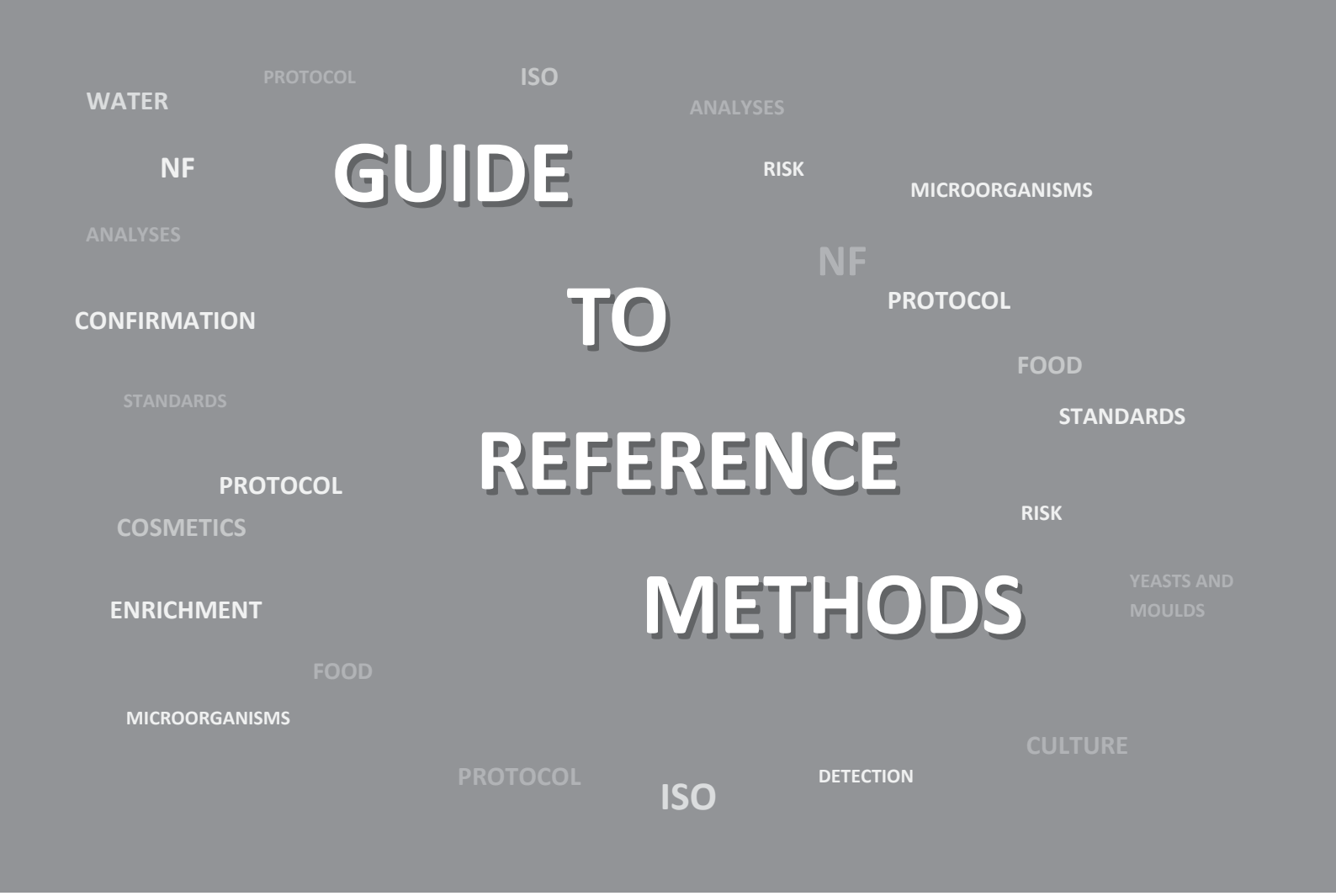


Water and environmental microbiology

Version dated March 2019

Biokar diagnostics

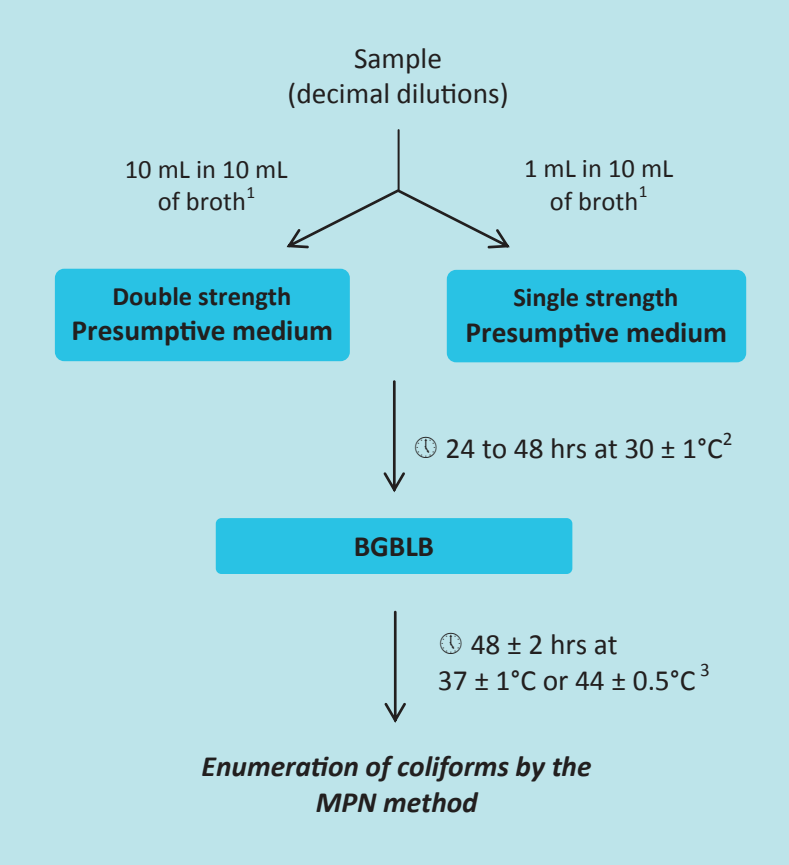
CONTENTS

Detection and enumeration of coliforms and thermotolerant coliforms

General method by culture in liquid media (MPN)

NF T 90-413: 10-1985
T 90-413

1. Protocol



[1] Lactose broth and sodium lauryl sulfate broth may be used as presumptive medium.
Inoculate each dilution in three tubes of double strength medium and three tubes of single strength medium.

[2] Prolong the incubation up to 48 hours if no gas is released after 24 hours of incubation.

[3] Incubate the BGBLB at 37°C for coliforms and at 44°C for thermotolerant coliforms. Schubert broth may be used for the detection of thermotolerant coliforms, with incubation for 24 hours.

2. Media and reagents

Section	Media and reagents	Compliance
6.2 Diluent	- *Ringer's solution* **Ringer's solution (1/4 strength)** 100 tablets - BR00108 - *Distilled water* **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408	Total Total
6.3.1 Presumptive media	- *Lactose broth* **BCP lactose broth** 500 g vial - BK119HA - *Sodium lauryl sulfate broth* **Tryptose lauryl sulfate broth** 50 x 10 mL tubes with Durham tubes (single strength) - BM09708 50 x 10 mL tubes with Durham tubes (double strength) - BM09808 500 g vial - BK010HA	Total [4] Total
6.3.2 Confirmation media	- *Brilliant green bile lactose broth* **Brilliant green bile lactose broth (BGBLB)** 50 x 10 mL tubes with Durham tubes - BM01108 500 g vial - BK002HA - *Schubert broth (modified by Fennel)* [5] -	Total [4] -

[4] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

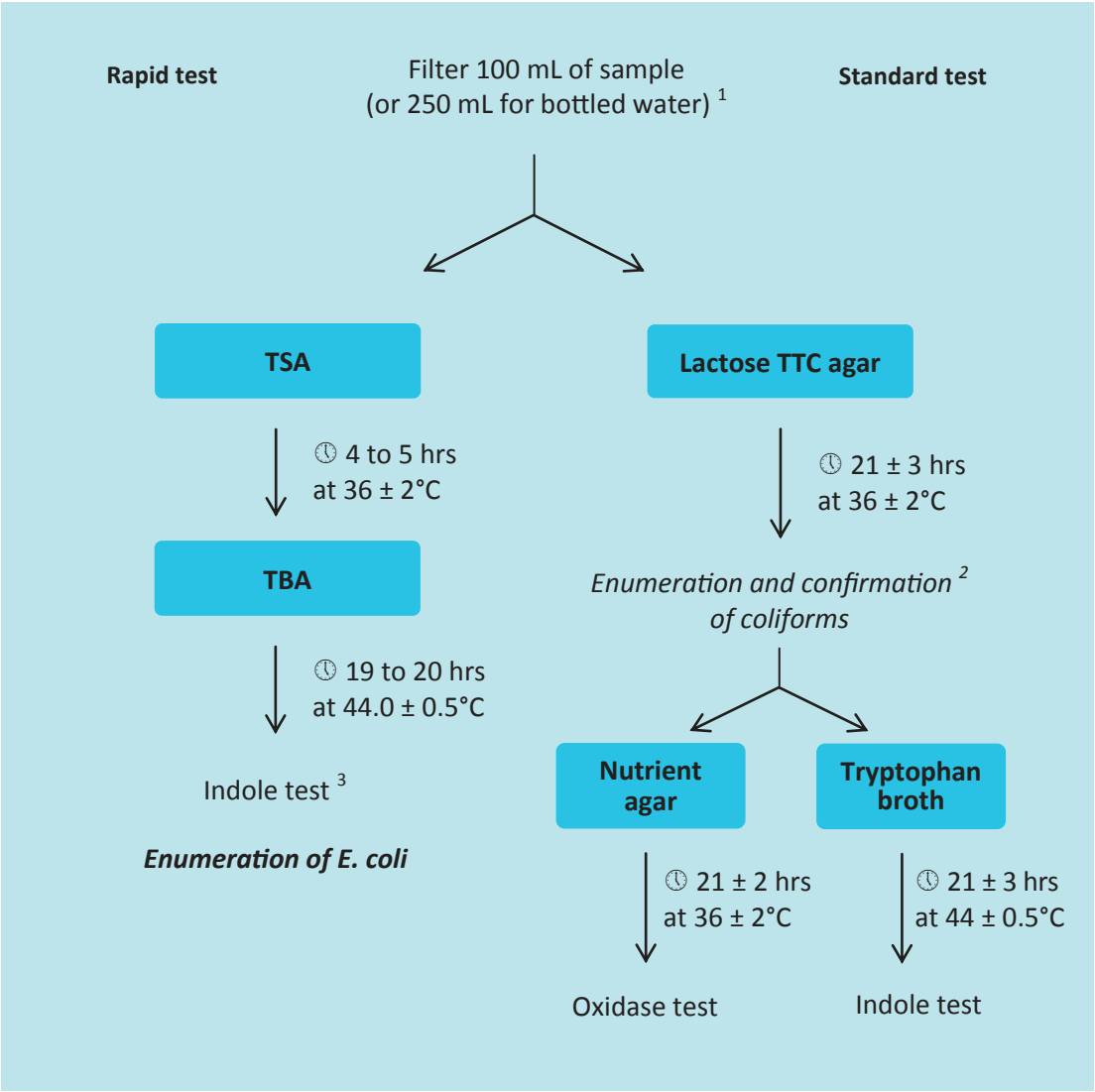
[5] Confirmation medium used for the detection of thermotolerant coliforms.

