thermoscientific



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.

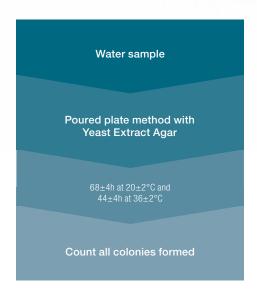
- Safe—media formulations that are compliant with ISO Standards for the testing of drinking water
- Conform to ISO standard—quality control testing methods are accredited in accordance with ISO 17025
- Proven and certified—all Certificates of Analysis (CoAs) confirm testing in accordance with the ISO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



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

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

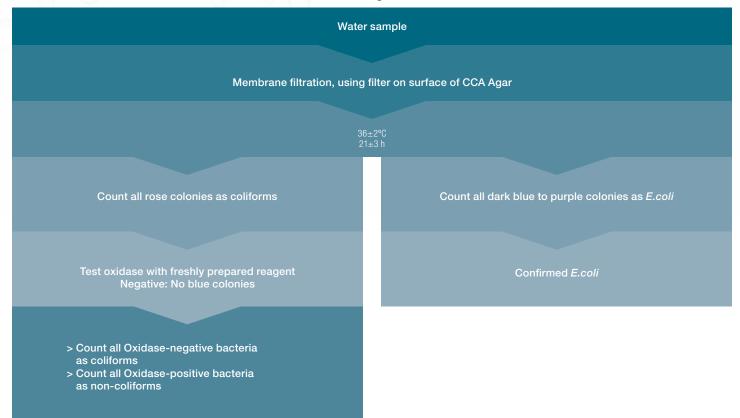
Method according to EN ISO 6222



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

Escherichia coli / Coliform bacteria

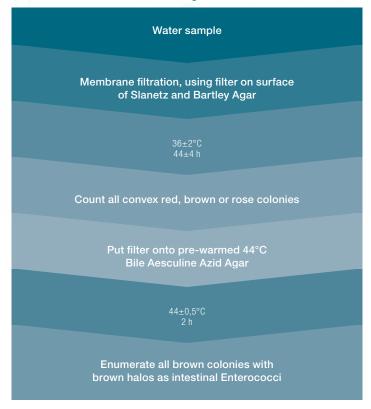
Method according to ISO 9308-1



Escherichia co	Escherichia coli and coliform						
Regulation	Method	Media	Product	Dehydrate culture m	edia	Prepared Product code	
EN ISO 9308-1 Standard test	Membrane filtration	CCA Agar	Thermo Scientific Chromogenic Coliform Agar	CM1205B	500 g	PO5318A PO5428J	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	PO5012A	10 x 90 mm plates
		Oxidase Reagent	Thermo Scientific [™] BactiDrop [™] Oxidase Reagent Thermo Scientific [™] Microbact [™] Oxidase Strips	R21540 MB0266A	50 vials 50 strips	PO5321A	10 x 90 mm plates

Enterococci

Method according to ISO 7899-2



Enterococci						
Regulation	Method	Media	Product	Dehydrated culture media Product code Format	Prepared n	
EN ISO 7899-2	Membrane filtration	Slanetz Bartley Agar	Slanetz Bartley Agar	CM0377B 500 g	PO5018A PO5410J* PO5423J*	10 x 90 mm plates 10 x 55 mm plates 10 x 55 mm plates
	Confirmation	Bile Aesculine Azide Agar	Enterococcus Selective Medium (Bile Aesculine Azid Agar)¹ or Kanamycin Aesculin Azide Agar Base²	CM0591B 500 g	PO5062A PO5059A	10 x 90 mm plates 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 Inspection under UV light (360±20 nm) Inspection under natural light Inspection under natural light Blue green (Pyocyanine forming) colonies: confirmed *Pseudomonas aeruginosa* presumptive 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 36±2°C up to 5 days 36±2°C 22±2 h Add Nessler's Reagent 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	aeruginosa				
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	R21540 50 vials MB0266A 50 strips	

^{*} 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 yellowbrownish colonies

Subculture all presumptive colonies onto blood agar plates

36±2°C, anaerobic 21+3 h >10 black or grey to yellowbrownish 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						
Regulation	Method	Media	Product	Dehydrat culture m	nedia	Prepared Product cod	
ISO 14189	Membrane filtration	TSC Agar	TSC Selective Agar			PO5315A	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
	atmosphere		Thermo Scientific™ AnaroJar™ 2,5 L Anaerobic System Thermo Scientific™ AnaeroBox™ Rectangular Anaerobic System			AG0025A AB0025A	1 pot 1 pot



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.

Samples with high concentration of Legionella species and Samples with low concentration of Legionella species and low concentration of interfering organisms² low concentration of interfering organisms2 0.1-0.5 mL of the sample; each Membrane filtration of 100 mL untreated, heat treated and acid sample¹. Place one untreated treated from the membrane 0.1-0.5 mL of the 0.1-0.5 of the sample membrane filter onto BCYE Agar; filtration with washing procedure onto BCYE with AB Place membrane filter with acid onto BCYE Agar and on one or sample onto BCYE solution on one or more selective more selective media: BCYE media: BCYE Agar with AB or Agar with AB or GVPC Agar or **GVPC Agar or MWY Agar** MWY Agar Subculture at least 3 typical Legionella colonies from each plate and inoculate on BCYE Agar without Cystein and BCYE Agar 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.

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	Prepare Product code	ed media Format
ISO 11731:2017 and EN ISO 111731-2	Sample preparation	Acid Buffer	Legionella Acid Buffer Solution		GFB01	6 x 1 L bottles
	Direct and membrane filtration	GVPC Agar	Legionella GVPC Selective Agar Legionella CYE Agar-Basis + Legionella BCYE Supplement + Legionella GVPC Selektive Supplement	CM0655B 500 g SR0110C 10 tubes (500 mL) SR0152E 10 tubes (500 mL)		10 x 90 mm plates 10 x 90 mm plates
		BCYE Agar with antibiotics	BCYE Agar with antibiotics		PO5325A	10 x 90 mm plates
		BCYE Agar	BCYE Agar		PO5072A	10 x 90 mm plates
		MWY Agar	MWY Agar		PO5071A	10 x 90 mm plates
	Subculture	BCYE Agar without Cystein	BCYE Agar without Cystein		PO5028A PO0255A*	10 x 90 mm plates 10 x 90 mm plates
		BCYE Agar	BCYE Agar		PO5072A	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, enum	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			
Legionella spp. <u>b</u>	y molecular methods only				
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 Disposa	able 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 μm, white/black	100 pieces
NG0210-6045	Membrane Filter for water testing, sterile, CN, 47 mm, 0,45 μm, 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

	0000	
Nalgene Reusabl	le 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

	Part days	
Product code	Product name	Sample number
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 a	eruginosa 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 per	ringens 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	
PO5321A	CASO Agar (ISO 11133)	



^{*} The ATCC Licensed Derivative Emblem, the ATCC Licensed Derivative word mark, and the ATCC catalog marks are trademarks of ATCC. Remel Inc. is licensed to use these trademarks and sell products derived from ATCC® cultures. The identity, purity, and authenticity of the Licensed Products are exclusively the responsibility of Remel Inc., and not ATCC.

© 2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. ATCC is a trademark of the American Type Culture Collection. Combisart, Microsart and Sartorius are trademarks of Sartorius Stedim Biotech GmbH. Microsart e.motion and Microsart e.jet are trademarks of Sartorius Lab Instruments GmbH & Co. KG.

