thermo scientific



Safe. Clean. ISO compliant.

Guide to microbiological testing of drinking water according to ISO standards



Simple and complete testing

As a food or beverage manufacturer, contract laboratory for testing drinking water or a hospital you know the importance of safeguarding the purity of the water you use in manufacturing processes and the products you deliver to your customers. Adherence to published standards is paramount. This Guide to microbiological testing of drinking 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 meets your testing needs.

Our range of products for water testing includes dehydrated and prepared media, membrane filters, quality control organisms and proficiency testing, as well as the equipment you need to deliver reliable results.

- Safe-media formulations that are compliant with ISO Standards for the testing of drinking water
- Conform to ISO standard-quality control testing methods are accredited in accordance with ISO 17025
- **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

thermoscientific			OXOID Deutschland GmbH Am Lippeglacis 4-8 D-46483 Wesel
CEI	RTIFICAT	E OF AN/	ALYSIS
PRODUCT POSIFIA LEGIONEL	LA GVPC SELEC	TIVE MEDIUS	4
LOT NUMBER 2133625			
EXPIRY DATE 2017.06.28			
General Characteristics	Results		Specification
Colour	Conforms		Jet black - Traffic black
Appearance	Conforms		Opaque
pro Packaging / Presentation	Conforms		5.7 - 7.1 Label & Print check
Sterility @ 25 & 36 ± 1°C for 72 hours	Conforms		Within Limits
Membrane filter 1	Lot 1603173		Product Code GFS11
Membrane filter 2			
	Lot 1600693		Product Code GPS12
Membrane filter 3	Lot 1004003		Product Code OPS12
S RELEVANCE LINES 3	Lot 1180883		Product Code NG147-0045
	Lot 1183847		Product Code NG147-0045
Membrane filter 4	Lot F6NA275B	8	Product Code NG02056045
Microbiological Performance	Control e.f.u	Test Result	Specification
Strains tested by membrane filtration			
Legionella pneumophila ATCC833152 WDCM 00107	50 - 120	Conforms	Recovery >= 50%, grey-blue colonies
Legionella anisa ATCC#35292 WDCM 00406	50 - 120	Conforms	Recovery >= 70%, grey-blue colonies
Strains tested by spread plate method Legionella pneumophila ATCC833152	67	51	2- 6mm, grey-blue colonies
WDCM 00107 Legionella anisa ATCC/835292 WDCM 00106	55	49	2-4mm, grey-blue colonies
Escherichia coli ATCC88739 WDCM 00012	1E+04 - 1E+05	Conforms	Total or partial inhibition
Pseudomonas aeraginosa ATCC#27853 WDCM 00025	IE+04 - 1E+05	Conforms	Total or partial inhibition
Enterococcus faecalis ATCC819433 WDCM 00009	IE+04 - 1E+05	Conforms	Total inhibition

The quality control methods meet requirements of 1SO 11133:2014.



The testing lateoratory of Coold Deutschland Gradiel is accrediated by the German accrediated by DA&S according to DNI EN ISO-EUC 17025 for the performance testing of media r microbiology to DIN EN ISO-E1133-2014 and registered under D-PL-2019D-01-00.

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

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

EN ISO 6222 Water sample Ourred plate method with
Yeast Extract Agar 68±4h at 20±2°C and
44±4h at 36±2°C Count all colonies formed

Method according to

Detection of microorganisms – Colony count number at 22°C and 36°C							
Regulation	Method	Media	Product	Dehydrated culture media Product code	l Format	Prepared I Product code	media 9 Format
EN ISO 6222	Pour plate	Yeast Extract Agar	Plate Count Agar for water testing (ISO) Plate Count Agar (ISO)	CM1012B	500 g	BO0055M	10 x 100 mL bottles 10 x 200 ml
						DO0000h	bottles