Detection and enumeration of *Escherichia coli* and coliform bacteria

Part 1: Membrane filtration method

NF EN ISO 9308-1: 09-2000
T 90-414

1. Protocol



[1] Carry out the rapid test and standard test simultaneously.

[2] Reinoculate at least 10 typical colonies on the nutrient medium, then carry out the confirmation tests.

[3] Place the membrane on a filter paper disc saturated with reagent for the detection of indole, and irradiate under an ultraviolet lamp for 10 to 30 minutes according to colour development.

2. Media and reagents

Section	Media and reagents	Compliance
B.1 Agar medium for standard test	- *Lactose TTC agar with sodium heptadecyl sulfate* **Lactose TTC agar with Tergitol 7** 20 plates Ø 55 mm - BM14708 120 plates Ø 55 mm - BM09308 **Lactose TTC agar with Tergitol-7 (dehydrated base)** 500 g vial - BK123HA **TTC supplement** 10 x 12.5 mg vials - BS02608 10 x 50 mg vials - BS02708	Total
B.3 Agar medium for rapid test	- *Tryptone soy agar* **Trypto-casein Soy Agar TSA** [4] 20 plates Ø 90 mm - BM05008 10 x 100 mL vials - BM01708 10 x 200 mL vials - BM04908 500 g vial - BK047HA	Total [5]
B.4 Agar medium for rapid test	- *Tryptone bile agar (TBA)* -	-
B.2 Confirmation medium	- *Tryptophan broth* **Tryptophan broth** 500 g vial - BK163HA 50 x 3 mL tubes - BM07608	Total [5]
B.5 Confirmation reagents	- *Kovac's reagent for indoles (standard test)* - - *Indole reagent (rapid test)* - - *Oxidase reagent* -	- - -

[4] pH 7.3 ± 0.2 instead of 7.2 ± 0.1.

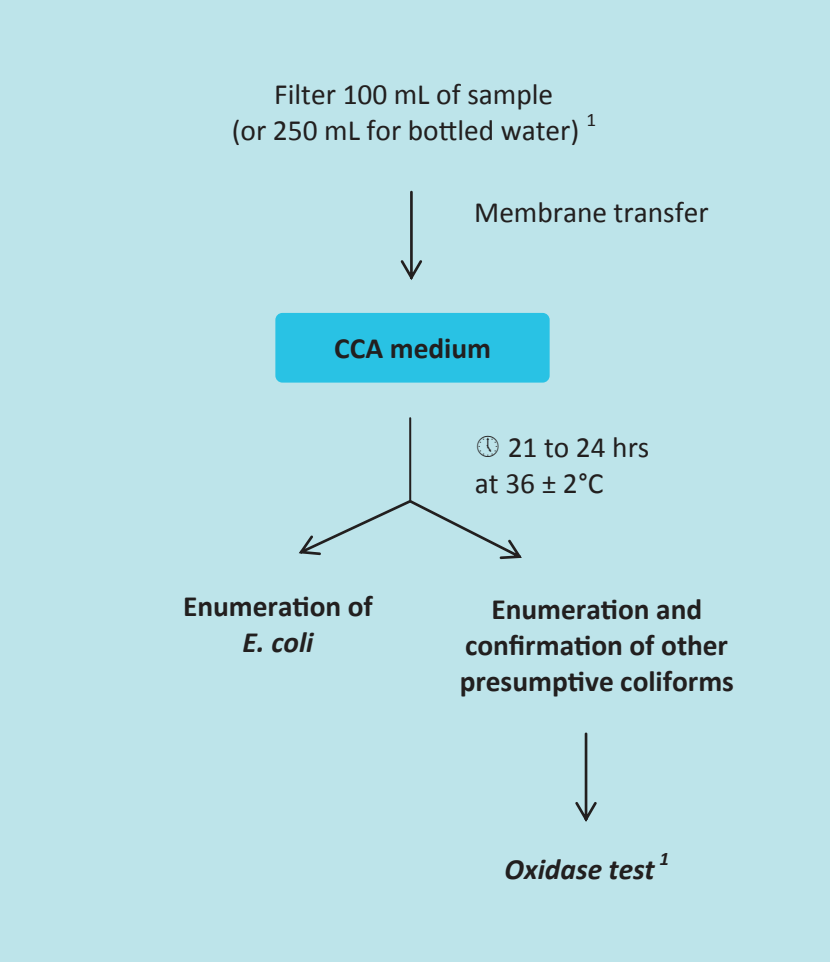
[5] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

Enumeration of *Escherichia coli* and coliform bacteria

Part 1: Membrane filtration method for water with low bacterial background flora

ISO 9308-1: 09-2014
Modified by amendment A1 (12-2016)

1. Protocol



[1] If necessary, a subculture may be prepared on medium to obtain isolated colonies.
Confirm all or at least 10 characteristic colonies

2. Media and reagents

Section	Media and reagents	Compliance
B.1	- *Chromogenic coliform agar (CCA)* **CCA agar** 20 Petri dishes - BM18208 500 g vial - BK204HA	Total
B.2	- *Oxidase reagent* -	-
B.3	- *Tryptone soy agar (TSA)* **Trypto-casein Soy Agar TSA** [2] 20 plates Ø 90 mm - BM05008 10 x 100 mL vials - BM01708 10 x 200 mL vials - BM04908 500 g vial - BK047HA	Total

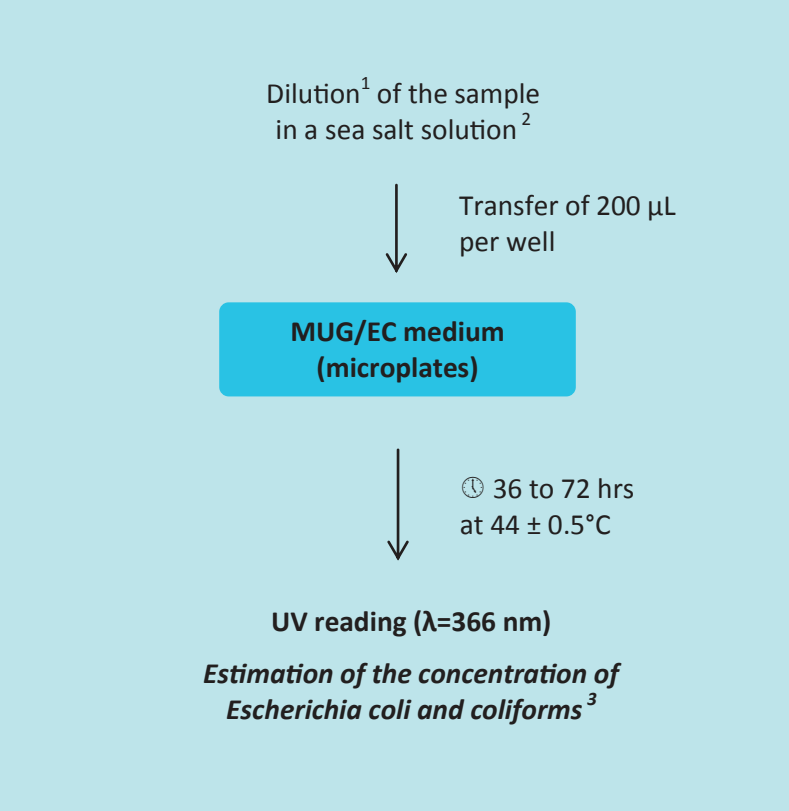
[2] pH 7.3 ± 0.2 instead of 7.2 ± 0.1.

Detection and enumeration of *Escherichia coli* and coliform bacteria in surface and waste water

Part 3: Miniaturized method (MPN) by inoculation in liquid medium

NF EN ISO 9308-3: 03-1999
T 90-433

1. Protocol



[1] The number of dilutions varies according to the type of sample.
For surface water, prepare decimal dilutions from 1:2 to 1:2000.
For waste water or water from sewage treatment plants, prepare decimal dilutions of 1:2 to 1:200,000.

