thermo scientific



Safe. Clean. ISO compliant.

Guide to bacteriological testing of water according to ISO standards



Simple and complete testing

As a manufacturer, service provider or a contract laboratory, you know the importance of safeguarding your customers from potential hazards associated with water whether in your products or used alongside them. Adherence to published standards is paramount. This Guide to bacteriological testing of water provides an overview of the standard procedures, and illustrates how our extensive range of microbiology products for the isolation, identification and enumeration of waterborne pathogens and quality indicators can meet your testing needs. Our range of products for water testing includes dehydrated and prepared media, membrane filters, and quality control organisms, as well as the equipment you need to deliver reliable results.

- **Safe** media formulations that are compliant with ISO Standards for the testing of water
- Conform to ISO standard quality control testing methods are accredited in accordance with ISO 17025:2017
- **Proven and certified** all Certificates of Analysis (CoAs) confirm testing in accordance with the ISO 11133:2014 standard, including membrane filter testing
- **Support** our team of microbiology experts is at hand to help with your technical queries

thermos	cientifi	-	TIFICATI	E OF ANA	OXOID Deutschland GmbH Am Lippeglacis 4-8 D-46483 Wesel
PRODUCT	PO5074A	LEGIONELL	GVPC SELEC	TIVE MEDIUM	τ
LOT NUMBER	2133625				
EXPIRY DATE	2017.06.28				
General Characteris	tics		Results		Specification
Colour Appearance pH Packaging / Presentati Sterility @ 25 & 36 ± Membrane filter 1 Membrane filter 2		IOUITS	Conforms Conforms 6.8 Conforms Conforms Lot 1603173 Lot 1600693 Lot 1604003		Jet black - Traffic black Opaque 6.7.7.1 Label & Print check Within Limits Product Code GFS11 Product Code GFS12 Product Code GFS12
Membrane filter 4			Lot 1180883 Lot 1183847 Lot F6NA27518	:	Product Code NG147-0045 Product Code NG147-0045 Product Code NG02056045
Microbiological Perf	ormance		Control c.f.u	Test Result	Specification
Strains tested by mem method with above lis Legionella pneumoph WDCM 00107 Legionella anisa ATC WDCM 00106	ited lots ila ATCC®3 C®35292	33152	50 - 120 50 - 120	Conforms Conforms	Recovery >= 50%, grey-blue colonies Recovery >= 70%, grey-blue colonies
Strains tested by sprea Legionella pneumoph WDCM 00107			67	51	2-6mm, grey-blue colonies
Legionella anisa ATC WDCM 00106	C®35292		55	49	2- 4mm, grey-blue colonies
Escherichia coli ATC WDCM 00012	C®8739		1E+04 - 1E+05	Conforms	Total or partial inhibition
Pseudomonas aerugin WDCM 00025 Enterococcus faecalis WDCM 00009			1E+04 - 1E+05 1E+04 - 1E+05		Total or partial inhibition Total inhibition

The quality control methods meet requirements of ISO 11133:2014

The testing laboratory of Oxoid Deutschland GmbH is accredited by the Ger body DAkKS according to DIN EN ISO/IEC 17025 for the performance testin for microbiology to DIN EN ISO11133:2014 and registered under D-PL-2015

DAkks

Figure 1. Example of Certificate of Analysis (Legionella GVPC Selective Medium)

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Detection of microorganisms – Colony counts at 22 °C and 36 °C

Method according to EN ISO 6222:1999

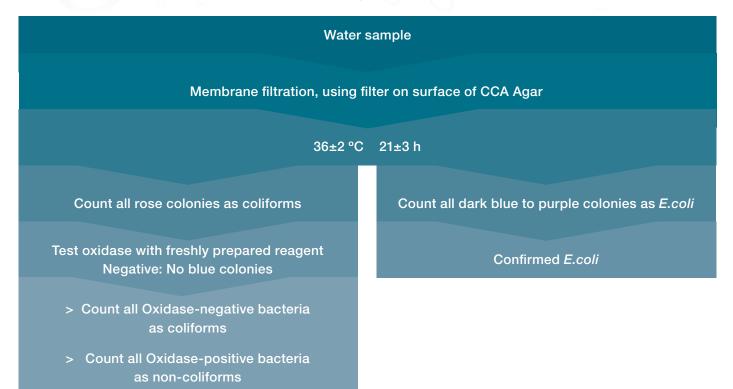
Water sample
Poured plate method with Yeast Extract Agar
68±4 h at 20±2 °C and 44±4 h at 36±2 °C
Count all colonies formed

