in Chemically Defined Media : MCD1991I

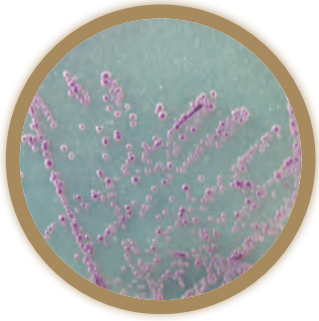
M1974 - HiCrome™ Rapid MRSA Agar



HiCrome™ RAPID MRSA AGAR BASE (M1974)

- » Selective Detection of Methicillin Resistant Staphylococcus
- » Inhibits Methicillin Sensitive Staphylococcus and other gram negative bacteria
- » Differentiates between Methicillin Resistant Staphylococcus aureus (greenish yellow) and Methicillin Resistant Staphylococcus epidermidis (blue)

M1985 - HiCrome™ Malessezia Agar



HiCrome™ MALESSEZIA AGAR (M1985)

- » Selective media for the detection of Malessezia
- » Can be isolated from clinical and veterinary samples

HiCrome™ Fluorogenic Media

HiCrome™ PA Broth

HiCrome™ PA Broth is recommended for the detection of presence and absence of coliform bacteria in water.

M1663

Composition **

Ingredients	Grams/Litre
Casein enzymic hydrolysate	20.000
Lactose	5.000
Bile salts mixture	1.500
Dipotassium hydrogen phosphate	3.000
Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000
2-Nitrophenyl β-D-galactopyranoside (ONPG)	1.250
4-methylumbelliferyl β-D-glucuronide (MUG)	0.100

Final pH (at 25°C) 7.0±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.35 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Dispense into sterile test tubes or as desired.

Principle and Interpretation

Examination of water for the presence of marker groups such as coliforms is one of the most common tests in food microbiology laboratory, partly because of the relative ease and speed with which these tests can be accomplished. Where it is claimed that water has been processed for safety, the finding of such organism demonstrates a failure of the process (1) HiCrome™ PA Broth is a modification of the medium originally devised by Hajna and Perry (4) and is used for the detection of presence and absence of coliform bacteria in water.

The fluorogenic compound 4-Methylumbelliferyl β-D-glucuronide (MUG) is incorporated in the medium for the fluorogenic detection of *Escherichia coli*, the main indicator organism for the faecal contamination of water. The enzyme β-glucuronidase possessed by *Escherichia coli* hydrolyses MUG to yield a fluorescent end product 4-Methylumbelliferone; which can be detected when the medium is observed for fluorescence under UV light (3,7) MUG also detects anaerogenic strains which may not be detected in the conventional procedure (3). ONPG test is used to determine the presence or absence of β-galactosidase in organisms (6) and is also important in differentiating *Enterobacteriaceae* which are commonly classified according to their ability to ferment lactose. ONPG is similar in structure to lactose. The presence of two enzymes, permease and β-D-galactosidase are required to demonstrate lactose fermentation. True lactose non fermenters do not possess either of these enzymes. Late lactose fermenting organisms do not have permease but do possess β-galactosidase. If β-galactosidase is present, the colourless ONPG is split into galactose and o-nitrophenol, a yellow compound (5).

Casein enzymic hydrolysate provides essential nutrients. Lactose is the fermentable carbohydrate, sodium choride maintains osmotic equilibrium. The medium has a strong buffering system to control the pH in the presence of fermentative action. Bile salts mixture inhibit gram-positive bacteria especially *Bacillus* species and faecal Streptococci. Mostly β-glucuronidase activity occurs within 4 hours but some weakly β-glucuronidase positive strains require overnight

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium : Light amber coloured, clear solution without any precipitate

Reaction : Reaction of 3.7% w/v aqueous solution at 25°C. pH : 7.0±0.2

Cultural Response : Cultural characteristics observed after incubation at 35-37°C for 18-an 24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	ONPG	Fluorescence at 366nm
Escherichia coli (25922)	50-100	luxuriant		positive reaction,
positive, throughout			yellow colour	the tube
Enterobacter aerogenes	50-100	luxuriant		positive reaction
negative (13048)			yellow colour	
Klebsiella pneumoniae	50-100	luxuriant		positive reaction
negative (13883)			yellow colour	
Proteus mirabilis	50-100	luxuriant		negative reaction
negative (25933)			no yellow colour or colourless	
Salmonella Typhimurium		50-100	luxuriant	negative reaction
negative (14028)			no yellow colour or colourless	
Staphylococcus aureus	>=10^3	inhibited		negative reaction,
negative (25923)			no yellow colour or colourless	
Enterococcus faecalis	>=10^3	inhibited		negative reaction,
negative (29212)			no yellow colour or colourless	

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Corry J. E. L., Curtis G. D. W., and Baird R. M., Culture Media For Food Microbiology, Vol. 34, Progress in industrial Microbiology, 1995, Elsevier, Amsterdam.
2. Eaton A. D., Clesceri L. S. and Greenberg A. W.,(Eds.), 2005, Standard Methods for the

M1663 — HiCrome™ PA Broth
1. Control 2. *Escherichia coli* 3. *Enterobacter aerogenes*
4. *Klebsiella pneumoniae* 5. *Salmonella* Typhimurium 6. *Proteus mirabilis*
7. *Staphylococcus aureus* 8. *Enterococcus faecalis*

HiColiform™ Broth, Modified

HiColiform™ Broth, Modified is used for the detection and confirmation of *Escherichia coli* and total coliforms from water samples, using a combination of chromogenic and fluorogenic substrates.

M1850

Ingredients	Grams/Litre
Peptone	5.000
Sodium chloride	5.000
Potassium sulfate	1.000
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate	1.000
Sodium lauryl sulphate	0.100
Sodium puruvate	1.000
Chromogenic substrate	0.100
Fluorogenic substrate	0.100
IPTG	0.100

Final pH (at 25°C) 6.8±0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17.4 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Mix well and Dispense in tubes or flasks or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle and Interpretation

HiColiform™ Broth, Modified was designed for detection and confirmation of *Escherichia coli* and total coliforms from water samples using a combination of chromogenic and fluorogenic substrates. *Escherichia coli* can be distinguished from other coliforms by its unique ability to fluoresce in the presence of fluorogenic substrate (1, 2). The fluorogenic substrate is split by enzyme β-glucuronidase especially present in *Escherichia coli*. The reaction is indicated by the development of a blue fluorescence under UV light. The presence of total coliforms is indicated by blue-green colourations due to the cleavage of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of β -galactosidase.

Peptone provides essential growth nutrients and is useful for the simultaneous detection of indole production. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium chloride helps to maintain the osmotic balance. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium : Light yellow coloured, clear to slightly opalescent solution in tubes

Reaction : Reaction of 1.74% w/v aqueous solution at 25°C. pH : 6.8±0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

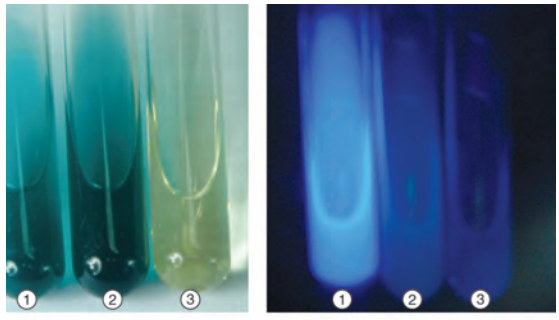
Organism (ATCC)	Inoculum (CFU)	Growth	Colour of medium	Fluorescence (under uv)
Enterobacter aerogenes (13048) negative reaction	50-100	luxuriant	blue-green	
Escherichia coli (25922)	50-100	luxuriant	blue-green	positive reaction

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References

1. Feng P.C.S. and Hartman P.A. ,1982, J.Appl. Environmental Microbiol. 43. 1320-1323.



) – HiColiform™ Broth, Modified
erichia coli 2. Enterobacter aerogenes 3. Control

Rapid HiColiform™ Agar / Broth

For detection and confirmation of *Escherichia coli* and total coliforms on the basis of enzyme substrate reaction from water samples, using a combination of chromogenic and fluorogenic substrates.