[2] For water with a salt content above 30 g/L, the first 1:2 dilution is prepared in sterile distilled water.
The sea salt solution is used for the subsequent dilutions.

[3] Count the fluorescent wells per dilution, and perform a statistical estimation of the microorganism concentration in the sample analysed (using the statistics table).

2. Media and reagents

Section	Media and reagents	Compliance
7.2 Diluent	- *Special (SD)* **Synthetic sea salt (DSM)** 50 x 18 mL tubes - BM08808 100 g vial - BR00308 - *Distilled water* **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408	Total Total
7.3 Culture medium	- *MUG/EC medium* **MUG/EC microplate** 25 opaque microplates - BT00108	Total

COMPASS® *cc* Agar

Alternative method for the enumeration of *Escherichia coli* and other coliform bacteria

BKR 23/08-06/12
Alternative water analysis method
www.afnor-validation.org

1. Protocol

Filter 100 mL of sample
(or 250 mL for bottled water)

↓ Transfer the membrane

COMPASS® *cc* Agar

↓ 24 ± 3 hrs at 36 ± 2°C

Enumeration of Escherichia coli (blue to dark blue colonies) and coliforms (pink colonies)

Without confirmation

2. Media and reagents

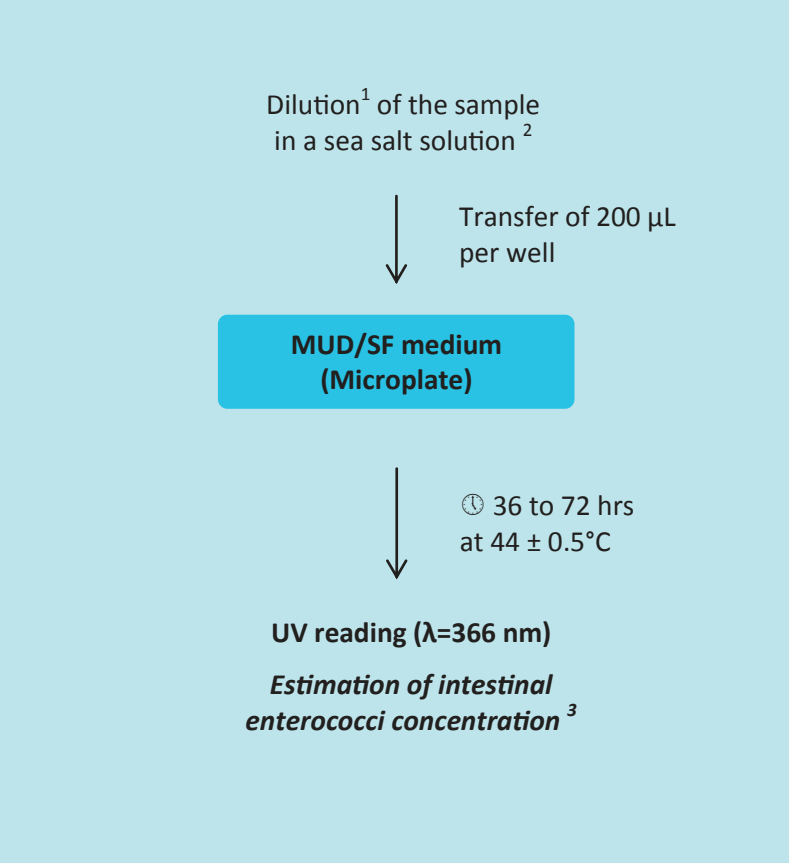
Selective medium	**COMPASS® *cc* Agar pre-poured** 20 Petri dishes Ø 55 mm - BM15308 **COMPASS® *cc* Agar (base)** 500 g vial - BK210HA **Supplement for COMPASS® *cc* Agar** 10 vials q.s. 500 mL - BS08408

Detection and enumeration of intestinal enterococci for surface and waste water

Part 1: Miniaturized method (MPN) by inoculation in liquid medium

NF EN ISO 7899-1: 03-1999
T 90-432

1. Protocol



[1] The number of dilutions varies according to the type of sample.
For surface water, prepare decimal dilutions from 1:2 to 1:2000.
For waste water or water from sewage treatment plants, prepare decimal dilutions of 1:2 to 1:200,000.

[2] For water with a salt content above 30 g/L, the first 1:2 dilution is prepared in sterile distilled water. The sea salt solution is used for the subsequent dilutions.

[3] Count the fluorescent wells per dilution, and perform a statistical estimation of the microorganism concentration in the sample analysed (using the statistics table).

2. Media and reagents

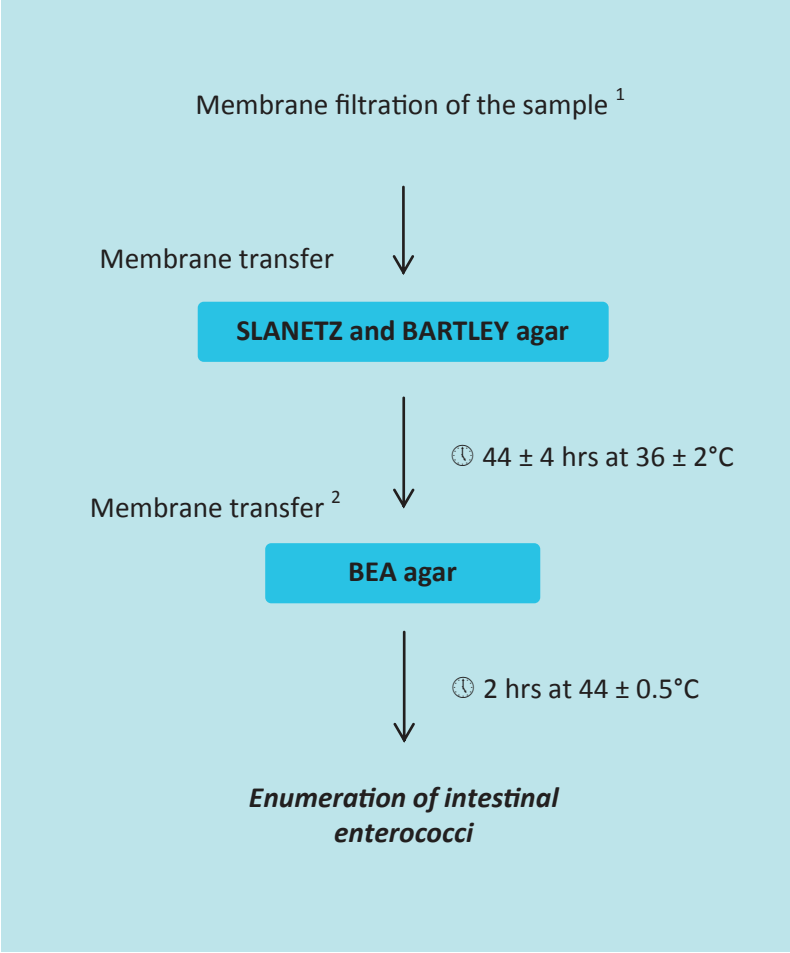
Section	Media and reagents	Compliance
7.2 Diluent	- *Special (SD)* **Synthetic sea salt (DSM)** 50 x 18 mL tubes - BM08808 100 g vial - BR00308 - *Distilled water* **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408	Total Total
7.3 Culture medium	- *MUD/SF medium* **MUD/SF microplate** 25 opaque microplates - BT00308	Total

Detection and enumeration of intestinal enterococci

Part 2: Membrane filtration method

NF EN ISO 7899-2: 08-2000
T 90-416

1. Protocol



[1] Filter a suitable volume of water for the type of water examined.

[2] Transfer the membrane to the confirmation medium (BEA agar), in the presence of characteristic colonies with a red, brown or pink colour in the centre or over the whole colony.

2. Media and reagents

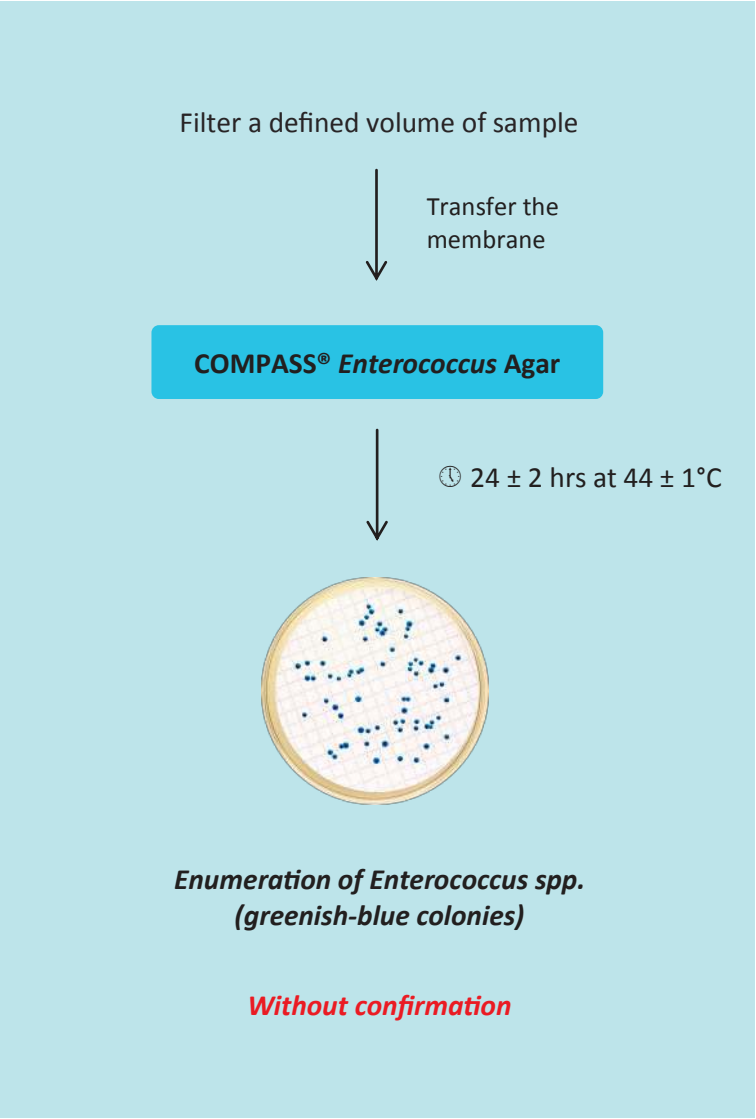
Section	Media and reagents	Compliance
6.2 Diluent	- *Distilled water* **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408	Total
6.3.1 Selective medium	- *Slanetz and Bartley medium* **Complete SLANETZ and BARTLEY agar** 20 Petri dishes Ø 55 mm - BM14608 120 Petri dishes Ø 55 mm - BM09408 500 g vial - BK037HA **SLANETZ and BARTLEY agar, dehydrated base** 500 g vial - BK129HA **TTC supplement** 10 x 12.5 mg vials - BS02608 10 x 50 mg vials - BS02708	Total
6.3.2 Confirmation medium	- *Bile Esculin Azide medium* **BEA agar** 10 x 100 mL vials - BM10408 500 g vial - BK158HA	Total

