

MBD-BT-SPEC-0512

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

HALF FRASER SELECTIVE SUPPLEMENT SR0166G

HALF FRASER SELECTIVE SUPPLEMENT

SR0166G

Formula

Vial contents (each vial is sufficient to supplement 2.25 litres of medium)

Ammonium iron (III) citrate	1.125 g
Nalidixic acid	22.5 mg
Acriflavine	28.125 mg

Description

A selective supplement for the isolation of *Listeria* spp.

Directions

Aseptically add 4ml 1:1 ethanol:sterile distilled water to 1 vial and invert gently to dissolve. Aseptically add the vial contents to 2.25 litres of sterile Fraser Broth Base (CM0895) prepared as directed and cooled to 50°C. Mix well and aseptically dispense into sterile containers.

Physical Characteristics

Orange/green pellet Sterility - passes test

Microbiological Tests Using Optimum Inoculum Dilution

Control Media: Chromogenic Listeria Agar (ISO), Tryptone Soya Agar or Columbia Blood Agar Base enriched with 5% v/v horse blood, where appropriate

Tested in Fraser Broth Base CM0895

Reactions after incubation at 30 ± 2°C for 24 ± 2 hours

Inoculate 10ml quantities of medium to achieve 1-10 colony-forming units/ml (cfu/ml) of *Listeria monocytogenes*. Incubate broths at $30 \pm 2^{\circ}$ C for 24 ± 2 hours. Subculture onto Chromogenic Listeria Agar (ISO) (CM1084, SR0226 & SR0228) and incubate plates at $37 \pm 2^{\circ}$ C for 24 ± 2 hours.

Listeria monocytogenes ATCC®7644 Listeria monocytogenes ATCC®13932

A satisfactory result is represented by recovery of positive strains equal to or greater than a 4 log(10) increase.



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Positive strains shall produce aesculin hydrolysis after 24 hours.

Reactions after incubation at 30 ± 2°C for 24 ± 2 hours

Inoculate 10ml quantities of medium to achieve >1E+03 cfu/ml. Incubate broths at $30 \pm 2^{\circ}$ C for 24 \pm 2 hours.

Bacillus cereus ATCC® 10876 No aesculin hydrolysis (no blackening)

Negative strains are inhibited or shall produce a negative diagnostic reaction.

Productivity determined by qualitative testing in accordance with the methods and criteria described in ISO 11133:2014

Inoculation with mixed cultures

Inoculate 10ml quantities of medium to achieve 1-10 colony-forming units/ml (cfu/ml) of *Listeria monocytogenes*, to each add 1E+02 to 1E+03 cfu/ml of *Escherichia coli* and 1E+02 to 1E+03 cfu/ml of *Enterococcus faecalis*. Incubate broths at $30 \pm 2^{\circ}$ C for 25 ± 1 hour. Subculture onto Chromogenic Listeria Agar (ISO) (CM1084, SR0226 & SR0228) and incubate plates at $37 \pm 2^{\circ}$ C for 24 ± 2 hours

Reactions after incubation at 30 ± 2°C for 25 ± 1 hour

Listeria monocytogenes + Escherichia coli	ATCC [®] 13932 ATCC [®] 8739	WDCM00012	•
+ Enterococcus faecalis	ATCC®19433	WDCM00009	
Listeria monocytogenes	ATCC®13932		0.5-1.0mm blue colonies with halo
+ Escherichia coli	ATCC®25922	WDCM00013	<u> </u>
+ Enterococcus faecalis	ATCC®19433	WDCM00009	No growth
Listeria monocytogenes	ATCC®13932	WDCM00021	0.5-1.0mm blue colonies with halo
+ Escherichia coli	ATCC®8739	WDCM00012	No growth
+ Enterococcus faecalis	ATCC®29212	WDCM00087	No growth
Listeria monocytogenes	ATCC®13932	WDCM00021	0.5-1.0mm blue colonies with halo
+ Escherichia coli	ATCC®25922	WDCM00013	No growth
+ Enterococcus faecalis	ATCC®29212	WDCM00087	No growth
Listeria monocytogenes	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ Escherichia coli	ATCC®8739	WDCM00012	No growth



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+ Enterococcus faecalis	ATCC®19433	WDCM00009	No growth
Listeria monocytogenes + Escherichia coli + Enterococcus faecalis		WDCM00109 WDCM00013 WDCM00009	0
Listeria monocytogenes + Escherichia coli + Enterococcus faecalis	ATCC®35152 ATCC®8739 ATCC®29212	WDCM00012	0
Listeria monocytogenes + Escherichia coli + Enterococcus faecalis	ATCC®25922	WDCM00109 WDCM00013 WDCM00087	0

A satisfactory result is represented by recovery of >10 cfu of *Listeria monocytogenes* on Chromogenic Listeria Agar (ISO).