M1465 / M1453

Composition **	M1465	M1453
Ingredients	**Grams/Litre**	**Grams/Litre**
Peptone, special	5.00	5.00
Sodium chloride	5.00	5.00
Sorbitol	1.00	1.00
Dipotassium hydrogen phosphate	2.70	2.70
Potassium dihydrogen phosphate	2.00	2.00
Sodium lauryl sulphate	0.10	0.10
Chromogenic substrate	0.08	0.08
Fluorogenic substrate	0.05	0.05
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.10	0.10
Agar	15.00	—

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.03 grams of M1465 and 16.03 grams of M1453 in 1000 ml distilled water. For double strength broth use 32.06 grams of M1453 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and dispense as desired. (for M1453) or pour into sterile Petri plates (for M1465)

Principle and Interpretation

The Rapid HiColiform™ Agar is modification of LMX Broth described by Manafi and Kneifel (2). These media are useful for the detection and confirmation of *Escherichia coli* and total coliforms in water samples on the basis of chromogenic and fluorogenic substrates (1-6).

Peptone special, which is rich in tryptophan provides essential growth nutrients and is useful for the simultaneous detection of indole production. Sorbitol provides the carbon source. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms. The fluorogenic substrate is split by enzyme β-D-glucuronidase, which is specifically found in *E. coli*. The reaction is indicated by a blue fluorescence under UV light. The presence of total coliforms is indicated by a blue-green colour of the broth due to cleavage of chromogenic substrate. IPTG, a highly stable synthetic analog of lactose induces synthesis of β-D-glucuronidase. In agar medium, 2-3 drops of Kovac's reagent is added over the suspected colonies. Change in the colour of colony to red confirms *E. coli*. Broth medium is overlayed with Kovac's reagent and formation of red ring confirms *E. coli*. If fluorescence is negative after 24 hours of incubation, continue incubation for another 24 hours without performing the indole test.

Quality Control

Appearance of powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel of M1465.

Colour and Clarity of prepared medium (M1465) / : Light yellow coloured, clear to slightly opalescent gel forms in Petri plates

clear solution having slight precipitate in tubes (M1453).

Reaction : Reaction of 3.1% w/v of M1465 or 1.6 % w/v of M1453 aqueous solution at 25°C. pH:6.8 ± 0.2

Cultural Response : M1453 : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour change in medium	Fluorescence (Under UV light)	Indole reaction
Escherichia coli (25922)	50-100	luxuriant	blue-green	+	+
Enterobacter aerogenes (13048)	50-100	luxuriant	blue-green	-	-

M1465 : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

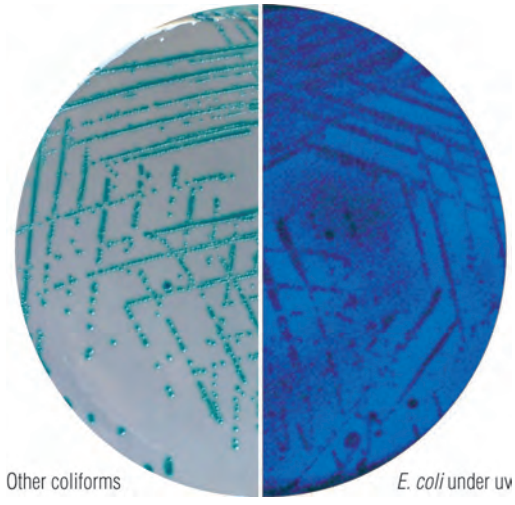
Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of colony	Fluorescence (Under UV light)	Indole reaction
Escherichia coli (25922)	50-100	luxuriant	blue-green	+	+
Enterobacter aerogenes (13048)	50-100	luxuriant	blue-green	-	-
Klebsiella pneumoniae (13883)	50-100	luxuriant	blue-green	-	-
Salmonella Typhimurium (14028)	50-100	luxuriant	yellow	-	-

Key : + : positive reaction, - : negative reaction.

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

References



M1465 Rapid HiColiform™ Agar

HiCrome™ ECD Agar w/ MUG

For the detection of *Escherichia coli* in water and food samples by using a combination of chromogenic and fluorogenic substrate.

M1488

Ingredients	Grams/Litre
Casein enzymic hydrolysate	20.00
Bile salts mixture	1.50
L-Tryptophan	1.00
Lactose	5.00
Sodium chloride	5.00
Dipotassium hydrogen phosphate	4.00
Potassium dihydrogen phosphate	1.50
Fluorogenic substrate	0.07
Chromogenic substrate	0.10
Agar	15.00

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 53.17 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ ECD Agar w/ MUG is recommended for rapid detection of *Escherichia coli* by using a combination of chromogenic and fluorogenic substrates. The presence of *Escherichia coli* is indicated by blue coloured colony formation due to cleavage of chromogenic substrate. Fluorogenic substrate permits rapid detection of *Escherichia coli* when medium is observed for fluorescence using UV light (1, 2). Fluorogenic substrate also detects anaerogenic strains, which may not be detected in conventional procedure (1). It is hydrolysed by enzyme β-D-glucuronidase, possessed by *Escherichia coli* to yield a fluorescent end product. The reaction is indicated by a blue fluorescence under UV light.

Casein enzymic hydrolysate provides essential nutrients. Lactose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. The medium has a strong buffering system to control the pH in the presence of fermentive action. The bile salt mixture inhibits gram-positive bacteria especially *Bacillus species* and *faecal Streptococci.*

Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Light amber coloured, clear gel forms in Petri plates.

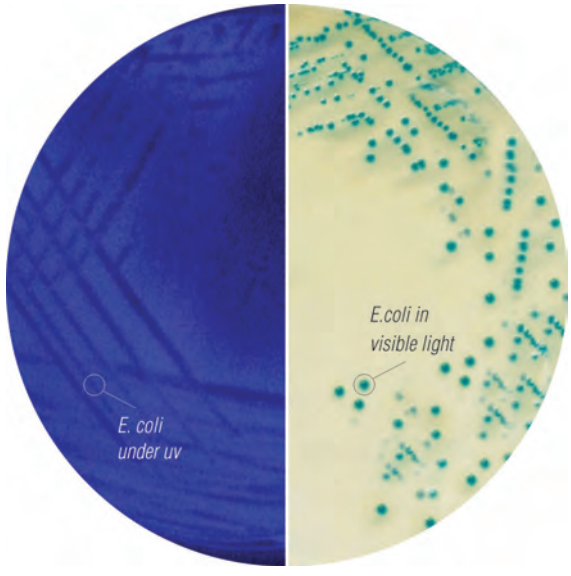
Reaction : Reaction of 5.32% w/v aqueous solution at 25°C. pH : 7.0 ± 0.2

Cultural Response : Cultural characteristics observed after an incubation at 44-45°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Cololur of colony	Fluorescence under UV	Indole
Escherichia coli (25922)	50-100	good	40-50%	bluish green	+	+
Klebsiella pneumoniae (13883)	50-100	good	40-50%	colourless	-	-
Staphylococcus aureus (25923)	>=10^3	inhibited		0%	-	-
Enterococcus faecalis (29212)	>=10^3	inhibited		0%	-	-
Pseudomonas aeruginosa (27853)	50-100	good	40-50%	colourless	-	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.



M1488 HiCrome ECD Agar w/ MUG

E. coli

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

HiCrome™ ECD Agar w/ MUG (M1488) is also available as HiCrome™ ECD HiVeg™ Agar w/ MUG (MV1488) wherein all the animal origin nutrients have been replaced by vegetable base dnutrients.