COMPASS® *Enterococcus* Agar

Alternative method for the enumeration of *Enterococcus* spp.

Alternative analysis method

1. Protocol



2. Media and reagents

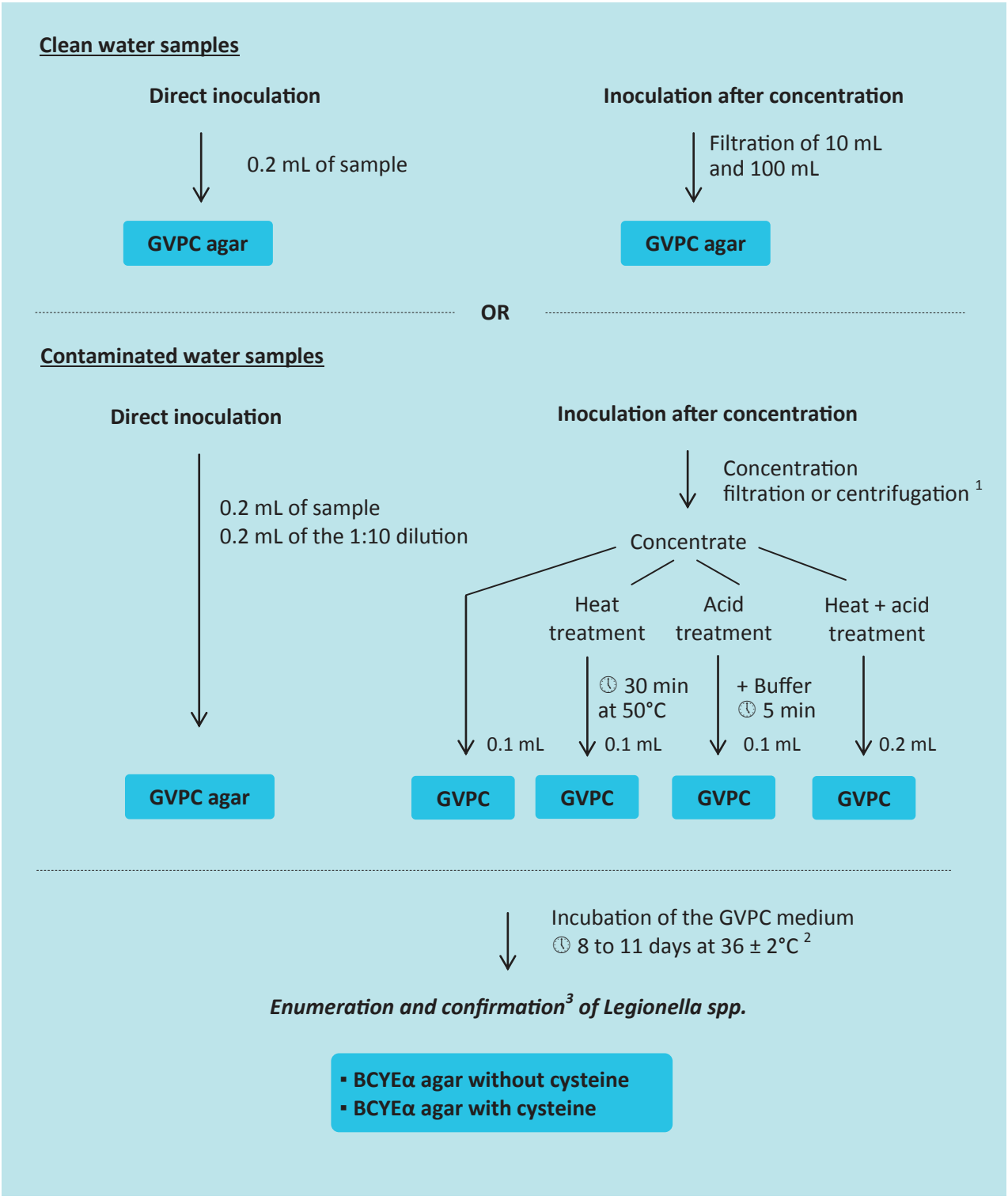
Selective medium	**COMPASS® *Enterococcus* Agar pre-poured** 20 Petri dishes Ø 55 mm - BM15708 **COMPASS® *Enterococcus* Agar ready-to-melt** 10 x 100 mL vials - BM11608 **COMPASS® *Enterococcus* Agar dehydrated** 500 g vial - BK183HA

Detection and enumeration of *Legionella* spp. and *Legionella pneumophila*

Method by direct inoculation and after concentration by membrane filtration or centrifugation

NF T 90-431: 08-2017
T 90-431

1. Protocol



2. Media and reagents

Section	Media and reagents	Compliance
7.2 Diluent	- *Purified water* **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408 - *Buffer pH 2.0* -	Total -
7.3. Culture media	- *Complete GVPC medium with antibiotics* **Selective GVPC agar for Legionella** 20 Petri dishes Ø 90 mm - BM07108 - *BCYE medium without cysteine* **BCYEα agar without cysteine** 20 Petri dishes Ø 90 mm - BM07308 - *BCYE medium without antibiotics* **BCYEα agar with cysteine** 20 Petri dishes Ø 90 mm - BM07208	Total Total Total

[1] For the contaminated water, carry out concentration of 500 mL of water by membrane filtration or centrifugation. In both cases, take up the filtrate or pellet in 5 mL of purified water.

[2] Examine the plates at least three times after 3 to 4 days, up to the end of the incubation period.

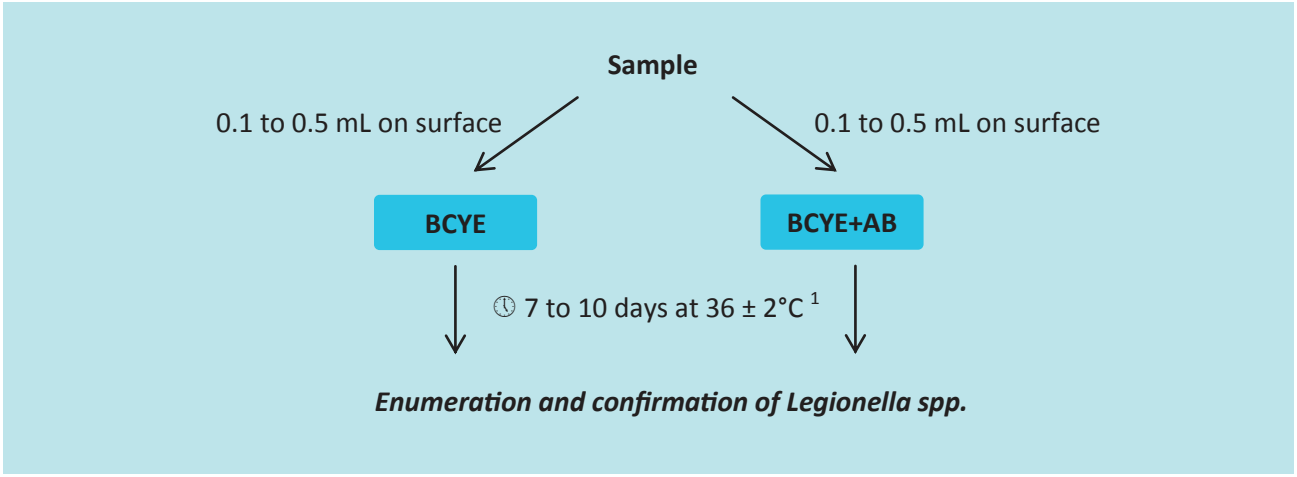
[3] Reinoculate 5 selected colonies on the BCYEα medium with cysteine and on the BCYEα medium with cysteine, then incubate for at least 2 days at 36 ± 2°C. The Legionella colonies are confirmed by growth on the BCYEα agar without cysteine and the absence of growth on the BCYEα agar with cysteine.