Selectivity determined by qualitative testing based on the methods described in ISO 11133:2014

Inoculation with pure cultures

Inoculate 10ml quantities of medium to achieve 1E+03 to 1E+04 colony-forming units/ml (cfu/ml) of *Escherichia coli* and *Enterococcus faecalis*. Incubate broths at $30 \pm 2^{\circ}$ C for 25 ± 1 hour. Subculture onto Chromogenic Listeria Agar (ISO) (CM1084, SR0226 & SR0228) and Tryptone Soya Agar (CM0131) and incubate plates at $37 \pm 2^{\circ}$ C for 24 ± 2 hours.

Reactions after incubation at 30 ± 2°C for 25 ± 1 hour

Escherichia coli	ATCC®8739	WDCM00012	No growth (CM1084, SR0226 & SR0228) No growth or cream colonies (CM0131)
Escherichia coli	ATCC®25922	WDCM00013	No growth (CM1084, SR0226 & SR0228) No growth or cream colonies (CM0131)
Enterococcus faecalis	ATCC®19433	WDCM00009	No growth (CM1084, SR0226 & SR0228) No growth or cream colonies (CM0131)
Enterococcus faecalis	ATCC®29212	WDCM00087	No growth (CM1084, SR0226 & SR0228) No growth or cream colonies (CM0131)

A satisfactory result is represented by no growth of *Escherichia coli* and *Enterococcus faecalis* on Chromogenic Listeria Agar (ISO).



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On Tryptone Soya Agar, a satisfactory result is represented by less than or equal to 1E+04 cfu/ml (equivalent to less than or equal to 100 cfu/10 μ l) for *Escherichia coli* and by less than or equal to 1E+06 cfu/ml (equivalent to less than or equal to 1E+04 cfu/10 μ l) for *Enterococcus faecalis*.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

HALF FRASER SELECTIVE SUPPLEMENT SR0166G

Section / Step	Description of Change	Reason for Change	Reference
Microbiological characteristics	Clarification of ISO 11133:2014 qualitative testing for mixed and pure cultures.	Change control	MOC-2022-0393

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

COOKED MEAT MEDIUM CM0081

COOKED MEAT MEDIUM	CM0081
Typical Formula*	
grams per litre	
Heart muscle	454.0
Peptone	10.0
'Lab-Lemco' powder	10.0
Sodium chloride	5.0
Glucose	2.0

^{*} adjusted as required to meet performance standards

Directions

Distribute 1.0g amounts of granules into tubes or bottles and add 10ml of distilled water. Increase the weight of granules and volume of water proportionally for larger quantities. Sterilize by autoclaving at 121°C for 15 minutes.

Physical Characteristics

Brown, free-flowing granules Colour on reconstitution - straw 4-5 Moisture level - less than or equal to 5% pH - 7.2 ± 0.2 at 25°C

Clarity - clear. Meat particles should occupy no less than 40ml in a 100ml volume of medium.

Microbiological Tests Using Optimum Inoculum Dilution

Control Media: Tryptone Soya Agar or Columbia Blood Agar Base enriched with 5% v/v horse blood, where appropriate

Reactions after incubation at 37°C for 48 hours

Medium is challenged with 1-10 colony-forming units/ml (cfu/ml)

Clostridium perfringens ATCC®13124 Proteolytic, saccharolytic, H₂S positive

Clostridium tetani ATCC®9441 Proteolytic, H₂S positive Clostridium histolyticum ATCC®19401 Proteolytic, H₂S positive

Peptostreptococcus anaerobius ATCC®27337 Saccharolytic Bacillus subtilis ATCC®6633 Surface growth

A satisfactory result is represented by reactions in accordance with the specification.