HIMEDIA®

HiFluoro™ Pseudomonas Agar Base

For selective isolation of *Pseudomonas aeruginosa* from clinical and non-clinical specimens by fluorogenic method.

M1469

Composition **

Ingredients	Grams/Litre
Gelatin peptone	18.00
Magnesium chloride	1.40
Potassium sulphate	10.00
Cetrimide	0.30
Fluorogenic mixture	2.05
Agar	15.00

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.75 grams in 1000 ml distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Pseudomonas aeruginosa (also known as *Pseudomonas pyocyanea*) is a gram-negative, aerobic, rod-shaped bacterium. Like other Pseudomonads, *P. aeruginosa secretes* a variety of pigments, including pyocyanin (blue-green), fluorescein (yellow-green and fluorescent), and pyorubin (red-brown). King et al developed Pseudomonas Agar P (i.e. King A media) for enhancing pyocyanin and pyorubin production and Pseudomonas Agar F (i.e. King B media) for enhancing fluorescein production (1). HiFluoro Pseudomonas Agar Base is devised based on the formula described by King et al. (1) except fluorogenic mixture. It is used as the selective medium for the isolation of *P. aeruginosa* from pus, sputum and drains etc.

Cetrimide (Cetyltrimethylammonium bromide) is incorporated in the medium to inhibit bacteria other than *P. aeruginosa.* It acts as a quaternary ammonium compound, cationic detergent that causes nitrogen and phosphorus to be released from bacterial cells other than *P. aeruginosa. P. aeruginosa* cleaves the fluorogenic compound to release the fluorogen which produces a visible fluorescence under long wave UV light.

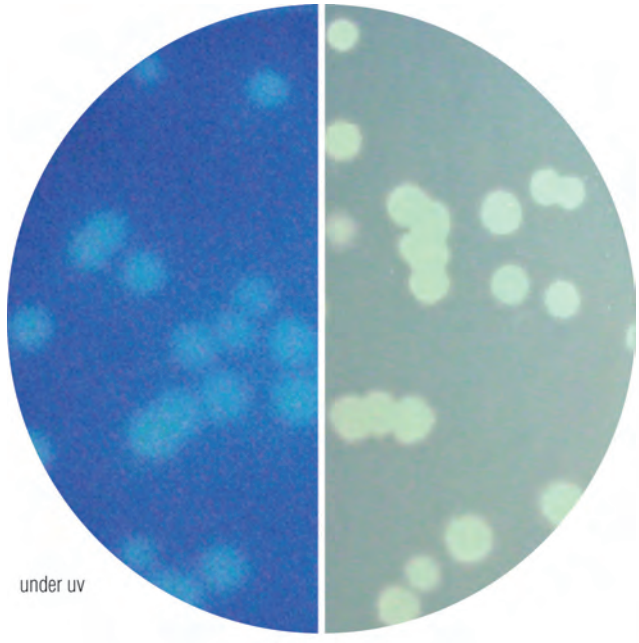
Quality Control

Appearance of powder : Cream to yellow coloured, homogeneous, free flowing powder.
Gelling : Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium : Light amber coloured, opalescent gel with slight precipitate forms in Petri plates.
Reaction : Reaction of 4.67% w/v aqueous solution at 25°C. pH:7.2 ± 0.2
Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Fluorescence
Pseudomonas aeruginosa (27853) +	50-100	good-luxuriant		>=50%
Escherichia coli (25922)	>=10^3	inhibited	0%	-
Stenotrophomonas maltophila (13637) -	>=10^3	inhibited	0%	
Staphylococcus aureus (25923)	>=10^3	inhibited	0%	-

Storage and Shelf-life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.



M1469 HiFluoro™ Pseudomonas Agar Base

® HiFluoro™ Pseudomonas Agar Base (M1469) is also available as HiFluoro™ Pseudomonas HiVeg™ Agar Base (MV1469) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

Supplement

Bacillus Selective Supplement — FD324

An antibiotic supplement recommended for the selective isolation of *Bacillus* species.

Formula

(Per vial sufficient for 1000 ml medium)

Polymyxin sulphate	10 mg
Bacitracin	10 mg

Directions

Rehydrate the contents of one vial aseptically with 10 ml sterile distilled water. Mix well and aseptically add it to 1000 ml sterile, molten HiCrome™ Bacillus Agar (M1651) / HiCrome™ Bacillus HiCynth™ Agar Base (MCD1651). Mix well and pour into sterile petri plates / tubes

Cefoxitin Supplement — FD259

An antimicrobial supplement recommended for the selective isolation of Methicillin Resistant *Staphylococcus aureus* from clinical specimens.

Formula

(Per vial sufficient for 500 ml medium)

Cefoxitin	3.000 mg

Directions

Rehydrate the contents of 1 vial with 5 ml of sterile distilled water and aseptically add to 500 ml of sterile molten cooled (45-50°C) MeReSa Agar Base (M1594)/ HiCrome™ MRSA Agar Base, Modified (M1953). HiCrome™ MeReSa Agar, Base (M1674)/ HiCrome™ MeReSa HiVeg™ Agar Base (MV1674). This supplement can either be used individually or in combination with (FD229) MeReSa Selective Supplement for more selectivity. Mix well and pour into sterile Petri plates.

Chromogenic Supplement — FD270

Chromogenic Supplement is recommended for the enumeration of faecal coliform by membrane filter technique.

Formula

(Per vial sufficient for 1000 ml medium)

Chromogenic Substrate	0.100g

Directions

Rehydrate the contents of one vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add it to 1000 ml of sterile, molten, cooled (45-50°C) MFC Basal Medium (M1812). Mix well and pour into sterile Petri plates.

Egg Yolk Tellurite Emulsion (50 ml/100 ml per vial) — FD046

Sterile stabilized tellurite emulsion of egg yolk recommended for identification of *Staphylococcus species.*

Formula

	(100ml per vial)	(50ml per vial)
Egg yolk	30.000 ml	15.000 ml
Sterile saline	64.000 ml	32.000 ml
Sterile 3.5% potassium tellurite solution	6.000 ml	3.000 ml

Directions

Warm up the refrigerated Egg Yolk Tellurite Emulsion to room temperature. Shake well to attain uniform emulsion (since on refrigeration emulsion has a tendency to form layers or small lumps). Aseptically add 50 ml in 950 ml of sterile, molten, cooled (45-50°C) Baird Parker Agar Base (M043 / M043S) / Baird Parker HiVeg™ Agar Base (MV043) / Baird Parker Agar Base w/ Sulpha (M1140) / HiCrome™ Aureus Agar Base (M1468). Mix well and pour into sterile Petri plates.

Enterococcus faecium Selective Supplement — FD226

Recommended to differentiate *Enterococcus faecium* from *Enterococcus faecalis.*

Formula

(Per vial sufficient for 500 ml medium)

Cephalexin	25.000 mg
Aztreonam	37.500 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500 ml of sterile, molten, cooled (45-50°C) Arabinose Agar Base (M1576) / HiCrome™ Enterococcus faecium Agar Base (M1580) / HiCrome™ Enterococccus faecium HiVeg™ Agar Base (MV1580). Mix well and pour into sterile Petri plates.

Supplement

HiCrome™ Candida Differential Selective Supplement — FD283R

An antibiotic supplement recommended for rapid and direct isolation and identification of *Candida species* from mixed cultures.

Formula

(per vial, sufficient for 500 ml medium)

Chloramphenicol	250.00 mg

Directions

Rehydrate the contents of 1 vial aseptically with 2 ml of 95% ethanol. Mix well and aseptically add to 500 ml of sterile, molten cooled (45-50°C) HiCrome™ Candida Differential Agar Base (M1297AR). Mix well and pour into sterile Petri plates.