Detection of microorganisms – Colony count number at 22 °C and 36 °C

Method	Media	Product	Format	Product code
EN ISO 6222:1	999			
Pour plate	Yeast Extract Agar	Plate Count Agar for water testing (ISO)	500 g Dehydrated culture media	CM1012B
		Plate Count Agar	10 x 100 mL bottles Prepared media	BO0055M
			10 x 200 mL bottles Prepared media	BO0055R

Escherichia coli / Coliform bacteria

Method according to ISO 9308-1:2014



Escherichia coli and coliform

Method	Media	Product	Format	Product code
EN ISO 9308-1:	2014 Standard test			
Membrane filtration	CCA Agar	Thermo Scientific [™] Chromogenic Coliform	500 g Dehydrated culture media	CM1205B
		Agar	10 x 90 mm plates Prepared media	P05318A
			10 x 55 mm plates Prepared media	P05428J
Oxidase test	Tryptone Soya Agar (TSA)	/a CASO AGEN (TSA)	500 g / 5 kg Dehydrated culture media	CM0131B/T
	0 ()		10 x 90 mm plates Prepared media	P05012A
	Oxidase Reagent	Thermo Scientific [™] BactiDrop [™] Oxidase	50 vials Dehydrated culture media	R21540
		Reagent Thermo Scientific [™] Microbact [™] Oxidase Strips	10 x 90 mm plates Prepared media	P05321A
			50 strips Dehydrated culture media	MB0266A

Enterococci

Method according to ISO 7899-2:2000

Water sample
Membrane filtration, using filter on surface of Slanetz and Bartley Agar
36±2 °C 44±4 h
Count all convex red, brown or rose colonies
Put filter onto pre-warmed 44°C Bile Aesculine Azid Agar
44±0,5°C 2 h
Enumerate all brown colonies with brown halos as intestinal Enterococci

Enterococci

Method	Media	Product	Format	Product code
EN ISO 7899-2	:2000			
Membrane filtration	Slanetz Bartley Agar	Slanetz Bartley Agar	500 g Dehydrated culture media	CM0377B
			10 x 90 mm plates Prepared media 10 x 55 mm plates Prepared media	PO5018A
				PO5410J*
			10 x 55 mm plates Prepared media	P05423J*
Confirmation	Bile Aesculine Azide Agar	Enterococcus Selective Medium (Bile Aesculine Azid Agar) ¹	10 x 90 mm plates Prepared media	PO5062A
		or Kanamycin Aesculin Azide Agar Base ²	500g Dehydrated culture media 10 x 90 mm plates Prepared media	CM0591B
				PO5059A

* Minimum order quantity required.

1. This medium is slightly different to the medium formulation according to ISO 7899-2:2000. The medium has additional sodium citrate and 20 g/l ox bile instead of 10 g/l and 0.55 g/l sodium azide instead of 0.15 g/l. 2. This medium is slightly different to the medium formulation according to ISO 7899-2:2000. It contains sodium citrate instead of ox bile and Kanamycin.

Pseudomonas aeruginosa

Method according to ISO 16266:2006

Water sample Membrane filtration, using filter on surface of Pseudomonas CN Agar 36±2 °C 44±4 h* Inspection under UV light Inspection under natural light Inspection under natural light (360±20 nm) Blue green (Pyocyanine Red brown colonies: Fluorescence colonies: forming) colonies: confirmed presumptive presumptive *Pseudomonas* Pseudomonas aeruginosa Pseudomonas aeruginosa aeruginosa Subculture to Nutrient Agar Subculture to Nutrient Agar 36±2°C 22±2 h 36±2°C 22±2 h Oxidase test: oxidase positive Add Nessler's Reagent ammonia development King's B Medium **Acetamide Solution** (red-yellow precipitation) 36±2°C 22±2 h 36±2°C up to 5 days Confirmed Pseudomonas aeruginosa Add Nessler's Reagent ammonia development Fluorescence under UV light (red-yellow precipitation) Confirmed Pseudomonas aeruginosa