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION COOKED MEAT MEDIUM CM0081

After incubation broth is subcultured onto a blood agar plate and incubated at 37°C for 24 hours

Medium is challenged with less than 1E+03 colony-forming units/ml (cfu/ml)

Streptococcus pneumoniae

ATCC®6303

0.5-4mm grey/green colonies, α haemolysis

A satisfactory result is represented by recovery equal to or greater than a 3 log (10) increase.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

COOKED MEAT MEDIUM CM0081

Section / Step	Description of Change	Reason for Change	Reference
Entire document	Update to new product specification template and update of organisms.	Change control	BT-CC-1485
Entire Document	Correction of typographical/minor errors. Addition of Control Media and Result Criteria.	Change control	BT-CC-1918

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA BROTH (CM0129)

	CM0129
grams per litre	17.0
	3.0
	5.0
	2.5
	2.5
	grams per litre

^{*} adjusted as required to meet performance standards

Directions

Dissolve 30g in 1 litre of water (purified, as required) and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

Physical Characteristics

Straw, free-flowing powder
Colour on reconstitution - straw 2-3
Moisture level - less than 7%
pH 7.3 ± 0.2 at 25°C
Clarity - clear

Thermophiles and mesophiles shall be absent after incubation at 55°C and 37°C for 3 days.

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA BROTH (CM0129)

Microbiological Tests Using Optimum Inoculum Dilution

Control Medium: Tryptone Soya Agar, Columbia Blood Agar Base enriched with 5% v/v horse blood or Sabouraud Dextrose Agar, where appropriate

Reactions after incubation at 30-35°C for 18-24 hours

Medium is challenged with 10-100 colony-forming units

Streptococcus pyogenes ATCC® 19615 Turbid growth

A satisfactory result is represented by visible growth.

Tested in accordance with current CLSI M22 A

Reactions after incubation at 33-37°C for 18-24 hours

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Escherichia coli ATCC® 25922 Turbid growth Staphylococcus aureus ATCC® 25923 Turbid growth

A satisfactory result is represented by visible growth.

Reactions after incubation at 33-37°C for 5 days

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Streptococcus pneumoniae ATCC® 6305 Turbid growth

A satisfactory result is represented by visible growth.

Reactions after incubation at 33-37°C for 5 days under anaerobic conditions (for details refer to Oxoid Manual - Atmosphere Generation Systems)

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Bacteroides fragilis ATCC® 25285 Turbid growth

A satisfactory result is represented by visible growth.

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA BROTH (CM0129)

Tested in accordance with current USP/EP/BP/JP

Reactions after incubation at 30-35°C for 24 hours

Medium is challenged with 10-100 colony-forming units

ATCC® 8739	Turbid growth
ATCC® 6538	Turbid growth
ATCC® 9027	Turbid growth
NCTC 6017	Turbid growth
ATCC® 14028	Turbid growth
	ATCC® 9027 NCTC 6017

A satisfactory result is represented by visible growth.

Reactions after incubation at 30-35°C for 3 days

Medium is challenged with 10-100 colony-forming units

Bacillus subtilis	ATCC® 6633	Flocculent/surface growth
Kocuria rhizophila	ATCC® 9341	Turbid growth

A satisfactory result is represented by visible growth.

Reactions after incubation at 20-25°C for 48 hours

Medium is challenged with 10-100 colony-forming units

Bacillus subtilis	ATCC® 6633	Flocculent/surface growth
Candida albicans	ATCC® 10231	Flocculent/surface growth

A satisfactory result is represented by visible growth.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA BROTH (CM0129)

Reactions after incubation at 20-25°C for 5 days

Medium is challenged with 10-100 colony-forming units

Aspergillus brasiliensis

ATCC® 16404

White mycelia, black spores /

no spores

A satisfactory result is represented by visible growth.