Escherichia coli / Coliform bacteria



Escherichia coli and coliform								
Regulation	Method	Media	Product	Dehydrate culture m Product cod	ed edia le Format	Prepared Product cod	media e Format	
EN ISO 9308-1 Standard test	Membrane filtration	CCA Agar	Thermo Scientific [™] Chromogenic Coliform Agar	CM1205B	500 g	P05318A P05428J	10 x 90 mm plates 10 x 55 mm plates	
	Oxidase test	Tryptone Soya Agar (TSA)	CASO Agar (TSA)	CM0131B/T	500 g / 5 kg	P05012A	10 x 90 mm plates	
		Oxidase Reagent	Thermo Scientific [™] BactiDrop [™] Oxidase Reagent Thermo Scientific [™] Microbact [™] Oxidase Strips	R21540 MB0266A	50 vials 50 strips	P05321A	10 x 90 mm plates	

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Enterococci

Method according to ISO 7899-2

Water sample

Membrane filtration, using filter on surface of Slanetz and Bartley Agar

Enumerate all brown colonies with brown halos as intestinal Enterococci

Enterococci					
Regulation	Method	Media	Product	Dehydrated culture media Product code Format	Prepared media Product code Format
EN ISO 7899-2	Membrane filtration	Slanetz Bartley Agar	Slanetz Bartley Agar	CM0377B 500 g	PO5018A 10 x 90 mm plates PO5410J* 10 x 55 mm plates PO5423J* 10 x 55 mm plates
	Confirmation	Bile Aesculine Azide Agar	Enterococcus Selective Medium (Bile Aesculine Azid Agar)' or Kanamycin Aesculin Azide Agar Base ²	CM0591B 500 g	PO5062A 10 x 90 mm plates PO5059A 10 x 90 mm plates

* Minimum order quantity required.

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

Pseudomonas aeruginosa

Method according to ISO 16266 Water sample Membrane filtration, using filter on surface of Pseudomonas CN Agar 36±2°C 44±4h* Inspection under UV light (360±20 nm) Inspection under natural light Inspection under natural light Blue green (Pyocyanine forming) colonies: confirmed Pseudomonas aeruginosa Subculture to Nutrient Agar Subculture to Nutrient Agar 36±2°C 22±2 h 36±2°C 22±2 h Acetamide solution Oxidase test: oxidase positive Acetamide Solution 36±2°C up to 5 days Add Nessler's Reagent 36±2°C 22±2 h ammonia development (red-yellow precipitation) Add Nessler's Reagent Fluorescence ammonia development (red-yellow precipitation) & under UV light Confirmed Pseudomonas aeruginosa *Check growth on membrane filter after 22 \pm 2 h and 44 \pm 4 h. Confirmed Pseudomonas aeruginosa

Pseudomonas a	eruginosa				
Regulation	Method	Media	Product	Dehydrated culture media Product code Format	Prepared media Product code Format
EN ISO 16266	Membrane filtration	Pseudomonas Selective Agar / CN Agar	Pseudomonas Centrimide Selective Agar Pseudomonas Agar Base CN Selective Supplement	CM0559B 500 g SR0102E 10 tubes	PO5076A 10 x 90 mm plates PO5413J* 10 x 55 mm plates
	Confirmation				
	Fluorescence	King's B Media			
	Hydrolysis of acetamide to ammonia	Acetamide Solution Nessler Reagent			
	Oxidase Test	Nutrient Agar	Nutrient Agar	CM0003B 500 g	
		Oxidase Reagent	BactiDrop Oxidase Reagent Microbact Oxidase Strips	R2154050 vialsMB0266A50 strips	

* Minimum order quantity required.

Clostridium perfringens (including spores)

Method according to ISO 14189:2013

Membrane filtration of (if necessary, o	100 mL onto TSC-Agar* dilute sample)	*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.			
44±1°C, a	anaerobic	Melt TSC Agar in a wat	er bath and put melted agar o	onto filter on the plate.	
21± Enumerate the presumptive color yellow brown staining with	^{3 h} nies which show black or grey to nin 30 minutes of opening	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	
Count all blac Clostridium	k colonies as perfringens				
1-10 black or grey to yellow- brownish colonies	>10 black or grey to yellow- brownish colonies				
Subculture all presumptive colonies onto blood agar plates	Subculture 10 different pre- sumptive colonies onto blood agar plates				
36±2°C, anaerobic 21±3 h	36±2°C, anaerobic 21±3 h				
Put colonies that grew under anac	erobic conditions onto filter paper acid. All colonies that turn purple				