Method	Media	Product	Format	Product code
EN ISO 16266:2	006			
Membrane filtration	Pseudomonas Selective Agar /	Pseudomonas Centrimide Selective Agar	10 x 90 mm plates Prepared media	PO5076A
	CN Agar	0	10 x 55 mm plates Prepared media 500 g Dehydrated culture media	PO5413J*
		Pseudomonas Agar Base		CM0559B
			10 tubes Dehydrated culture media	SR0102E
Confirmation				
Fluorescence	King's B Media			
Hydrolysis of acetamide to ammonia	Acetamide Solution Nessler Reagent			
Oxidase Test	Nutrient Agar	Nutrient Agar	500 g Dehydrated culture media	CM0003B
	Oxidase Reagent	BactiDrop Oxidase Reagent	50 vials Dehydrated culture media	R21540
		Microbact Oxidase Strips	50 strips Dehydrated culture media	MB0266A

* Minimum order quantity required.

Clostridium perfringens (including spores)

Method according to ISO 14189:2013

Membrane filtration of 100 mL onto TSC-Agar* (if necessary, dilute sample)

44±1°C, anaerobic 21±3 h

Enumerate the presumptive colonies which show black or grey to yellow brown staining within 30 minutes of opening

Count all black colonies as Clostridium perfringens

1-10 black or grey to yellow-brownish colonies

Subculture all presumptive colonies onto blood agar plates

36±2°C, anaerobic 21±3 h

>10 black or grey to yellow-brownish colonies

Subculture 10 different presumptive colonies onto blood agar plates

36±2°C, anaerobic 21±3 h

Put colonies that grew under anaerobic conditions onto filter paper and add 2-3 drops of phosphate acid. All colonies that turn purple within 3-4 minutes are confirmed positive.

*ATTENTION: Alternatively, a thin layer (approximately 5 mL to 10 mL) of molten TSC Agar (TV5204G TSC Agar Base), as an overlay on the filter can be used. Allow to solidify before anaerobic incubation. This method may enhance the blackening of the colonies. Medium without cycloserine; e.g. TV5204G TSC Agar Base, 20 mL.

Melt TSC Agar in a water bath and put melted agar onto filter on the plate.

Melt agar in a water bath at 95°C for approx. 10-15 minutes

Cool medium to 45°C

Shake tube carefully and pour a thin layer of molten agar

Clostridium p	erfringens
Method	Media

Method	Media	Product	Format	Product code
ISO 14189:2013	3			
Membrane filtration	TSC Agar	TSC Selective Agar 10 x 90 mm plates Prepared media		P05315A
		TSC Agar Base +	Dehydrated culture media 50 tubes Prepared media	CM0587B
				TV5204G
		TSC Selective Supplement	10 tubes Dehydrated culture media	SR0088E
		10 x 100 r Prepared media	10 x 100 mL bottles Prepared media	BO0634M
			10 tubes Prepared media	SR0088E
Anaerobic atmosphere		Thermo Scientific [™] AnaroGen [™] System	10 sachets Prepared media	AN0025A
		Thermo Scientific [™] AnaroJar [™] 2,5 L Anaerobic System	1 pot Prepared media	AG0025A
		Thermo Scientific [™] AnaeroBox [™] Rectangular Anaerobic System	1 pot Prepared media	AB0025A

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Legionella

Methods according to ISO 11731:2017

Sample preparation

0.1-0.5 mL of the

sample onto BCYE

Heat treated - Put sample into water bath for 30+/-2 minutes at 50 °C
Acid treated - 1 part sample to 9 parts of acid buffer. Mix well and leave it for 5+/- 0.5 minutes.
Acid treated filter - Put 30 mL of acid solution onto the membrane filter. Leave it for 5 +/- 0.5 minutes and remove the solution by filtration. Wash with 20 mL of either sterile Aqua dest., Pages Saline, Ringer Solution or PBS.