The Microbiological Quality Control of this product complies with the following pharmacopoeia;

- 1. European Pharmacopoeia: Current version.
 - 2.6.12 Microbiological Examination of Non-Sterile Products: Harmonised Method: Microbial Enumeration tests
 - 2.6.13 Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms. B. Harmonised Method
- 2. United States Pharmacopoeia: Current version.
 - 61 Microbiological Examination of Non-Sterile Products: Microbial Enumeration tests.
 - 62 Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms
- 3. Japanese Pharmacopoeia: Current version.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA BROTH (CM0129)

Section / Step	Description of Change	Reason for Change	Reference
N/A	Addition of CLSI testing	Change control	BT-CC-1475
	Update to USP/EP/BP/JP testing		



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA AGAR CM0131

TRYPTONE SOYA AGAR CM0131

(Casein soya bean digest agar)†

† EP, USP, JP, BP

Typical Formula*

Pancreatic digest of casein	grams per litre	15.0
Enzymatic** digest of soya bean		5.0
Sodium chloride		5.0
Agar		15.0
** contains papain		

^{*} adjusted as required to meet performance standards

Directions

Suspend 40g in 1 litre of water (purified, as required). Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile Petri dishes.

Physical Characteristics

Straw, free-flowing powder
Colour on reconstitution - straw 1-2
Moisture level - less than or equal to 7%
pH 7.3 ± 0.2 at 25°C
Clarity - clear
Gel strength - firm, comparable to 15.0g/litre of agar

The medium is tested for compatibility using 7% v/v oxalated horse blood, defibrinated horse blood or defibrinated sheep blood. There shall be no evidence of lysis or darkening, after incubation at 37°C, 25°C and 4°C for 72 hours.

Microbiological Tests using Optimum Inoculum Dilution

Control Medium: Tryptone Soya Agar

Plain plates

Reactions after incubation at 30-35°C for 18-24 hours

Medium is challenged with 10-100 colony-forming units



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA AGAR CM0131

Streptococcus viridans NCTC1080 0.25-0.5mm pale straw colonies

Staphylococcus aureus ATCC®9144 0.5-1mm straw colonies
Staphylococcus epidermidis ATCC®1228 1-2mm white/grey colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Enriched with 7% v/v horse blood

Reactions after incubation at 37°C for 24 hours

Medium is challenged with 10-100 colony-forming units

Streptococcus pyogenes ATCC®19615 0.25-0.5mm pale straw colonies,

β haemolysis

Streptococcus viridans NCTC1080 0.5-1mm grey/green colonies,

α haemolysis

Streptococcus pneumoniae ATCC®6305 0.5-1mm grey/green colonies,

α haemolysis

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Reactions after incubation at 37°C for 48 hours under microaerophilic conditions

Haemophilus influenzae ATCC® 19418 Pinpoint-0.5mm colourless colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Zones of growth/no growth surrounding X, V and X+V factor discs (DD0003, DD0004 and DD0005) when plain plates are inoculated with the following organisms and incubated at 37°C for 18 hours:

		Χ	V	X+V
Haemophilus influenzae	ATCC®9334	0	0	≥ 15mm
Haemophilus influenzae	ATCC®19418	0	0	≥ 15mm
Haemophilus influenzae	ATCC®49247	0	0	≥ 15mm
Haemophilus parainfluenzae	ATCC®33392	0	≥ 20mm	≥ 20mm

Zones of inhibition with Bacitracin discs (DD0002) shall be 10-20mm when 7% v/v horse blood plates are inoculated with *Streptococcus pyogenes* ATCC® 19615 and incubated at 37°C for 18 hours.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA AGAR CM0131

Testing performed in accordance with ISO11133:2014

Plain plates

Reactions after incubation at 30 ± 2°C for 24 ± 2 hours

Medium is challenged with 50-120 colony-forming units

Bacillus cereus	ATCC®11778	WDCM00001	3-5mm irregular, straw colonies
Bacillus subtilis	ATCC®6633	WDCM00003	2-4mm irregular, straw colonies

Escherichia coli ATCC®8739 WDCM00012 1-3mm cream colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Reactions after incubation at 36 ± 2°C for 20 ± 2 hours

Medium is challenged with 50-120 colony-forming units

Escherichia coli	ATCC®25922	WDCM00013	1-3mm cream colonies
Escherichia coli	ATCC®11775	WDCM00090	1-3mm cream colonies
Escherichia coli	NCTC13167	WDCM00179	1-3mm cream colonies
Pseudomonas aeruginosa	ATCC® 10145	WDCM00024	1-4mm straw colonies
Enterococcus faecalis	ATCC®29212	WDCM00087	0.5-2mm straw colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Reactions after incubation at 37 ± 2°C for 24 ± 2 hours