HiCrome™ Candida Selective Supplement — FD192

An antibiotic supplement recommended for the selective isolation of *Candida species* from mixed cultures.

Formula

(Per vial sufficient for 500 ml medium)

Gentamicin	50.000 mg

Directions

Rehydrate the content of 1 vial with 5 ml of sterile distilled water. Mix well and aseptically add to 500 ml of sterile, molten, cooled (45-50°C) HiCrome™ Candida Differential Agar Base, Modified (M1456A) / HiCrome™ Candida Differential HiVeg™ Agar Base, Modified (MV1456A). Mix well and pour into sterile Petri plates.

HiCrome™ ECC Selective Supplement — FD190

An antibiotic supplement recommended for the selective isolation of *Escherichia coli* and coliforms from water and food samples.

Formula

(per vial sufficient for 1000 ml medium)

Cefsulodin	10.000 mg

Directions

Rehydrate the contents of one vial aseptically with 2 ml sterile distilled water. Mix well and aseptically add to 1000 ml of sterile, molten, cooled (45-50°C) HiCrome™ ECC Selective Agar Base (M1294) / HiCrome™ ECC Selective Agar Base, HiVeg™ (MV1294). Mix well and pour into sterile Petri plates.

HiCrome™ EC 0157:H7 Selective Supplement — FD187

Recommended for selective isolation and easy detection of *Escherichia coli* O157:H7 from food samples.

Formula

(per vial sufficient for 1000 ml medium)

Novobiocin	10.000 mg
Potassium tellurite	1.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 10 ml of sterile distilled water. Mix well and add aseptically to 990 ml sterile, molten, cooled (45-50°C) HiCrome™ EC O157:H7 Selective Agar Base (M1575) / HiCrome™ EC O157:H7 Selective HiVeg™ Agar Base (MV1575). Mix well and pour into sterile Petri plates.

HiCrome™ EC 0157: H7 Selective Supplement I — FD230

An antimicrobial supplement recommended for isolation and easy detection of Escherichia coli O157:H7 from food and environmental samples.

Formula

(Per vial sufficient for 500 ml medium)

Novobiocin	15.000 mg
Potassium tellurite	1.500mg
Distilled water	5.000 ml

Directions

Warm up refrigerated contents to 45-50°C and aseptically add one vial to 495 ml of sterile, cooled (45-50°C) HiCrome™ Enrichment Broth Base for EC O157:H7 (M1598). Mix well and dispense in sterile test tubes.

Supplement

HiCrome™ EC O157:H7 Selective Supplement, Modified — FD295

A selective supplement recommended for presumptive enumeration of *Escherichia coli* O157:H7 by membrane filtration technique.

Formula

(per vial, sufficient for 1000 ml medium)

Monensin	0.038 gm
Novobiocin	0.0075 gm

Directions

Rehydrate the contents of one vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add it to 1000 ml of sterile, molten cooled (45-50°C) HiCrome™ Modified EC O157:H7 Selective Agar Base (M1862). Mix well and pour into sterile Petri plates.

HiCrome™ ESBL Agar Supplement — FD278

Recommended for the detection of Extended Spectrum β-Lactamase (ESBL) producing organisms.

Formula

(per vial, sufficient for 500 ml medium)

Ceftazidime	1.500 mg
Cefotaxime	1.500 m
Ceftriazone	1.000 mg
Aztreonam	1.000 mg
Fluconazole	5.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500ml of sterile, molten, cooled (45-50°C) HiCrome™ ESBL Agar (M1829). Mix well and pour into sterile Petri plates.

HiCrome™ KPC Agar Supplement — FD279

HiCrome™ KPC Agar Supplement is recommended for the detection of Carbapenem resistant gram negative bacteria.

Formula

(Per vial sufficient for 500 ml medium)

Selective Mix	0.200 g

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500ml of sterile, molten, cooled (45-50°C) HiCrome™ KPC Agar Base (M1831). Mix well and pour into sterile Petri plates.

HiCrome™ Listeria Selective Supplement — FD181

An antimicrobial supplement recommended for rapid and direct identification of *Listeria species.*

Formula

(per vial sufficient for 500 ml medium)

Ceftazidime	2.000 mg
Amphotericin B	2.500 mg

Directions

Rehydrate the contents of 1 vial with 5 ml of sterile distilled water. Mix well and aseptically add to 500 ml sterile, molten, cooled (45-50°C) HiCrome™ Listeria Agar Base, Modified (M1417/SM1417) / HiCrome™ Listeria Agar Base (M1417F) / HiCrome™ L. mono Rapid Differential Agar Base (M1924). Mix well and pour into sterile Petri plates.

Supplement

HiCrome™ Nickels & Leesment Selective Supplement — FD245

An antibiotic supplement used for the selective isolation of *Leuconostoc species.*

Formula

(Per vial sufficient for 500 ml medium)

Vancomycin	100.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml of sterile distilled water. Mix well and aseptically add it to sterile, molten, cooled (45-50°C) HiCrome™ Nickels and Leesment Medium (M1712) / HiCrome™ Nickels and Leesment HiVeg™ Agar (MV1712). Mix well and pour into sterile Petri plates.

HiCrome™ Selective Salmonella Agar Supplement — FD274

For the selective isolation and differentiation of *Salmonella species* from coliforms by chromogenic method.

Formula

(Per vial sufficient for 1000 ml medium)

Novobiocin	10.000 mg
Cefsulodin	24.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5ml of sterile distilled water. Mix gently to dissolve the contents completely. Aseptically add the rehydrated contents to 1000 ml of sterile, cooled (45-50°C) HiCrome™ Selective Salmonella Agar Base (M1842). Mix well and pour into sterile Petri plates.

HiCrome™ Strep B Selective Supplement — FD273

An antibiotic supplement recommended for the selective isolation of Group B Streptococci from clinical samples.

Formula

(per vial, sufficient for 1000 ml medium)

Colistin	10.000 mg
Nalidixic Acid	10.000 mg
Gentamicin	2.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml of sterile distilled water. Mix well and aseptically add to 1000 ml of sterile, molten cooled (45-50°C) HiCrome™ Strep B Selective Agar Base (M1840). Mix well and pour into sterile Petri plates.

HiCrome™ VRE Agar Supplement — FD277

HiCrome™ VRE Agar Supplement is recommended for selective isolation of Vancomycin Resistant Enterococci (VRE).

Formula

(Per vial sufficient for 500 ml medium)

Vancomycin	4.000 mg
Fluconazole	5.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500ml of sterile, molten, cooled (45-50°C) HiCrome™ VRE Agar Base (M1830) / HiCrome™ VRE Agar Base, Modified (M1925). Mix well and pour into sterile Petri plates.

Klebsiella Selective Supplement — FD225

Recommended for the selective isolation and easy detection of *Klebsiella species* from water and other sources.

Formula

(Per vial sufficient for 500 ml medium)

Carbenicillin	25.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 2 ml of sterile distilled water. Mix well and aseptically add it to 500 ml of sterile, molten, cooled (45-50°C) HiCrome™ Klebsiella Selective Agar Base (M1573) / HiCrome™ Klebsiella Selective HiVeg™ Agar Base (MV1573). Mix well and pour into sterile Petri plates.

Supplement

L. mono Enrichment Supplement I — FD214

For selective differentiation of *Listeria monocytogenes* from other *Listeria* species, as per ISO Committee.

Formula

(Per vial sufficient for 500 ml medium)

L – phosphatidylinositol	1.000 g
Distilled water	25.000 ml

Directions

Thaw the contents of 1 vial of L. mono Enrichment Supplement I at room temperature. Aseptically add the sterile contents to 460 ml of sterile, molten, cooled (45-50°C) L. mono Differential Agar Base (M1540) / L. mono Differential HiVeg™ Agar Base (MV1540) alongwith sterile rehydrated contents of 1 vial each of L. mono Selective Supplement I (FD212) and L. mono Selective Supplement II (FD213) / or 470ml of HiCrome™ L. mono Rapid Differential Agar Base (M1924). Mix well and pour into sterile Petri plates.