Enumeration of *Legionella*

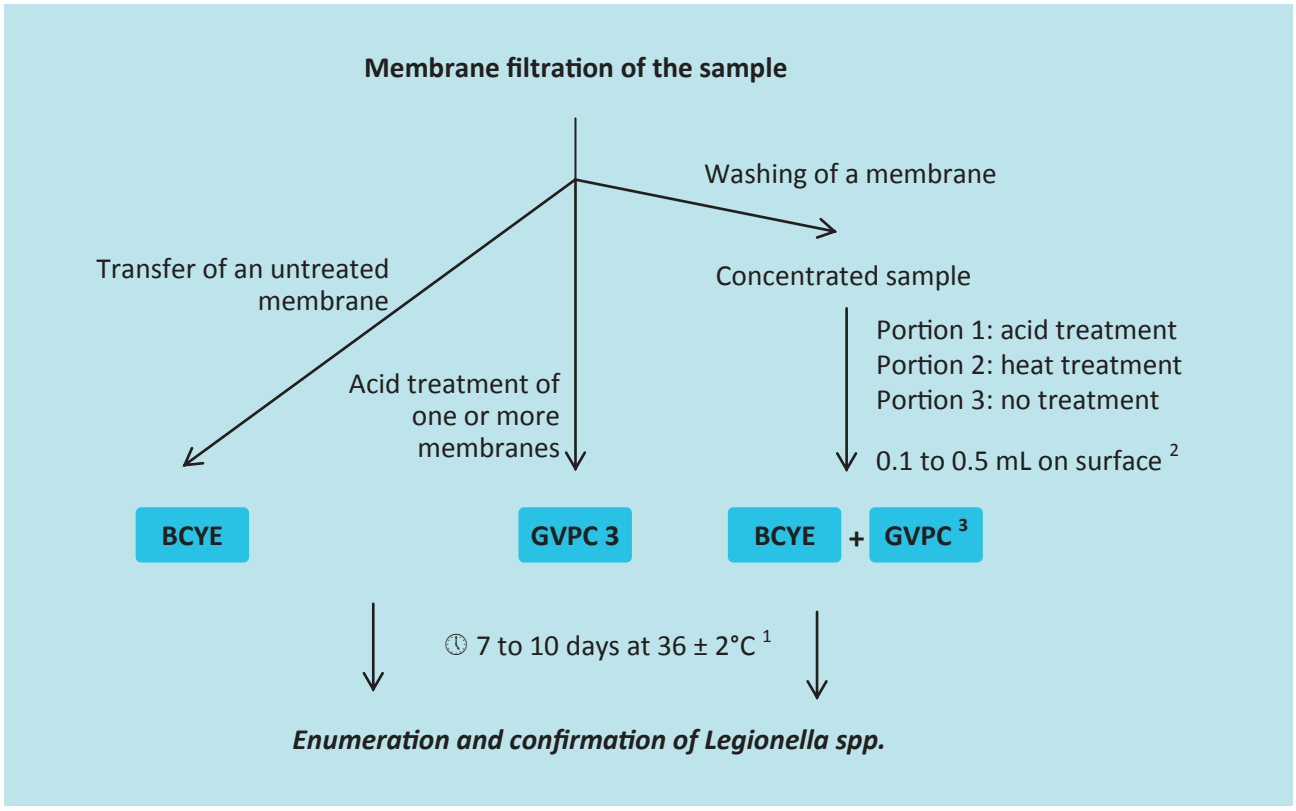
NF EN ISO 11731: 07-2017
T 90-430

1. Protocol

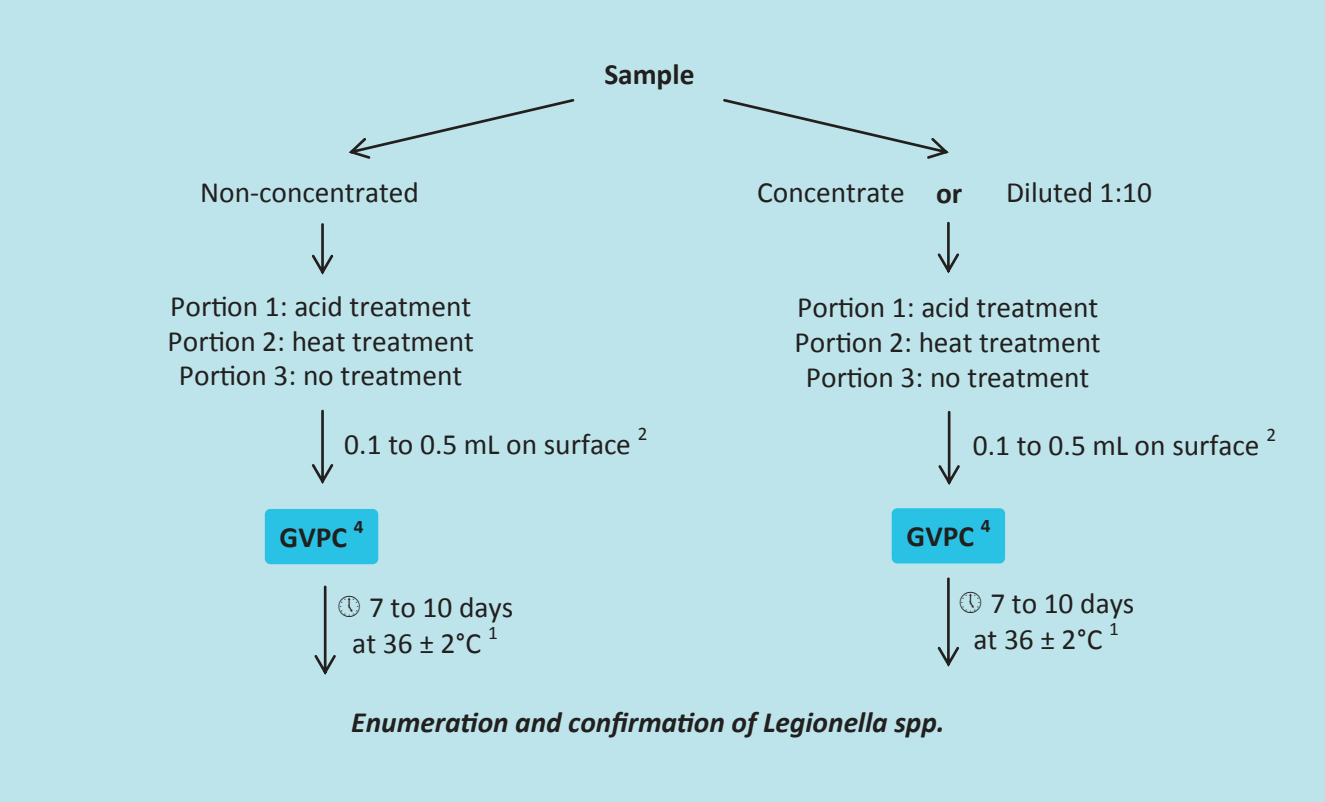
A- Sample with a high concentration of Legionella (>10^4 CFU/L) and a low concentration of interfering microorganisms



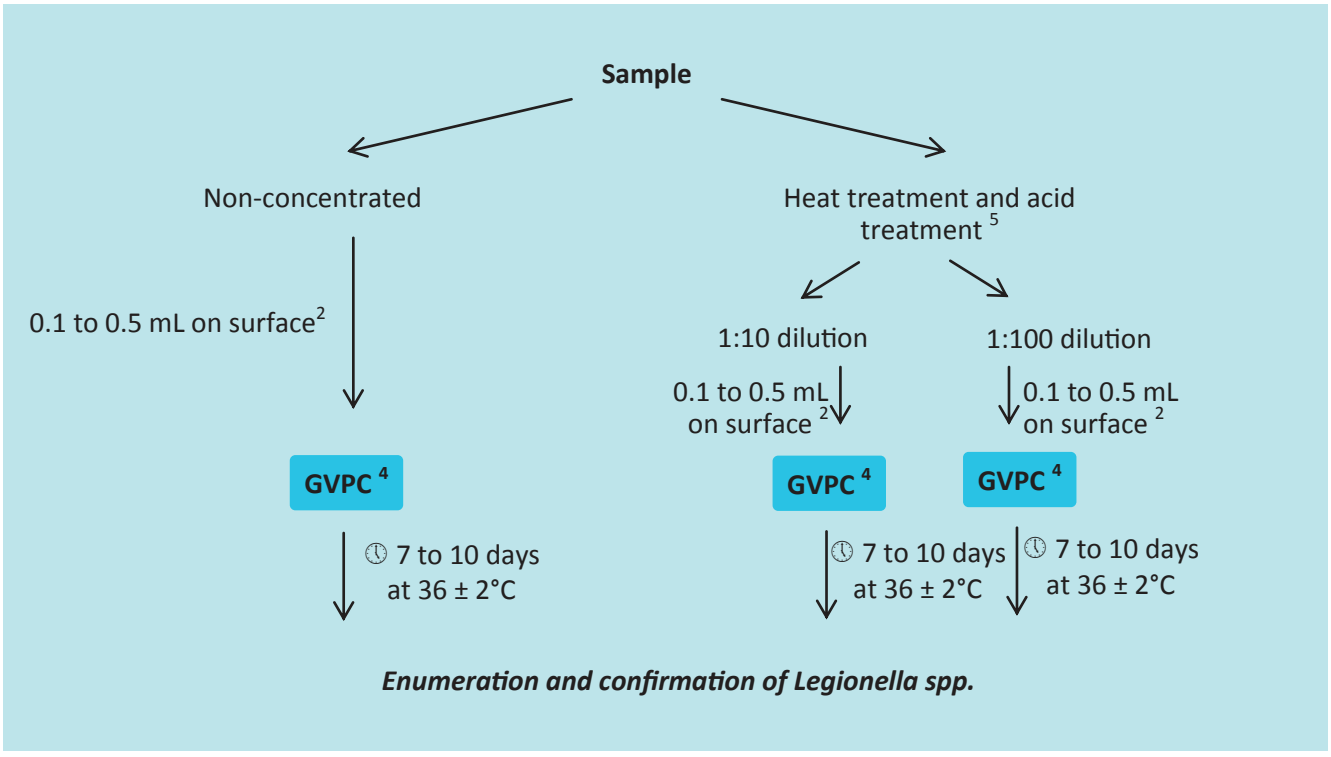
B- Sample with a low concentration of *Legionella* and a low concentration of interfering microorganisms