Medium is challenged with 50-120 colony-forming units

Staphylococcus aureus	ATCC®25923	WDCM00034	0.5-1mm straw colonies
Listeria monocytogenes	ATCC®13932	WDCM00021	0.25-2mm straw colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA AGAR CM0131

Reactions after incubation at 44 ± 2°C for 21 ± 3 hours

Medium is challenged with 50-120 colony-forming units

Escherichia coli ATCC®8739 WDCM00012 1-3mm cream colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Reactions after anaerobic incubation at 44 ± 2°C for 21 ± 3 hours

Medium is challenged with 50-120 colony-forming units

Clostridium perfringens ATCC®13124 WDCM00007 1-2mm straw colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Testing performed in accordance with current CLSI M22 A

Enriched with 5% Sheep Blood

Reactions after incubation at 35 ± 2°C for 21 ± 3 hours

Medium is challenged with 1E+02 to 1E+04 colony-forming units

Streptococcus pyogenes	ATCC®19615	0.5-1mm pale straw colonies,
		β haemolysis
Streptococcus pneumoniae	ATCC®6305	0.5-2mm grey/green colonies,
		lpha haemolysis
Staphylococcus aureus	ATCC [®] 25923	1-2mm white/grey colonies
Escherichia coli	ATCC®25922	1-2mm straw colonies



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA AGAR CM0131

Testing performed in accordance with current USP/EP/BP/JP

Plain plates

Reactions after incubation at 30-35°C for 24 hours

Medium is challenged with 10-100 colony-forming units

Staphylococcus aureus	ATCC®6538	0.5-1mm straw colonies
Escherichia coli	ATCC®8739	1-3mm cream colonies
Bacillus subtilis	ATCC®6633	2-4mm irregular, straw colonies
Pseudomonas aeruginosa	ATCC®9027	1-4mm straw colonies
Salmonella typhimurium	ATCC®14028	1-3mm straw colonies
Salmonella abony	NCTC6017	1-3mm straw colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Reactions after incubation at 30-35°C for 5 days

Medium is challenged with 10-100 colony-forming units

Canaida albicans	ATCC*10231	1-3mm cream colonies
Aspergillus brasiliensis	ATCC®16404	Greater than 10mm colonies, white
		mycelia, with/without black spores

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

The Microbiological Quality Control of this product complies with the following pharmacopoeia;

- 1. European Pharmacopoeia: Current version.
 - 2.6.12 Microbiological Examination of Non-Sterile Products: Harmonised Method: Microbial Enumeration tests
 - 2.6.13 Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms. B. Harmonised Method
- 2. United States Pharmacopoeia: Current version.
 - 61 Microbiological Examination of Non-Sterile Products: Microbial Enumeration tests.
 - 62 Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms
- 3. Japanese Pharmacopoeia: Current version.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

TRYPTONE SOYA AGAR CM0131

Section / Step	Description of Change	Reason for Change	Reference
Entire document/ Microbiological Characteristics	Update to current format. Removal of duplicate results and obsolete statements/ Change Haemophilus influenzae from ATCC9344 to 9334. Change 44°C incubation time from 21 ± 2 hours to ± 3 hours.	Minor - Implementation of IVDR (2017746)	MOC-2022-0167

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

BISMUTH SULPHITE AGAR CM0201

BISMUTH SULPHITE AGAR	CM0201
Typical Formula*	
grams per litre	
Peptone	5.0
'Lab-Lemco' powder	5.0
Glucose	5.0
Di-sodium phosphate	4.0
Iron (II) sulphate	0.3
Bismuth sulphite indicator	8.0
Brilliant green	0.016
Agar	12.7

^{*} adjusted as required to meet performance standards

Directions

Suspend 20g in 500ml of distilled water in a 1 litre flask. With frequent agitation, bring to the boil to dissolve completely. Cool to 50°C. Mix well to ensure even dispersion of the medium and pour 25ml into sterile Petri dishes. Allow the medium to solidify with the dish uncovered. Larger volumes may be prepared if great care is taken and adequate headspace is provided. DO NOT AUTOCLAVE. DO NOT OVERHEAT.