L. mono Enrichment Supplement II — FD227

Recommended for selective differentiation of *Listeria monocytogenes* from other *Listeria* species.

Formula

(Per vial sufficient for 500 ml medium)

L-phosphatidylinositol	0.500 g
Distilled water	15.000 ml

Directions

Thaw the contents of 1 vial of L. mono Enrichment Supplement II at room temperature. Aseptically add the sterile contents of one vial to 470 ml of sterile, molten, cooled (45-50°C) L. mono Confirmatory Agar Base (M1552) / L. mono Confirmatory HiVeg™ Agar Base (MV1552) along with sterile rehydrated contents of one vial each of L. mono Selective Supplement I (FD212) and L. mono Selective Supplement II (FD213). Mix well and pour into sterile Petri plates.

L. mono Selective Supplement I — FD212

A selective supplement recommended by ISO Committee for the isolation of *Listeria species.*

Formula

(Per vial sufficient for 500 ml medium)

Polymyxin B sulphate	38350 IU

Directions

Rehydrate the contents of 1 vial aseptically with 10 ml sterile distilled water. Mix well and aseptically add it to 460 ml of sterile, molten, cooled (45-50°C) L. mono Differential Agar Base (M1540) / L. mono Differential HiVeg™ Agar Base (MV1540) along with sterile contents of one vial of L. mono Enrichment Supplement I (FD214) and sterile rehydrated contents of one vial of L. mono Selective Supplement II (FD213) or add in 470 ml of sterile, molten, cooled (45-50°C) L. mono Confirmatory Agar Base (M1552) / L. mono Confirmatory HiVeg™ Agar Base (MV1552) along with sterile contents of one vial of L. mono Enrichment Supplement II (FD227) and rehydrated contents of one vial of L. mono Selective Supplement II (FD213). Mix well and pour into sterile Petri plates.

L. mono Selective Supplement II — FD213

A selective supplement recommended by ISO Committee for the isolation of *Listeria species.*

Formula

(Per vial sufficient for 500 ml medium)

Ceftazidime	10.000 mg
Amphotericin B	5.000 mg
Nalidixic acid, sodium salt	10.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 2 ml of 0.2 N Sodium hydroxide, further add 3 ml of sterile distilled water. Mix well and aseptically add it to 460 ml of sterile, molten, cooled (45-50°C) L. mono Differential Agar Base (M1540) / L. mono Differential HiVeg™ Agar Base (MV1540) along with sterile contents of 1 vial of L. mono Enrichment Supplement I (FD214) and sterile rehydrated contents of 1 vial of L. mono Selective Supplement I (FD212) or add in 470 ml of sterile, molten, cooled (45-50°C) L. mono confirmatory Agar Base (M1552) / L. mono Confirmatory HiVeg™ Agar Base (MV1552) along with sterile contents of 1vial of L. mono Enrichment Supplement II (FD227) and sterile rehydrated contents of 1vial of L. mono selective Supplement I (FD212). Mix well and pour into sterile Petri plates.

Supplement

M-CP Selective Supplement - I — FD153

An antibiotic supplement recommended by the Directive of the Council of the European Union 98/83/EC for the selective isolation of *Clostridium perfringens*

Formula

(per vial sufficient for 500 ml medium)

D-cycloserine	200.000 mg
Polymyxin B sulphate	12.500 mg

Directions

Rehydrate the contents of one vial aseptically with 5 ml of sterile distilled water. Mix well and aseptically add it to 485 ml sterile, molten, cooled (45-50°C) M-CP Agar Base (M1354) / M-CP HiVeg™ Agar Base (MV1354) along with one vial of M-CP Selective Supplement II (FD154) / M-CP Selective Supplement II, Modified (FD154A). Mix well and pour into sterile Petri plates.

M-CP Selective Supplement - II — FD154

Filter sterilized 0.5% solution of phenolphthalein diphosphate recommended by the Directive of the Council of the European Union 98/83/EC for the selective isolation of *Clostridium perfringens.*

Formula

(per vial sufficient for 500 ml medium)

Phenolphthalein diphosphate	0.050g
Distilled water	10.000 ml

Directions

Warm up the refrigerated 0.5% phenolphthalein diphosphate solution to room temperature and add aseptically 10 ml of solution to 485 ml sterile, molten, cooled (45-50°C) M-CP Agar Base (M1354) / M-CP HiVeg™ Agar Base (MV1354) alongwith rehydrated contents of one vial of M-CP Selective Supplement I (FD153). Mix well and pour into sterile Petri plates.

M-CP Selective Supplement - II , Modified — FD154A

This supplement is recommended for selective isolation of *Clostridium perfringens.*

Formula

(per vial sufficient for 500 ml medium)

Phenolphthalein diphosphate	0.050 g

Directions

Rehydrate the contents of 1 vial aseptically with 10 ml of sterile distilled water. Mix well. Add aseptically to 485 ml sterile, molten, cooled to 45-50°C M-CP Agar Base (M1354)/ M-CP HiVeg™ Agar Base (MV1354) along with rehydrated contents of one vial of M-CP Selective Supplement I (FD153). Mix well and pour into sterile Petri plates.

MDR Acinetobacter Selective Supplement — FD271

This antibiotic supplement is recommended for the selective isolation of MDR strains of *Acinetobacter species.*

Formula

(Per vial sufficient for 500 ml medium)

Ampicillin, sodium salt	5.000 mg
Ceftazidime	5.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5ml of sterile distilled water. Mix gently to dissolve the contents completely. Aseptically add the rehydrated contents to 500ml of sterile, molten cooled (45-50°C) Leeds Acinetobacter Agar Base (M1839) / HiCrome™ Acinetobacter Agar Base (M1938). Mix well and pour into sterile Petri plates.

MeReSa Selective Supplement — FD229

An antimicrobial supplement recommended for the selective isolation of Methicillin Resistant *Staphylococcus aureus* from clinical specimens.

Formula

(Per vial sufficient for 500 ml medium)

Methicillin	2.000 mg

Directions

Rehydrate the contents of 1 vial with 5 ml of sterile distilled water and aseptically add to 500 ml of sterile molten cooled (45-50°C) MeReSa Agar Base (M1594)/ HiCrome™ MeReSa Agar, Base (M1674)/ HiCrome™ MeReSa HiVeg Agar Base (MV1674) / HiCrome™ MRSA Agar Base Modified (M1953). This supplement either be used individually or in combination with (FD259) Cefoxitin Supplement. Mix well and pour into sterile Petri plates.

Supplement

Monensin Selective Supplement — FD309

Recommended for the selective and differential isolation of coliform bacteria using membrane filtration technique.

Formula

(per vial sufficient for 1000 ml medium)

Monensin	0.038 gm

Directions

Rehydrate the contents of one vial aseptically with 2 ml of methanol. Mix well and aseptically add to 1000 ml sterile cooled (45-50°C) HiCrome M-Coliform Differential Agar Base (M1951). Mix well and pour into sterile Petri plates.

MUG Supplement (50 mg per vial) — FD092

A fluorogenic substrate recommended for measuring β - glucuronidase activity, for rapid and sensitive identification of *Escherichia coli.*

Formula

(per vial sufficient for 500 ml / 1000 ml medium)

4-Methylumbeliferyl β-D-Glucuronide (MUG)	50.000 mg

Directions

Rehydrate the contents of one vial aseptically with 5 ml methanol. One vial is sufficient for 500 ml agar media or 1000 ml of broth media.