Samples with high concentration of Legionella species and low concentration of interfering organisms²

0.1-0.5 of the sample

onto BCYE with AB

Samples with low concentration of Legionella species and low concentration of interfering organisms²

Membrane filtration of 100 mL sample'. Place one untreated membrane filter onto BCYE Agar; Place membrane filter with acid solution on one or more selective media: BCYE Agar with AB or GVPC Agar or MWY Agar 0.1-0.5 mL of the sample; each untreated, heat treated and acid treated from the membrane filtration with washing procedure³ onto BCYE Agar and on one or more selective media: BCYE Agar with AB or GVPC Agar or MWY Agar

36 ± 2 °C, humid atmosphere, 7-10 days

Subculture at least 3 typical Legionella colonies from each plate and inoculate on BCYE Agar without Cystein and BCYE Agar

 36 ± 2 °C, humid atmosphere, 2-5 days

Colonies that grow on BCYE but not on BCYE without cystein are considered to be Legionella³

- 1. 10 1000 mL sample volume according to ISO 11731-2.
- The choice of the method for the enumeration of Legionella species depends on the origin/characteristics of the sample and the reason for sampling or investigation. A
 decision matrix can be found in the ISO 11731:2017, Annex J.
- 3. Further information, please see ISO 11731:2017.

Method	Media	Product	Format	Product co
ISO 11731:201	7 and EN ISO 11731-2	2		
Sample preparation	Acid Buffer	Legionella Acid Buffer Solution	6 x 1 L bottles Prepared media	GFB01
Direct and membrane	Legionella GVPC Agar	Legionella GVPC Selective Agar	10 x 90 mm plates Prepared media	P05074A
filtration	-		10 x 90 mm plates Prepared media	P00245A
		Legionella CYE Agar-Base +	500 g Dehydrated culture media	CM1203B
		Legionella BCYE Supplement +	10 tubes (500 mL) Dehydrated culture media	SR0251C
		Legionella GVPC Selective Supplement	10 tubes (500 mL) Dehydrated culture media	SR0252E
	Legionella GVPC Agar with antibiotics	Legionella BCYE Agar with antibiotics	10 x 90 mm plates Prepared media	P05325A
	Legionella BCYE Agar	Legionella BCYE Agar	10 x 90 mm plates Prepared media	PO5072A
		Legionella CYE Agar Base +	500 g Dehydrated culture media	CM1203B
		Legionella BCYE Supplement	10 tubes (500 mL) Dehydrated culture media	SR0251C
			10 tubes (500 mL) Dehydrated culture media	SR0255B
	Legionella MWY Agar	Legionella MWY Agar	10 x 90 mm plates Prepared media	PO5071A
	0	Legionella CYE Agar Base +	500 g Dehydrated culture media	CM1203B
		Legionella BCYE Supplement +	10 tubes (500 mL) Dehydrated culture media	SR0251C
Subculture	Legionella BCYE Agar	Legionella BCYE Agar without Cystein	10 x 90 mm plates Prepared media	P05028A
	without Cystein		10 x 90 mm plates Prepared media	P00255A*
		Legionella CYE Agar Base +	500 g Dehydrated culture media	CM1203B
		Legionella BCYE without Cystein Supplement	10 tubes (100 mL) Dehydrated culture media	SR0253A
	Legionella BCYE Agar	Legionella BCYE Agar	10 x 90 mm plates Prepared media	P05072A
		Legionella CYE Agar Base +	500 g Dehydrated culture media	CM1203B
		Legionella BCYE Supplement	10 tubes (500 mL) Dehydrated culture media	SR0251C

* Minimum order quantity required.

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Filter Funnels and Membranes

Thermo Scientific[™] Nalgene[™] Membrane Filters are cellulose nitrate membranes that are certified for microbiological QC testing and analysis of water.