Physical Characteristics

Light green, free-flowing powder
Colour on reconstitution - light green
Moisture level - less than or equal to 7%
pH - 7.6 ± 0.2 at 25°C
Clarity - opaque
Gel strength - firm, comparable to 12.7g/litre of agar

Microbiological Tests Using Optimum Inoculum Dilution

Control Medium: Tryptone Soya Agar

Reactions after incubation at 37°C for 48 hours

Medium is challenged with 10-100 colony-forming units

Salmonella typhi	ATCC®19430	0.5-2mm black 'rabbit-eye' colonies
		with sheen
Salmonella typhimurium	ATCC®14028	0.25-2mm black colonies with sheen
Salmonella virchow	NCTC5742	0.25-2mm black colonies with sheen



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

BISMUTH SULPHITE AGAR CM0201

Salmonella abonyNCTC60170.25-2mm black colonies with sheenSalmonella poonaNCTC48400.25-2mm black colonies with sheen

Salmonella enteritidis ATCC®13076 0.25-1.5mm green colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Medium is challenged with 50-200 colony-forming units

Escherichia coli	ATCC®25922	No growth to 1.5mm green colonies
Escherichia coli	ATCC®8739	No growth to 1.5mm green colonies
Klebsiella pneumoniae	ATCC®13883	No growth to 3.5mm green colonies
Citrobacter freundii	ATCC®8090	0.5-1.5mm dark green colonies

A satisfactory result is represented by recovery equal to or less than 100% of the control medium.

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Staphylococcus aureus	ATCC®6538	No growth
Enterococcus faecalis	ATCC®29212	No growth

Pseudomonas aeruginosa ATCC®9027 No growth to 1.0mm green colonies

Negative strains are inhibited. For *Pseudomonas aeruginosa* ATCC®9027, a satisfactory result is represented by a negative diagnostic reaction.

Equivalent results are obtained after incubation at 30-35°C for 48 hours.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION BISMUTH SULPHITE AGAR CM0201

Section / Step	Description of Change	Reason for Change	Reference
Reactions	Clarifying acceptable colony	Change Control	MOC-2022-
after	sizes for Klebsiella pneumoniae		1108
incubation at	ATCC®13883		
37°C for 48			
hours'			



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

PLATE COUNT AGAR (ISO) (CM0325)

PLATE COUNT AGAR (ISO)	CM0325
(Tryptone Glucose Yeast Agar)	

Formula

Enzymatic digest of casein	grams per litre	5.0
Yeast extract		2.5
Glucose		1.0
Agar		9.0

Directions

Suspend 17.5g in 1 litre of distilled water. Dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

Physical Characteristics

Straw, free flowing powder
Colour on reconstitution - straw 1-2
Moisture level - less than 7%
pH - 7.0 ± 0.2 at 25 °C
Clarity - clear
Gel Strength - firm, comparable to 9.0g/litre Agar

Thermophiles and Mesophiles shall be absent after incubation at 55°C and 37°C for 3 days.

Microbiological Tests Using Optimum Inoculum Dilution

Control Medium: Tryptone Soya Agar

Medium is challenged with 10-100 colony forming units

Standard plate counts are performed using Quality Control Organisms

Reactions after incubation at 30 ± 2°C for 48 ± 2 hours

Pour plate technique

Staphylococcus aureus	ATCC® 6538	0.5-2mm straw colonies
Staphylococcus aureus	ATCC® 6538P	0.5-2mm straw colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

PLATE COUNT AGAR (ISO) (CM0325)

Testing performed in accordance with ISO11133:2014

Reactions after incubation at 30 ± 2°C for 72 ± 3 hours

Pour plate technique

Medium is challenged with 50-120 colony forming units

Escherichia coli ATCC® 25922 WDCM00013 1-3mm straw colonies

Escherichia coli ATCC® 8739 WDCM00012 1-3mm straw colonies

Staphylococcus aureus ATCC® 25923 WDCM00034 0.5-2mm straw colonies

Bacillus subtilis ATCC® 6633 WDCM00003 0.5-2mm straw colonies

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

PLATE COUNT AGAR (ISO) (CM0325)