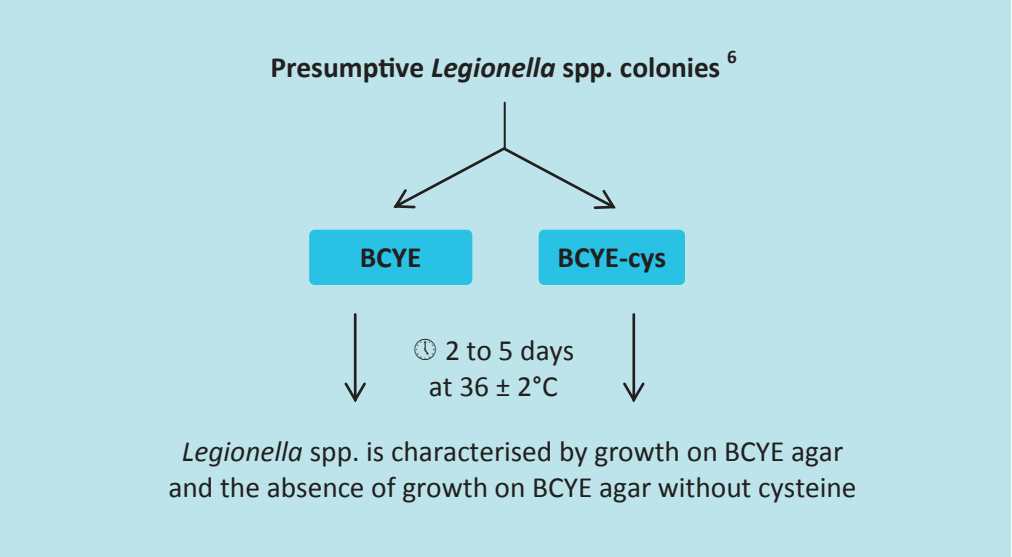
C- Sample with a HIGH concentration of interfering microorganisms



D- Sample with a VERY HIGH concentration of interfering microorganisms



Confirmation of presumptive *Legionella* colonies



[1] Carry out a first reading on the plates on the second, third, fourth, or fifth day, then a final reading at the end of the incubation period.
[2] Using each portion (acid-treated, heat-treated and untreated), inoculate a defined volume on BCYE medium and on a second selective medium.
[3] Other selective media may also be used, such as BCYE agar+AB or MWY agar.
[4] MWY medium may also be used as a selective medium.
[5] For the associated treatment, first carry out the heat treatment, then the acid solution treatment .
[6] Reinoculate at least one colony for each morphological type. If there is only one type of colony, sample three colonies.

2. Media and reagents

Section	Media and reagents	Compliance
Confirmation culture media B.1/B.2/B.3/ B.4/B.5	- *Buffered charcoal yeast extract agar (BCYE)* **BCYEα agar with cysteine** 20 Petri dishes Ø 90 mm - BM07208	Total
	- *Buffered charcoal yeast extract agar without cysteine (BCYE-cys)* **BCYEα agar without cysteine** 20 Petri dishes Ø 90 mm - BM07308	Total
	- *Buffered charcoal yeast extract agar with selective supplements (BCYE+AB)* -	-
	- *Glycine vancomycin polymyxin B cycloheximide agar (GVPC)* **GVPC agar for Legionella** 20 Petri dishes Ø 90 mm - BM07108	Total
	- *Modified Wadowsky Yee (MWY) agar* -	-
Subculture media B.6/B.7/B.8	- *Blood agar* **Tryptone soy (blood agar base)** 500 g vial - BK028HA	Equivalent
	- *Nutrient agar* **2.5% nutrient agar** [7] 50 x 18 mL tubes - BM12508	Total
	- *Tryptone soy agar (TSA)* **Trypto-casein Soy Agar TSA** [7] 20 plates Ø 90 mm - BM05008 10 x 100 mL vials - BM01708 10 x 200 mL vials - BM04908 500 g vial - BK047HA	Total

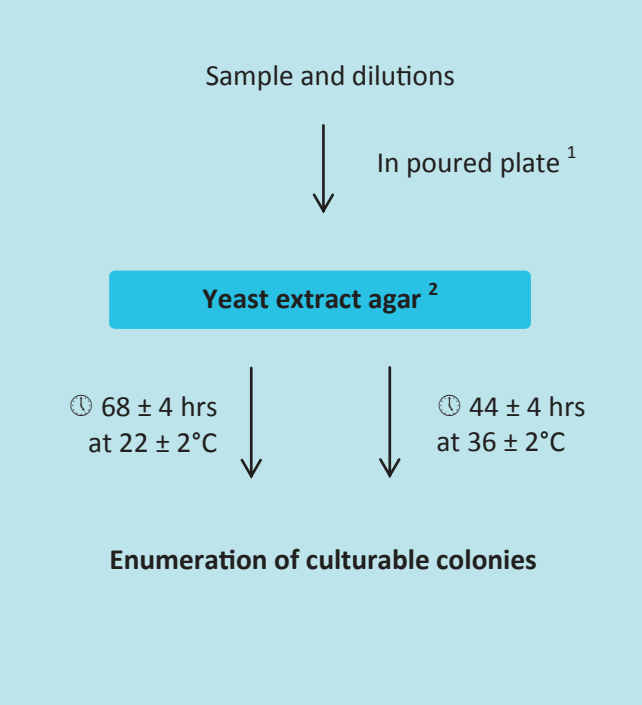
[7] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

Enumeration of culturable microorganisms

Colony count by inoculation in a nutrient agar culture medium

NF EN ISO 6222: 07-1999
T 90-401

1. Protocol



[1] Inoculate a volume of not more than 2 mL in the Petri dish, and add 15 to 20 mL of melted medium. Carry out inoculation in duplicate.
[2] Incubate a set of plates at 36°C for 44 hours and another set simultaneously at 22°C for 68 hours.

2. Media and reagents

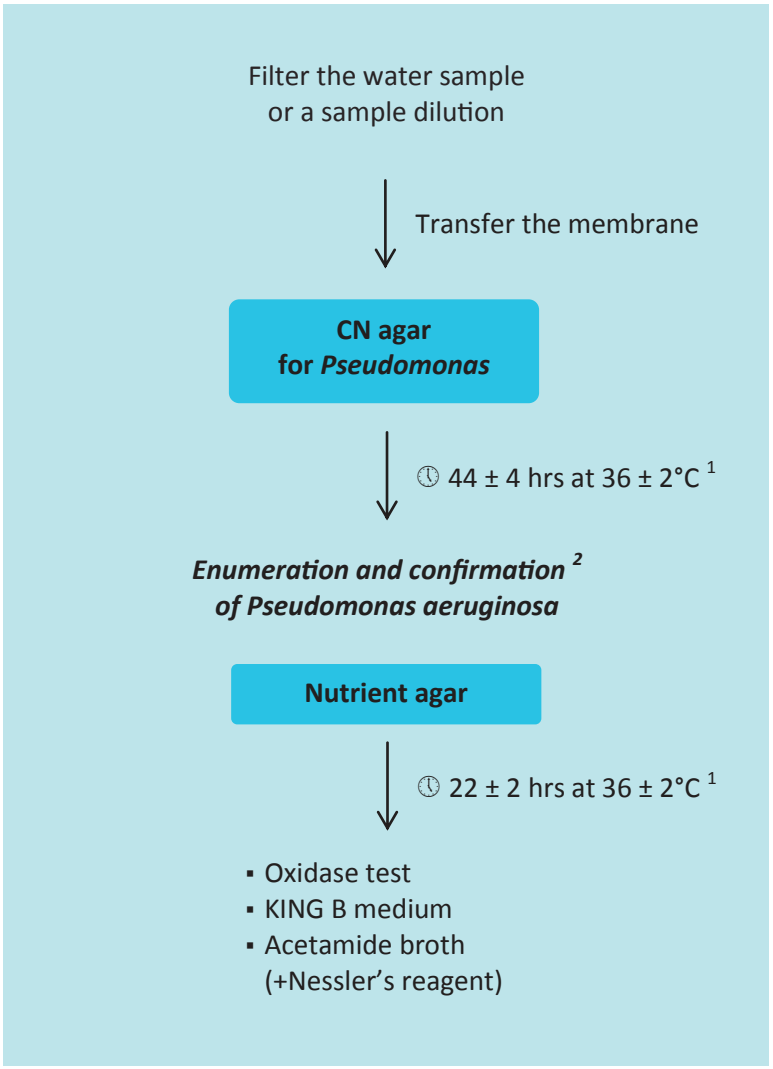
Section	Media and reagents	Compliance
7.2 Diluent	Refer to standard NF EN ISO 8199, page 44.	
7.3 Culture medium	- *Yeast extract agar* **Yeast extract agar (PCA without glucose)** 10 x 200 mL vials - BM06808 500 g vial - BK153HA	Total

Detection and enumeration of *Pseudomonas aeruginosa*

Method by membrane filtration

NF EN ISO 16266: 08-2008
T 90-419

1. Protocol



[1] Check the growth of the cultures after 22 ± 2 hours and 44 ± 4 hours. The final enumeration result is obtained after 44 ± 4 hours of incubation.

[2] Reinoculate all colonies requiring confirmation or as many colonies as possible, if this is an option.