Oxytetra Selective Supplement — FD032

An antibiotic supplement recommended for the selective isolation and cultivation of yeasts and moulds.

Formula

(per vial, sufficient for 500 ml medium)

Oxytetracycline	50.000 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml of sterile distilled water. Mix well and aseptically add to 500 ml of sterile, molten, cooled (45-50°C) Oxytetra Glucose Yeast Extract Agar Base (M639 / M639I) / Oxytetra Glucose Yeast Extract Agar Base with Biotin (M1136) / HiCrome™ OGYE Agar Base (M1467). Mix well and pour into sterile Petri plates.

Polymyxin B Selective Supplement — FD003

An antibiotic supplement recommended for the selective isolation of various microorganisms

Formula

(per vial, sufficient for 500 ml/1000 ml medium)

Polymyxin B sulphate	50000 Units

Directions

Rehydrate the contents of one vial aseptically with 2 ml sterile distilled water. Mix well and aseptically add it to 475 ml of sterile, molten Bacillus Cereus Agar Base (M833) / Bacillus Cereus HiVeg™ Agar Base (MV833) / or to 450 ml of KG Agar Base (M658) / KG HiVeg™ Agar Base (MV658) / MYP Agar Base (M636/M636S) /MYP HiVeg™ Agar Base (MV636) / Modified MYP Agar Base (M1139) / Modified MYP HiVeg™ Agar Base (MV1139) along with 25 ml / 50 ml Egg Yolk Emulsion (FD045) to make a total volume of 500 ml or to 500 ml of SDS Agar (M1155) / SDS HiVeg™ Agar (MV1155) / Salt Polymyxin Broth Base (M821/M821I) / Salt Polymyxin HiVeg™ Broth Base (MV821) / HiCrome™ Staph Agar Base, Modified (M1837) / Soyabean Casein Digest Medium Base (M011F) or to 1000 ml of HiCrome™ Bacillus Agar (M1651). Mix well and pour into sterile Petri plates / tubes.

Supplement

Potassium Tellurite 1% — FD052

Recommended for the selective isolation of *Staphylococci* and *Corynebacteria.*

Formula

(to achieve 1% solution dilute the contents in 8.9 ml sterile distilled water)

Potassium tellurite concentrate	1.1 ml

Directions

Warm up the refrigerated contents of one vial to room temperature. Add aseptically 8.9 ml sterile distilled water, mix well and add in sterile, molten, cooled (45-50°C) Baird Parker Agar Base (M043B / MM043 / MU043 / ME043)/ Vogel Johnson Agar Base w/o Tellurite (M023 / MM023 / MU023) / Vogel Johnson HiVeg™ Agar Base w/o Tellurite (MV023) / Mycoplasma Broth Base w/ CV (M268) / Mycoplasma HiVeg™ Broth Base w/ CV (MV268) / TPEY Agar Base (M402)/ TPEY HiVeg™ Agar Base (MV402)/ Tellurite Glycine Agar Base (M448) / Cholera Medium Base (M558) / Cholera HiVeg™ Medium Base (MV558) / Giolitti-Cantoni Broth Base (M584I) /Dextrose Proteose Peptone Agar Base (M734) / Dextrose Proteose Peptone HiVeg™ Agar Base (MV734) / Cystine Tellurite Agar Base (M881) / Diphtheria Virulence Agar Base (M882) / Diphtheria Virulence HiVeg™ Agar Base (MV882) / Tryptone Tellurite Agar Base (M1056) / Baird Staphylococcus Enrichment Broth Base (M1091) / Tellurite Blood Agar Base (M1260) / Mitis Salivarius Agar Base (M259)/ Mitis Salivarius HiVeg™ Agar Base (MV259)/ Monsur Medium Base (M474) as desired. Mix well and dispense in sterile Petri plates or tubes.

Tellurite - Cefixime Supplement — FD147

A selective supplement recommended by ISO Committee for the isolation of *Escherichia coli 0157:H7* .

Formula

(Per vial sufficient for 500 ml medium)

Potassium tellurite	1.250 mg
Cefixime	0.025 mg

Directions

Rehydrate the contents of 1 vial aseptically with 5 ml of sterile distilled water. Mix gently to dissolve the contents completely. Aseptically add the contents to 495 ml of sterile, molten, cooled (45-50°C) MacConkey Sorbitol Agar Base (M298I) / HiCrome™ MacConkey Sorbitol Agar Base (M1340). Mix well and pour into sterile Petri plates.

Supplement

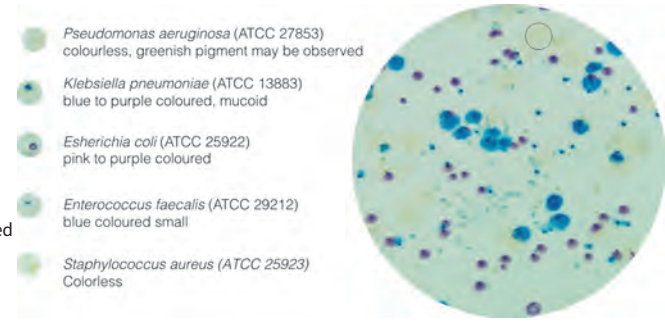
DT001 HiDtect™ UTI Identification Disc

For rapid detection and confirmation of microrganisms mainly causing urinary tract infections, For eg. *E.coli, Proteus, Klebsiella, Pseudomonas, Staphylococcus aureus* and *Enterococcus species.*

Appearance : White coloured sterile identification disc.

Cultural Response : identification observed within 1-4 hours on replication and incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Escherichia coli (25922)	Pink-purple
Staphylococcus aureus (25923)	colourless to green
Pseudomonas aeruginosa (27853)	colourless (greenish pigment is observed
Enterococcus faecalis (29212)	blue - blue green (small)
Klebsiella pneumoniae (13883)	blue to purple mucoid
Proteus mirabilis (12453)	light brown



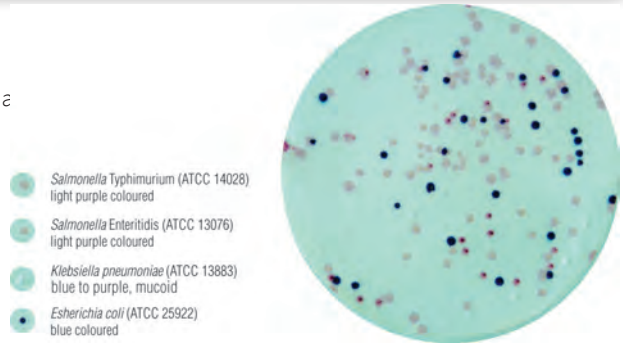
DT002 HiDtect™ Salmonella Identification Disc

For rapid detection of *Salmonella* species from coliforms

Appearance : White coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours on replication a incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Escherichia coli (25922)	blue - greenish blue
Salmonella Typhimurium (14028)	light purple
Salmonella Enteritidis (13076)	light purple
Klebsiella pneumoniae (13883)	blue to purple mucoid



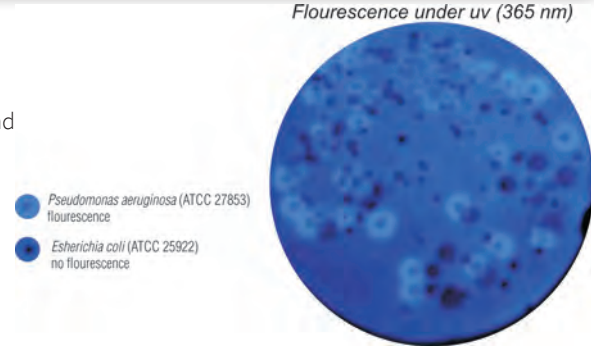
DT003 HiDtect™ Pseudomonas Identification Disc

For rapid detection of Pseudomonas aeruginosa from clinical and nonclinical specimen