Section / Step	Description of Change	Reason for Change	Reference
N/A	Update to ISO	Change control	BT-CC-1902

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

X.L.D. MEDIUM CM0469

X.L.D. MEDIUM		CM0469
Typical Formula*		
Yeast extract	grams per litre	3.0
L-Lysine HCl		5.0
Xylose		3.75
Lactose		7.5
Sucrose		7.5
Sodium desoxycholate		1.0
Sodium chloride		5.0
Sodium thiosulphate		6.8
Ammonium iron (III) citrate		0.8
Phenol red		0.08
Agar		12.5

^{*} adjusted as required to meet performance standards

Directions

Suspend 53g in 1 litre of distilled water. With frequent agitation, bring to the boil to dissolve completely. Cool to 50°C. Mix well and pour into sterile Petri dishes. DO NOT AUTOCLAVE. DO NOT OVERHEAT.

Physical Characteristics

Straw/pink, free-flowing powder
Colour on reconstitution - red
Moisture level - less than or equal to 7%
pH 7.4 ± 0.2 at 25°C
Clarity - clear
Gel strength - firm, comparable to 12.5g/litre of agar

Microbiological Tests Using Optimum Inoculum Dilution

Control Medium: Tryptone Soya Agar

Reactions after incubation at 37 ± 2°C for 24 ± 3 hours

Inoculation with mixed cultures using diminishing sweep technique

Medium is challenged with 1E+03 to 1E+05 colony-forming units (cfu) of *Salmonella* and *Shigella* spp. and 1E+05 to 1E+07 cfu for *Escherichia coli* ATCC® 8739.

Salmonella abony

NCTC6017

1-3mm red colonies, black centre

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

X.L.D. MEDIUM CM0469

Salmonella enteritidis	ATCC®13076	1-2mm red colonies, black centre
Salmonella typhimurium	ATCC®14028	1-2mm red colonies, black centre
Salmonella virchow	NCTC5742	1-2mm red colonies, black centre
Salmonella arizonae	ATCC®13314	1-3mm red colonies, black centre
Salmonella nottingham	NCTC7832	1-3mm red colonies, black centre
Shigella sonnei	ATCC®9290	0.5-7mm irregular/smooth red colonies
Shiqella flexneri	ATCC®12022	0.5-2mm irregular, red colonies

In mixed culture, using the diminishing sweep technique, a satisfactory result is represented by diagnostic reactions of Salmonellae and Shigellae strains and Escherichia coli. Clear differentiation must be seen and is based on the colour and morphology of the colonies.

Inoculation with pure cultures

Medium is challenged with 10-100 colony-forming units

Pseudomonas aeruginosa ATCC®9027 No growth or 0.5-2mm red colonies

For Pseudomonas aeruginosa ATCC®9027, a satisfactory result is represented by recovery equal to or less than 90% of the control medium.

Proteus mirabilis	ATCC®12453	0.5-2mm orange/red colonies, with or without
		black centre, no swarming
Proteus mirabilis	ATCC®29906	0.5-2mm orange/red colonies, with or without
		black centre, no swarming
Serratia marcescens	ATCC®8100	1-2mm orange/yellow colonies
Citrobacter freundii	ATCC®8090	0.5-2mm yellow colonies
Klebsiella pneumoniae	ATCC®29665	2-4mm yellow, mucoid colonies

Other pure cultures are inhibited or shall produce colonies with a negative diagnostic reaction.

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Staphylococcus aureus ATCC®6538 No growth

Negative strains are inhibited.

Inoculation using diminishing sweep technique

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Escherichia coli ATCC® 11775 No growth or 0.5-4mm yellow colonies

Escherichia coli ATCC®11775 is inhibited or shall produce colonies with a negative diagnostic reaction.

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

X.L.D. MEDIUM CM0469

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Shigella sonnei ATCC®25931 0.5-7mm irregular/smooth red colonies

Shigella sonnei ATCC® 25931 shall produce colonies with a positive diagnostic reaction.

Equivalent results are obtained after incubation at 30-35°C for 24 hours.

Testing performed in accordance with ISO11133:2014

Reactions after incubation at 37 ± 2°C for 24 ± 3 hours

Medium is challenged with 50-120 colony-forming units

Salmonella enteritidis ATCC®13076 WDCM00030 1-3mm red colonies, black centre Salmonella typhimurium ATCC®14028 WDCM00031 1-3mm red colonies, black centre

A satisfactory result is represented by recovery of equal to or greater than 70% of the control medium.