Colonies producing pyocyanin (greenish-blue pigmentation) or positive oxidase, which are fluorescent in UV radiation and capable of producing ammonia from acetamide should be considered to be *Pseudomonas aeruginosa.*

2. Media and reagents

Section	Media and reagents	Compliance
5.1 Culture medium	- *Pseudomonas agar base (CN-agar)* **CN agar for *Pseudomonas*** 20 Petri dishes Ø 55 mm - BM14508 120 Petri dishes Ø 55 mm - BM19608 500-g vial - BK165HA [3]	Total
5.2 Confirmation culture media and reagents	- *King B medium* **KING B agar** 7 x 7 mL tubes - BM10508	Total
	- *Acetamide broth* **Acetamide broth** 7 x 5 mL tubes - BM09508	Total
	- *Nutrient agar* **2.5% nutrient agar** 50 x 18 mL tubes - BM12508	Total [4]
	- *Oxidase reagent* -	-
	- *Nessler's reagent* -	-

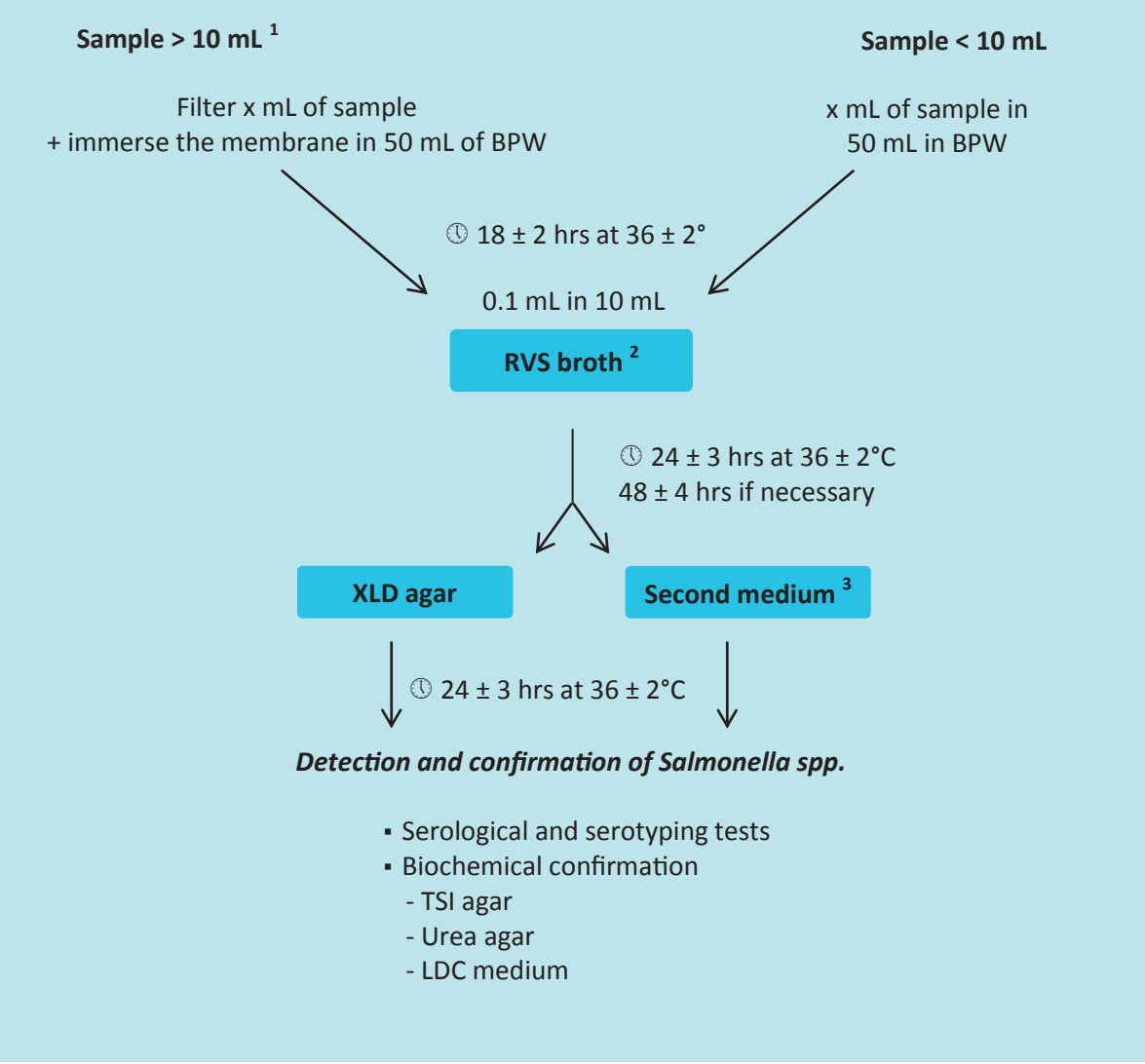
[3] 10 mL of glycerol per litre of agar base medium needs to be added to the dehydrated medium.

[4] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

Detection of *Salmonella* spp.

NF EN ISO 19250: 06-2013
T 90-435

1. Protocol



[1] For turbid or polluted water, a sterile filtration adjuvant may be added and a sample may be filtered through a sterile absorbent buffer acting as a support instead of using the membrane.

[2] MKTTn broth may also be used at the same time; inoculate 1 mL of the pre-enrichment obtained in 10 mL, and incubate for 24 ± 3 hrs at 36 ± 2°C.

[3] Its intrinsic properties should complement those of XLD agar and enable detection of lactose + *Salmonella* (including *typhi* and *paratyphi*).

[4] Formula including 9.0 g/L of disodium phosphate dodecahydrate (molecular mass = 358.14).

[5] Formula including 3.56 g/L of anhydrous disodium phosphate (molecular mass = 141.96).

[6] Formula including anhydrous magnesium chloride instead of the hexahydrate form described.

[7] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

[8] Contains 14.0 g of tryptone instead of 20.0 g of peptone as described.

2. Media and reagents

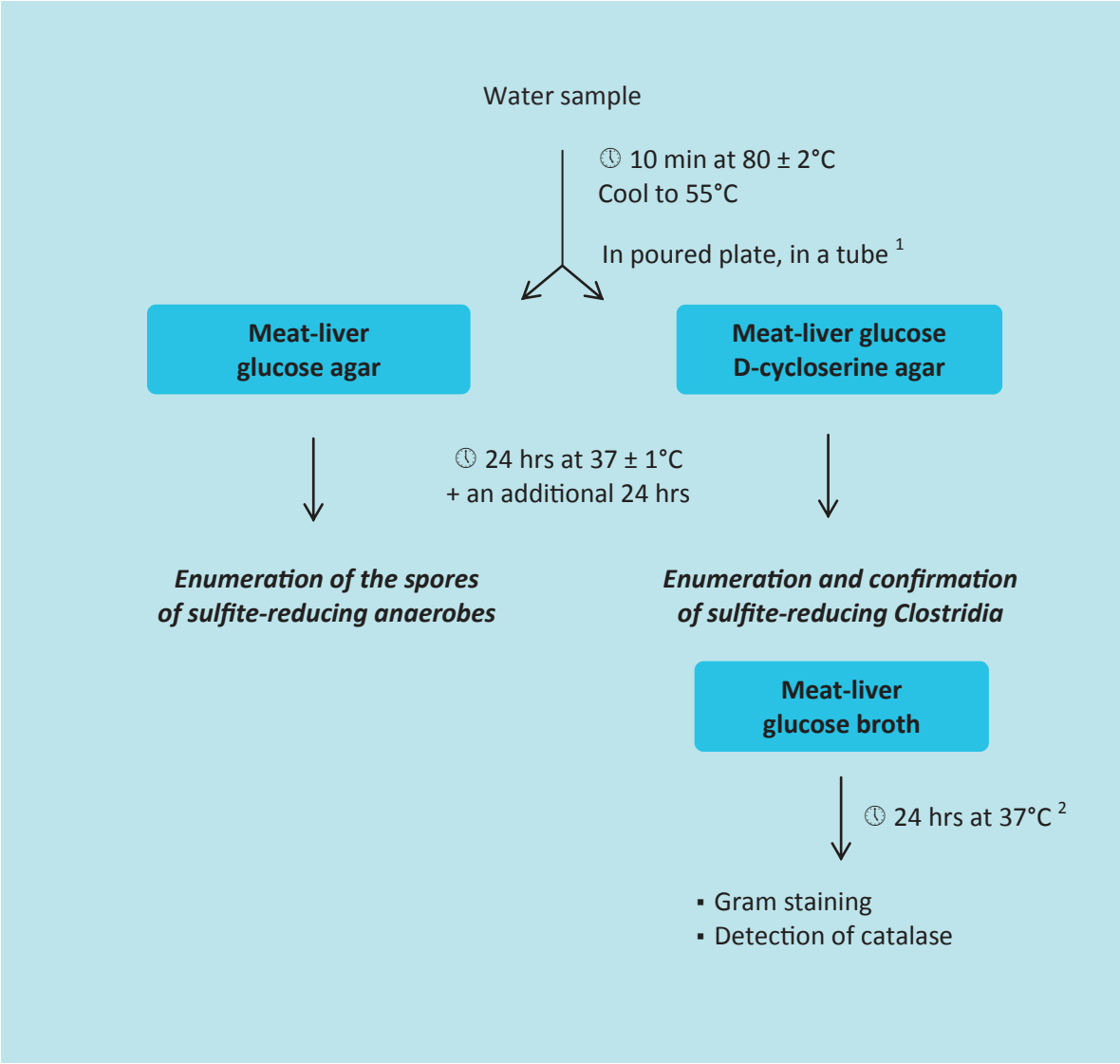
Section	Media and reagents	Compliance
B.1 Pre-enrichment medium	- *Buffered peptone water (BPW)* **BPW (20 g/L)** [4] 500 g vial - BK131HA; 5 kg drum - BK131GC **BPW (25.5 g/L)** [5] 50 x 9 mL tubes - BM05608 10 x 90 mL vials - BM05708 10 x 225 mL vials - BM01008 3 x 3 L flexible bags - BM13108 2 x 5 L flexible bags - BM13208 500 g vial - BK018HA; 5 kg drum - BK018GC	Total
B.2 First enrichment medium	- *Rappaport-Vassiliadis broth with soya (RVS)* **RAPPAPORT-VASSILIADIS broth with soya (RVS)** 50 x 18 mL tubes - BM07408 500 g vial - BK148HA	Total [6]
B.9 Second enrichment medium (optional)	- *MULLER-KAUFFMANN tetrathionate-novobiocin (MKTTn) broth* **MKTTn broth (ready-to-use)** 50 x 10 mL tubes - BM07808 **MKTTn broth (base without iodine)** 500 g vial - BK208HA **MKTTn broth (base without iodine, novobiocin)** 500 g vial - BK169HA **Selective novobiocin supplement** 10 x 10 mg vials - BS03308 8 x 40 mg vials - BS05608	Total [7]
B.3 Second plating out	- *Xylose lysine deoxycholate (XLD) agar* **XLD agar (ISO 6579)** 20 Petri dishes Ø 90 mm - BM08708 500 g vial - BK168HA	Total
	The choice of the second medium is left to the discretion of the testing laboratory [3]	
B.4 Confirmation reagent and medium	- *Nutrient agar* **2% nutrient agar** 50 x 18 mL tubes - BM11808 500 g vial - BK185HA **2.5% nutrient agar** 50 x 18 mL tubes - BM12508 - *Triple sugar and iron (TSI) agar* **TSI agar** 500 g vial - BK221HA - *Urea agar (Christensen)* - - *L-lysine decarboxylation (LDC) medium* - - *Selenite cystine broth* **Selenite cystine broth** 500 g vial - BK00908	Equivalent Total [7, 8] - - Total