Clostridium perfringens

Regulation	Method	Media	Product	Dehydrated culture media Product code Format		Prepared Product cod	media e Format
ISO 14189	Membrane filtration	TSC Agar	TSC Selective Agar			P05315A	10 x 90 mm plates
			TSC Agar Base +	CM0587B	500 g	TV5204G	50 tubes
			TSC Selective Supplement	SR0088E	10 tubes	BO0634M	10 x 100 mL bottles
						SR0088E	10 tubes
	Anaerobic		Thermo Scientific" AnaroGen" System			AN0025A	10 sachet
			Thermo Scientific" AnaeroBox" Rectangular Anaerobic System			AB0025A	1 pot

Legionella

Methods according to ISO 11731:2017

Sample preparation

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.



10 - 1000 mL sample volume according to ISO 11731-2.

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 ISO11731:2017.

Legionella						
Regulation	Method	Media	Product	Dehydrated culture media Product code Format	Prepar Product code	ed media Format
ISO 11731:2017 and EN ISO 111731-2	Sample preparation	Acid Buffer	Legionella Acid Buffer Solution		GFB01	10 x 6 L bottles
	Direct and membrane filtration	GVPC Agar	Legionella GVPC Selective Agar		P05074A P00245A	10 x 90 mm plates 10 x 90 mm plates
			Legionella CYE Agar-Basis + Legionella BCYE Supplement + Legionella GVPC Selektive Supplement	CM0655B 500 g SR0110C 10 tubes SR0152E 10 tubes	(500 mL) (500 mL)	
		BCYE Agar with antibiotics	BCYE Agar with antibiotics		P05325A	10 x 90 mm plates
		BCYE Agar	BCYE Agar		P05072A	10 x 90 mm plates
		MWY Agar	MWY Agar		P05071A	10 x 90 mm plates
	Subculture	BCYE Agar without Cystein	BCYE Agar without Cystein		P05028A P00255A	10 x 90 mm plates 10 x 90 mm plates
		BCYE Agar	BCYE Agar		P05072A	10 x 90 mm plates

. UK only

Legionella proficiency testing and QC organisms

Thermo Scientific[™] Culti-Loops[™] Reference Strains for the quality control testing of GVPC Agar according to ISO 11133:2014

Legionella Quality Control Organisms					
Product code	Product name	WDCM no.			
R4603950	Legionella pneumophila ATCC® 33152™*	WDCM 00107			
R4601990	Enterococcus faecalis ATCC [®] 19433 [™] *	WDCM 00009			
R4607030	Enterococcus faecalis ATCC [®] 29212 [™] *	WDCM 00087			
R4607060	Pseudomonas aeruginosa ATCC® 27853 [™] *	WDCM 00025			
R4605210	Pseudomonas aeruginosa ATCC® 9027™*	WDCM 00026			
R4607085	Escherichia coli ATCC® 8739™*	WDCM 00012			
R4607050	Escherichia coli ATCC [®] 25922 [™] *	WDCM 00013			

External quality assessment (EQA) of *Legionella* isolation for laboratories that examine waters for *Legionella* spp.

Detection, enumeration and identification of Legionella spp.						
Product code	Product name	Sample numbers				
LEG01D01	Legionella isolation scheme LEG01 sample 1	2 each				
LEG01D02	Legionella isolation scheme LEG01 sample 2	2 each				
LEG01D03	Legionella isolation scheme LEG01 sample 3	2 each				
LEG01D04	Legionella isolation scheme LEG01 sample 4	2 each				
<i>Legionella</i> spp. <u>by m</u>	<i>Legionella</i> spp. <u>by molecular methods only</u>					
LM01D01	Legionella Molecular Scheme LM01 sample 1	2 each				
LM01D02	Legionella Molecular Scheme LM01 sample 2	2 each				

Not available in all EU countries. Please check availability with your local sales office.