Appearance : White coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours on replication and incubation at 35-37°C , when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony	Fluorescence
Escherichia coli (25922)	colourless	Negative
Pseudomonas aeruginosa (27853)	colourless	Positive
Enterococcus faecalis (29212)	colourless	Negative
Klebsiella pneumoniae (13883)	colourless, mucoid	Negative



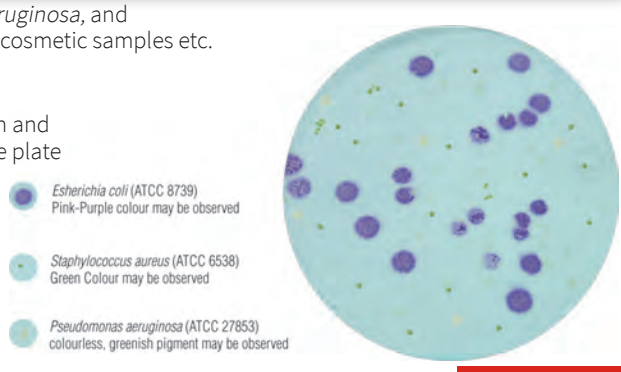
DT005 HiDtect™ Universal Microbial Limit Test Disc

For detection of pathogenic microrganisms such as *E.coli, S.aureus, P.aeruginosa,* and *Salmonella* species from pharmaceutical preparations, raw materials and cosmetic samples etc.

Appearance : White coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours on replication and incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Pseudomonas aeruginosa (9027)	colourless (greenish pigment is observed)
Escherichia coli (8739)	Pink-purple
Staphylococcus aureus (6538)	green to bluish green
Salmonella Typhimurium (14028)	colourless
Salmonella Abony (NCTC 6017)	colourless



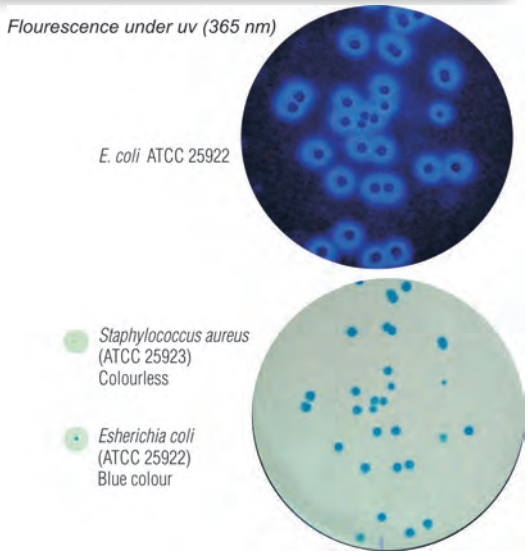
DT008 HiDtectTM Dual Confirmation of E. coli Identification Disc

For rapid detection and confirmation of *Escherichia coli* in water and food samples based on chromogenic and fluorogenic confirmation.

Appearance : White coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours on replication and incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony	Fluoroscence under uv light
Pseudomonas aeruginosa (27853)	colourless	negative
Escherichia coli (25922)	blue	positive
Staphylococcus aureus (25923)	colourless	negative
Salmonella Typhimurium (14028)	colourless	negative



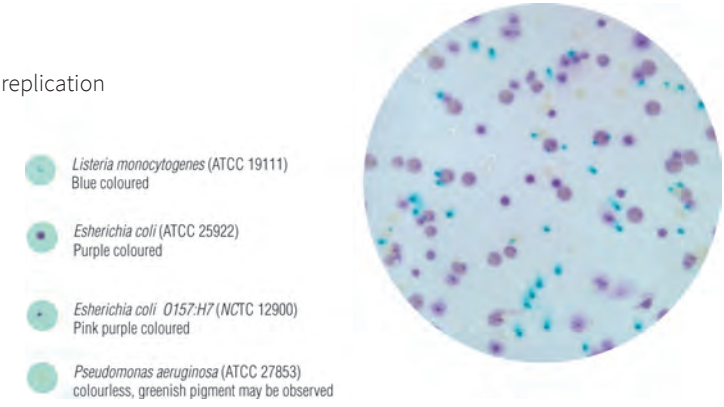
DT010 HiDtect™ Universal Food pathogen Identification Disc

For rapid detection of food pathogens such as *E.coli, E.coli O157:H7, Staphylococcus aureus, Salmonella, Listeria* and *Shigella* species etc. from various food, dairy, fish and meat products.

Appearance : Light pink coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours after replication and incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Escherichia coli (25922)	purple
Staphylococcus aureus (25923)	colourless - green
Salmonella Typhimurium (14028)	colourless
Listeria monocytogenes (19111)	blue - green
Escherichia coli O157:H7 (NCTC 12900)	purple - pink
Shigella flexneri (12022)	colourless



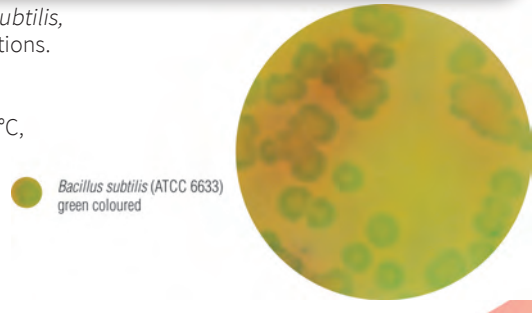
DT011 HiDtect™ Bacillus Identification Disc

For rapid detection and differentiation between various species of Bacillus such as *B.subtilis, B.cereus, B.thuringiensis*, from food, meat, fish, cosmetic and pharmaceutical preparations.

Appearance : Pale pink coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours after incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Bacillus cereus (10876)	light blue
Bacillus subtilis (6633)	yellowish green to green
Bacillus thuringiensis (10792)	light blue



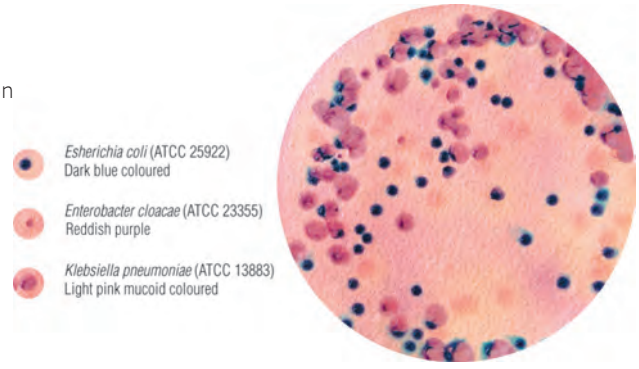
DT012 HiDtect™ Total Coliform Identification Disc

For qualitative detection of coliforms from water, pharmaceutical preparations, dairy and food products.

Appearance : Pink coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours after replication and incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Escherichia coli (25922)	dark blue
Enterobacter cloacae (23355)	reddish to purple
Citrobacter freundii (8090)	reddish to purple
Klebsiella pneumoniae (13883)	pink to purple



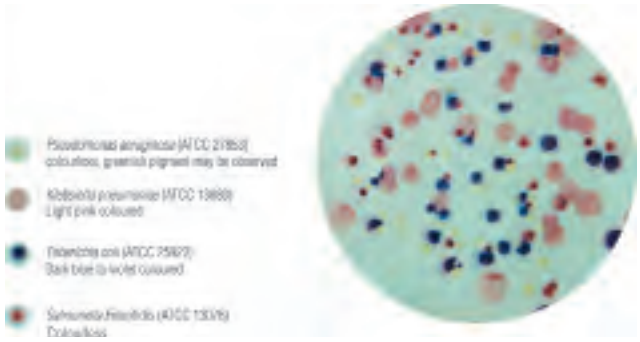
DT013 HiDtect™ Differential Coli-E. coli Identification Disc

For or rapid detection of *E.coli, Klebsiella, Pseudomonas and Salmonella species* in food, environmental and water samples.