Product	Size or Quantity	Cat. No.
Nalgene Disposable Analytical Funnels equipped with membrane fi	lter	
Sterile Analytical Filter Units, 100 mL, 47 mm, 0.45 µm, white/black	50 pieces	NG145-0045
Sterile Analytical Filter Units, 250 mL, 47 mm, 0.45 µm, white/black	50 pieces	NG145-2045
Sterile Analytical Filter Units, 100 mL, 47 mm, 0.45 µm, grey/black	50 pieces	NG147-0045
Sterile Test Filter Funnel, 250 mL, 0.45 uL	50 pieces	NG147-2045
Membrane filter		
Membrane Filter for water testing, sterile, CN, 47 mm, 0,45 µm, white/ black	100 pieces	NG0205-4045
Membrane Filter for water testing, sterile, CN, 47 mm, 0,45 µm, grey/black	100 pieces	NG0210-6045
Accessories		
Vacuum Manifold	1 piece	NG0345-0001
Filter Stopper, non-sterile, no. 8 rubber	3 pieces	NG0396-0080
Filter Forceps, bent tip	1 piece	NG0399-0001
Filter Forceps, straight tip	1 piece	NG0399-0002
Filter Funnel Adapter, non-sterile	25 pieces	NG0397-0010
Vacuum Gasket, non-sterile thermoplastic elastomer	6 pieces	NG0395-0708
Nalgene Reusable Analytical Filters and accessories		
Filter Funnels with Clamp, 250 mL	1 piece	NG0315-0047
Filter Holders with Receiver, 500 mL, 500 mL	4 pieces	NG0300-4000
Filter Holders with Receiver, 250 mL, 250 mL	4 pieces	NG0300-4050
Filter Holders with Receiver, 500 mL, 1000 mL	4 pieces	NG0300-4100
Filter Holders with Funnel, 250 mL	1 piece	NG0310-4000
Filter Holders with Funnel, 500 mL	1 piece	NG0310-4050
Reusable Bottle Top Filters, 250 mL	1 piece	NG0320-2545
Reusable Bottle Top Filters, 500 mL	1 piece	NG0320-5033
Reusable Bottle Top Filters, 500 mL	1 piece	NG0320-5045

Culti-Loops QC Organisms

For quality control testing according to ISO 11133:2014

Product	Sample numbers	Cat. No.
Escherichia coli QC Organisms for testing of CCA Agar		
Escherichia coli ATCC® 8739™*	WDCM 00012	R4607085
Escherichia coli ATCC® 25922™*	WDCM 00013	R4607050
Enterobacter aerogenes ATCC [®] 13048 [™] *	WDCM 00175	R4607080
Pseudomonas aeruginosa ATCC® 27853™*	WDCM 00025	R4607060
Pseudomonas aeruginosa ATCC® 9027™*	WDCM 00026	R4605210
Enterococcus faecalis ATCC® 19433™*	WDCM 00009	R4601990
Enterococcus faecalis ATCC [®] 29212 [™] *	WDCM 00087	R4607030
Enterococci QC Organisms for testing of Slanetz and Bartley	Agar	
Escherichia coli ATCC® 8739™*	WDCM 00012	R4607085
Escherichia coli ATCC® 25922™*	WDCM 00013	R4607050
Staphylococcus aureus subsp. aureus ATCC® 6538™*	WDCM 00032	R4607016
Staphylococcus aureus subsp. aureus ATCC® 25923 [™] *	WDCM 00034	R4607010
Enterococcus faecalis ATCC® 29212™*	WDCM 00087	R4607030
Enterococcus faecalis ATCC® 19433™*	WDCM 00009	R4601990
Pseudomonas aeruginosa QC Organisms for testing of Pseud	omonas CN Agar	
Pseudomonas aeruginosa ATCC® 27853™*	WDCM 00025	R4607060
Pseudomonas aeruginosa ATCC® 9027™*	WDCM 00026	R4607060
Pseudomonas aeruginosa ATCC® 10145 [™] *	WDCM 00024	R4607065
Escherichia coli ATCC® 8739 [™] *	WDCM 00012	R4607085
Escherichia coli ATCC® 25922 [™] *	WDCM 00013	R4607050
Enterococcus faecalis ATCC® 19433™*	WDCM 00009	R4601990
Enterococcus faecalis ATCC® 29212™*	WDCM 00087	R4601990
Clostridium perfringens QC Organisms for testing of TSC Aga	ır	
Bacillus subtilis ATCC 6633	WDCM 00003	R4601221
QC organisms for testing GCVP Agar (Legionella method)		
Legionella pneumophila ATCC® 33152 [™] *	WDCM 00107	R4603950
Legionella anisa NCTC 11974™	WDCM 00106	R4601315
Enterococcus faecalis ATCC® 19433™*	WDCM 00009	R4601990
Enterococcus faecalis ATCC® 29212™*	WDCM 00087	R4607030
Pseudomonas aeruginosa ATCC® 27853™*	WDCM 00025	R4607060
Pseudomonas aeruginosa ATCC® 9027™*	WDCM 00026	R4605210
Escherichia coli ATCC® 8739™*	WDCM 00012	R4607085
Escherichia coli ATCC® 25922™*	WDCM 00013	R4607050
Prepared Media for the Quality Control testing according to Is	SO 11133:2014	
CASO Agar (ISO 11133)		P05321A

CASO Agar (ISO 11133)

PO5321A

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