Medium is challenged with 1E+04 to 1E+05 colony-forming units

Escherichia coli ATCC® 8739 WDCM00012 No growth or 0.5-4mm yellow cols Escherichia coli ATCC® 25922 WDCM00013 No growth or 0.5-4mm yellow cols

Inhibited strains shall produce no growth or at least a 1 log (10) reduction with a negative diagnostic reaction when compared to the control medium.

Inoculation using diminishing sweep technique

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Enterococcus faecalis ATCC®29212 WDCM00087 No growth Enterococcus faecalis ATCC®19433 WDCM00009 No growth

Negative strains are inhibited.

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

X.L.D. MEDIUM CM0469

Testing performed in accordance with current CLSI M22 A

Reactions after incubation at 35°C for 18-24 hours

Medium is challenged with 10-100 colony-forming units

Shigella flexneri ATCC®12022 0.5-2mm irregular, red colonies
Salmonella typhimurium ATCC®14028 1-2mm red colonies, black centre

A satisfactory result is represented by recovery of equal to or greater than 70% of the control medium.

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Enterococcus faecalis ATCC® 29212 No growth

Negative strains are inhibited.

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Escherichia coli ATCC® 25922 No growth or 0.5-4mm yellow cols

Inhibited strains shall produce no growth or at least a 1 log (10) reduction with a negative diagnostic reaction when compared to the control medium.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

X.L.D. MEDIUM CM0469

Section / Step	Description of Change	Reason for Change	Reference
Microbiological Tests	Update to specification for Shigella sonnei	Change control	BT-CC-1911
Microbiological Tests	Salmonella and Shigella mixed culture testing changed from low number quantitative to high number qualitative testing.	Change control	BT-CC-2398



BT-SPEC-0164

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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

BUFFERED PEPTONE WATER (CM0509)

BUFFERED PEPTONE WATER		CM0509
Formula		
Peptone	grams per litre	10.0
Sodium chloride		5.0
Di-sodium phosphate		3.5
Potassium dihydrogen phosphate		1.5

Directions

Add 20g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

Physical Characteristics

Straw, free flowing powder
Colour on reconstitution - straw 2-3
Moisture level - less than 7%
pH - 7.2 ± 0.2 at 25°C
Clarity - clear (single and double strength broth)
Buffering Capacity Test - passes test

Microbiological Tests Using Optimum Inoculum Dilution

Control Medium: Tryptone Soya Agar

Reactions after incubation at 37 ± 2°C for 18 ± 2 hours

Medium is challenged with 10-100 colony forming units

Salmonella nottingham NCTC 7832 Turbid growth Escherichia coli ATCC® 11775 Turbid growth

A satisfactory result is represented by visible growth.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

BUFFERED PEPTONE WATER (CM0509)

Testing performed in accordance with ISO11133:2014

Reactions after incubation at 37 \pm 2°C for 18 \pm 2 hours

Medium is challenged with 10-100 colony forming units

Salmonella typhimurium	ATCC® 14028 WDCM 00031	Turbid growth
Salmonella enteritidis	ATCC® 13076 WDCM 00030	Turbid growth
Escherichia coli	ATCC® 8739 WDCM 00012	Turbid growth
Escherichia coli	ATCC® 25922 WDCM 00013	Turbid growth

A satisfactory result is represented by visible growth from an inoculum of 10-100 colony forming units.

Testing performed in accordance with ISO22964:2017

Reactions after incubation at 36 ± 2°C for 18 ± 2 hours

Medium is challenged with 10-100 colony forming units

Cronobacter sakazakii	ATCC® 29544	WDCM 00214	Turbid growth
Cronobacter muytjensii	ATCC® 51329	WDCM 00213	Turbid growth

A satisfactory result is represented by visible growth from an inoculum of 10-100 colony forming units.



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OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION BUFFERED PEPTONE WATER (CM0509)

Section / Step	Description of Change	Reason for Change	Reference
Entire document	Update to new template and addition of ISO22964:2017 section	Change control	BT-CC-1531