Appearance : White coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours after replication and incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Escherichia coli (25922)	dark blue to violet
Klebsiella pneumoniae (13883)	light pink
Pseudomonas aeruginosa (27853)	colourless
Salmonella Enteritidis (13076)	colourless



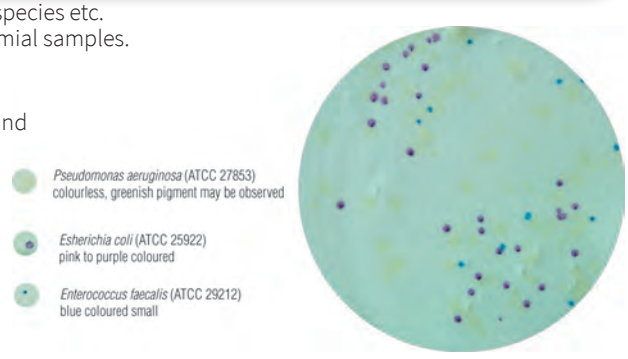
DT015 HiDtect™ Universal Enviro Identification Disc

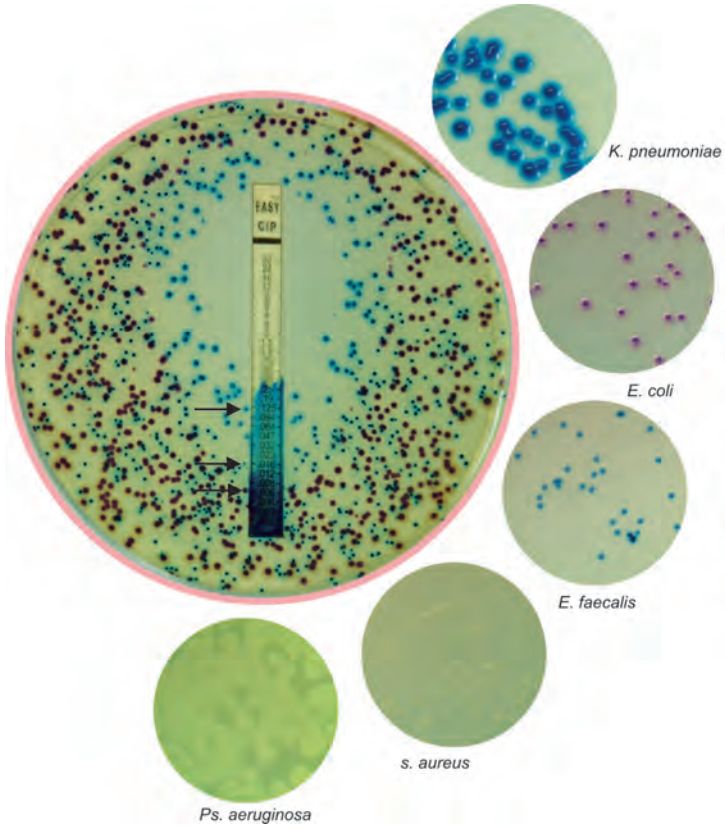
For rapid detection of *Pseudomonas, Enterococcus, E.coli* and *Salmonella* species etc. from food environmental samples, samples of clinical origin such as nosocomial samples.

Appearance : Light pink coloured sterile identification disc.

Cultural Response : Identification observed within 1-4 hours on replication and incubation at 35-37°C, when disc is placed on an 18 hour old grown culture plate of any general media.

Organisms (ATCC)	Colour of Colony
Escherichia coli (25922)	Pink-purple
Staphylococcus aureus (25923)	colourless - green
Pseudomonas aeruginosa (27853)	colourless (greenish pigment is observed)
Enterococcus faecalis (29212)	blue - blue green (small)
Salmonella Typhimurium (14028)	colourless





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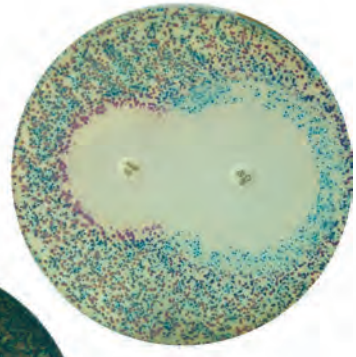
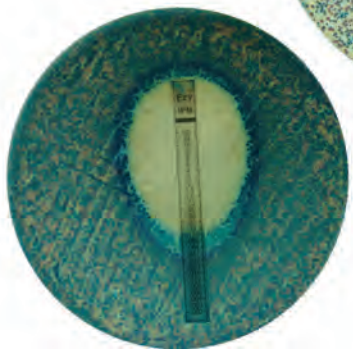
- Can be employed in clinical testing of urinary tract infection



Organism identification

- *Escherichia coli* – Pink to purple
- *Enterobacter* or *Klebsiella* – Metallic blue
- *Enterococcus faecalis* - Blue,
- *Staphylococcus aureus* – Colourless to Golden yellow
- *Pseudomonas aeruginosa* – Greenish pigment
- *Proteus* species – Brown colouration
- Other yeasts- Colourless



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- CORPORATE OFFICE -

A-516, Swastik Disha Business Park, Via Vadhani Indl Est, LBS Marg,
Mumbai - 400 086, India.
Tel : +91-22-6147 1919 / 2500 3747 | Fax : +91-22-6147 1920 / 2500 5764
Email : info@himedialabs.com

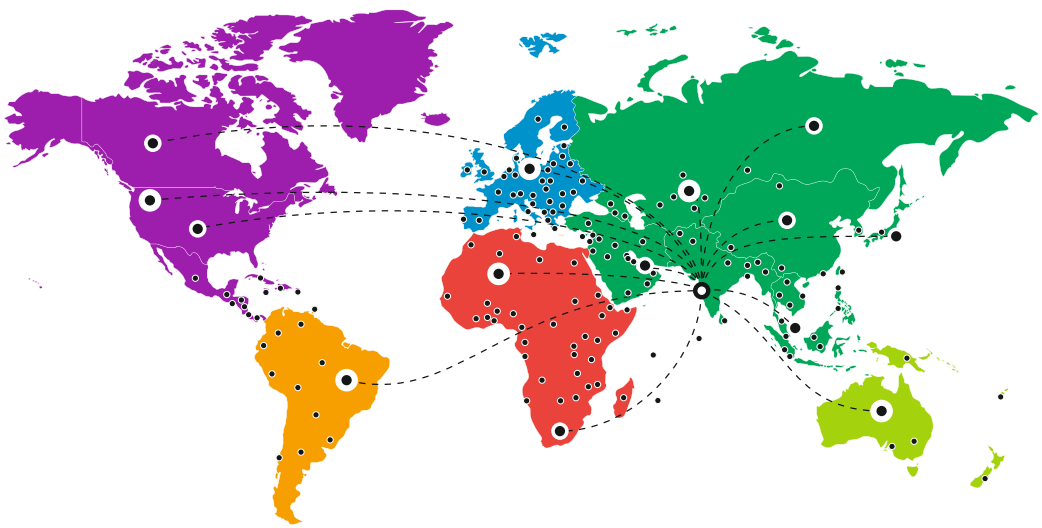
- OVERSEAS OFFICES -

USA & Canada

HiMedia Laboratories LLC, 107 W Dorothys Way, Lincoln University, West Chester,
Pennsylvania 19352, USA.
Tel : +1-484-734-4401 | Fax : +1-484-734-4402
Email : infous@himedialabs.com

Europe

HiMedia Laboratories GmbH, Marie-Curie-Str. 3, 64683,
Einhausen, Germany.
Tel : +49 6251 989 24 26 | Fax : +49 6251 989 24 27
Email : infoeu@himedialabs.com



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