Thermo Scientific Nalgene Analytical Test Filter Funnels and Membranes

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

Nalgene Disposable Analytical Funnels equipped with membrane filter					
Product code	Product name	Pack size			
NG145-0045	Sterile Analytical Filter Units, 100 mL, 47 mm, 0.45 µm, white/black	50 pieces			
NG145-2045	Sterile Analytical Filter Units, 250 mL, 47 mm, 0.45 µm, white/black	50 pieces			
NG147-0045	Sterile Analytical Filter Units, 100 mL, 47 mm, 0.45 µm, grey/black	50 pieces			
NG147-2045	Sterile Test Filter Funnel, 250 mL, 0.45 uL	50 pieces			
Membrane filter					
NG0205-4045	Membrane Filter for water testing, sterile, CN, 47 mm, 0,45 μ m, white/black	100 pieces			
NG0210-6045	Membrane Filter for water testing, sterile, CN, 47 mm, 0,45 μ m, grey/black	100 pieces			
Accessories					
NG0345-0001	Vacuum Manifold	1 piece			
NG0396-0080	Filter Stopper, non-sterile, no. 8 rubber	3 pieces			
NG0399-0001	Filter Forceps, bent tip	1 piece			
NG0399-0002	Filter Forceps, straight tip	1 piece			
NG0397-0010	Filter Funnel Adapter, non-sterile	25 pieces			
NG0395-0708	Vacuum Gasket, non-sterile thermoplastic elastomer	6 pieces			

Nalgene Reusable	Analytical Filters and accessories	
Product code	Product name	Sample numbers
NG0315-0047	Filter Funnels with Clamp, 250 mL	1 piece
NG0300-4000	Filter Holders with Receiver, 500 mL, 500 mL	4 pieces
NG0300-4050	Filter Holders with Receiver, 250 mL, 250 mL	4 pieces
NG0300-4100	Filter Holders with Receiver, 500 mL, 1000 mL	4 pieces
NG0310-4000	Filter Holders with Funnel, 250 mL	1 piece
NG0310-4050	Filter Holders with Funnel, 500 mL	1 piece
NG0320-2545	Reusable Bottle Top Filters, 250 mL	1 piece
NG0320-5033	Reusable Bottle Top Filters, 500 mL	1 piece
NG0320-5045	Reusable Bottle Top Filters, 500 mL	1 piece

Culti-Loops QC Organisms

for quality control testing according to ISO 11133:2014

Escherichia coli QC Organisms for testing of CCA Agar		
Product code	Product name	Sample numbers
R4607085	Escherichia coli ATCC® 8739"*	WDCM 00012
R4607050	Escherichia coli ATCC [®] 25922 ^{™*}	WDCM 00013
R4607080	Enterobacter aerogenes ATCC [®] 13048 ^{™*}	WDCM 00175
R40607060	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
Enterococci QC Organisms for testing of Slanetz and Bartley Agar		
R4607085	Escherichia coli ATCC® 8739"*	WDCM 00012
R4607050	Escherichia coli ATCC® 25922"*	WDCM 00013
R4607016	Staphylococcus aureus subsp. aureus ATCC [®] 6538 [™] *	WDCM 00032
R4607010	Staphylococcus aureus subsp. aureus ATCC [®] 25923™*	WDCM 00034
R4607030	Enterococcus faecalis ATCC® 29212 [™] *	WDCM 00087
R4601990	Enterococcus faecalis ATCC [®] 19433 [™] *	WDCM 00009
Pseudomonas aeruginosa QC Organisms for testing of Pseudomonas CN Agar		
R4607060	Pseudomonas aeruginosa ATCC® 27853™*	WDCM 00025
R4605210	Pseudomonas aeruginosa ATCC® 9027™*	WDCM 00026
R4607065	Pseudomonas aeruginosa ATCC® 10145™*	WDCM 00024
R4607085	Escherichia coli ATCC [®] 8739 [™] *	WDCM 00012
R4607050	Escherichia coli ATCC [®] 25922 ^{™*}	WDCM 00013
R4601990	Enterococcus faecalis ATCC [®] 19433 [™] *	WDCM 00009
R4601990	Enterococcus faecalis ATCC® 29212 [™] *	WDCM 00087
Clostridium perfringens QC Organisms for testing of TSC Agar		
R4601221	Bacillus subtilis ATCC 6633	WDCM00003
Prepared Media for	the Quality Control testing according to ISO 11133:2014	
P05321A	CASO Agar (ISO 11133)	



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