Detection and enumeration of the spores of sulfite-reducing anaerobes and sulfite-reducing *Clostridia*

General method by the standing tube technique

NF T 90-415: 10-1985
T 90-415

1. Protocol



[1] The volumes of the test samples and media should be selected such that any appearing black colonies are clearly separated and easy to count. If necessary, prepare the appropriate decimal dilutions.

[2] In the context of identification of *Clostridium* colonies, reinoculate the culture on tryptose-sulfite D-cycloserine agar and incubate for 18 to 24 hrs at 37°C. Then carry out the confirmation tests.

2. Media and reagents

Section	Media and reagents	Compliance
6.2 Diluents	- *Ringer's solution* **Ringer's solution (1/4 strength)** 100 tablets - BR00108 **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408	Total
6.3 Medium for detection of the spores of sulfite-reducing anaerobes	- *Meat-liver glucose agar* **Meat-liver glucose agar** 500 g vial - BK157HA	Total
6.4 Medium and reagent for identification of the spores of *Clostridium*	- *Tryptose-sulfite D-cycloserine agar* **TSC (base) agar** 50 x 20 mL tubes - BM03908 10 x 200 mL vials - BM07708 500 g vial - BK031HA **D-cycloserine 200 mg selective supplement** 10 vials q.s. 200 mL - BS00608 **D-cycloserine liquid selective supplement** 10 vials q.s. 9 litres 1 vial q.s. 5 litres - *Meat-liver glucose broth* - - *Hydrogen peroxide* -	Total [3] - -

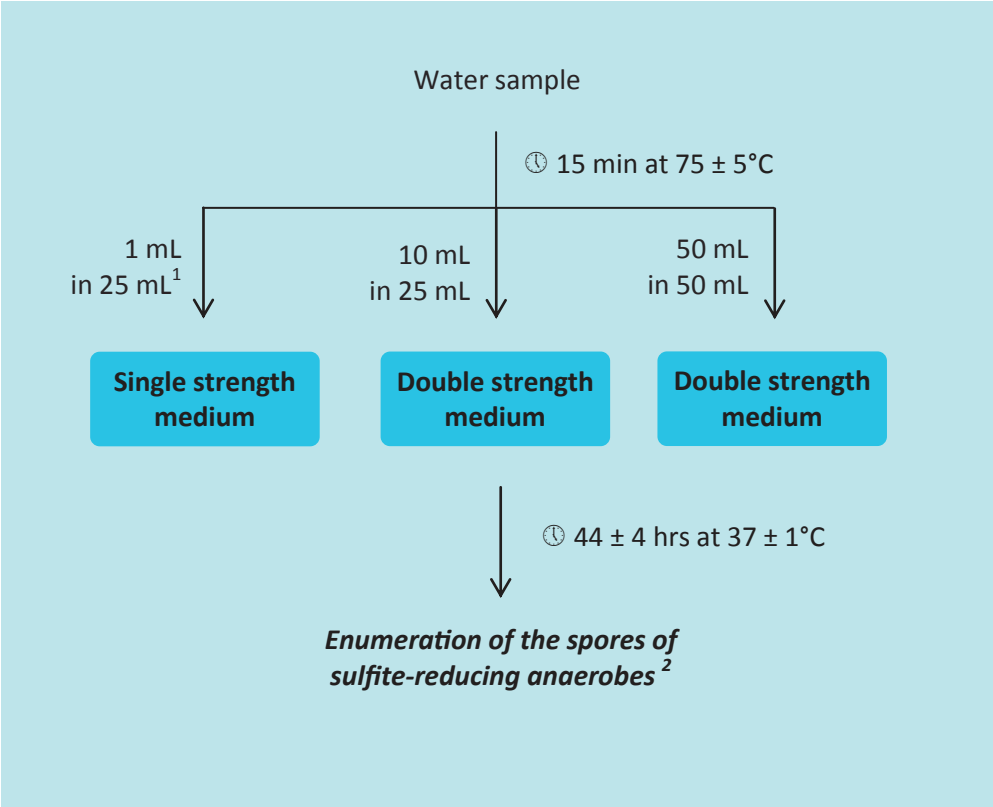
[3] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

Detection and enumeration of the spores of microorganisms (*Clostridia*)

Part 1: Method by enrichment in a liquid medium

NF EN 26461-1/ISO 6461-1: 07-1993
T 90-434

1. Protocol



[1] If necessary, add 1 mL of a 1:10 dilution of sample to a series of five capped vials containing 25 mL of the single strength medium.

[2] Express the results according to the standard ISO 8199.

2. Media and reagents

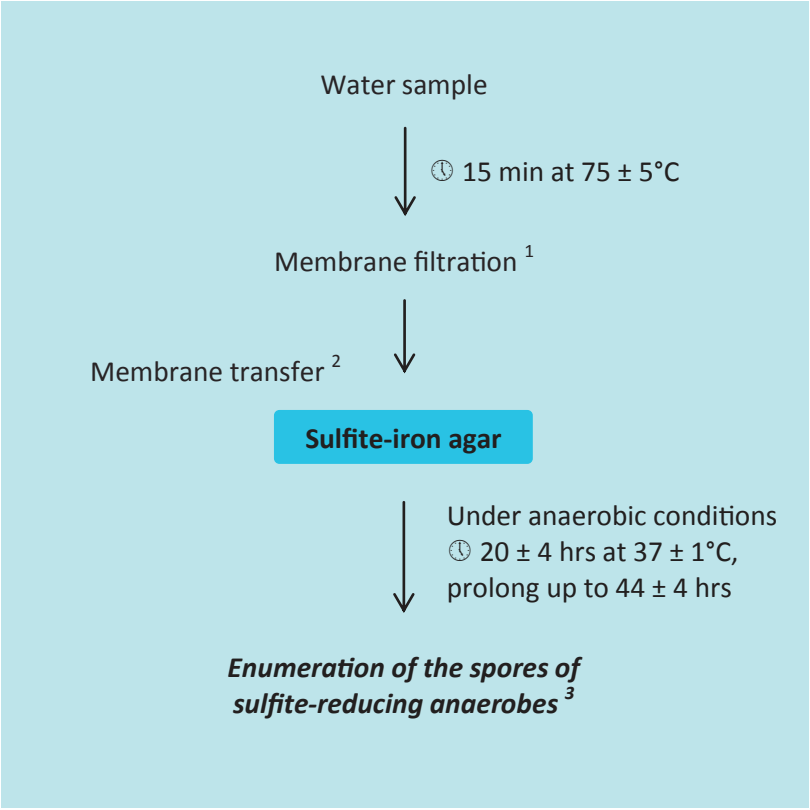
Section	Media and reagents	Compliance
6.2.1 Diluents	Refer to standard NF EN ISO 8199, page 44.	
6.2.2 Differential reinforced clostridial medium (DRCM)	*- Single strength basal medium* - *- Double strength basal medium* - *- Sodium sulfite (Na_2SO_3), 4% (m/m) solution* - *- Iron (III) citrate ($C_6H_5O_7Fe$), 7% (m/m) solution* -	- - - -

Detection and enumeration of the spores of microorganisms (*Clostridia*)

Part 2: Method by membrane filtration

NF EN 26461-2/ISO 6461-2: 07-1993
T 90-417

1. Protocol



[1] Filter 100 mL of drinking water, spring water and well water, mineral water, sea water and surface water with low Clostridia pollution.
Filter smaller quantities of water highly polluted by Clostridia or sewer water. When volumes less than 10 mL are filtered, add 10 mL to 100 mL of sterile water or diluent.

[2] Transfer the membrane, with the top facing downwards, to the bottom of the empty Petri dish, and pour on 18 mL of melted medium.

[3] Express the results according to the standard ISO 8199.

2. Media and reagents

Section	Media and reagents	Compliance
6.2 Selective medium [4]	- *Sulfite-iron agar* **2% nutrient agar (base)** 50 x 18 mL tubes - BM11808 500 g vial - BK185HA **2.5% nutrient agar (base)** 50 x 18 mL tubes - BM12508 - *Sodium sulfite (Na_2SO_3), 4% (m/m) solution* - - *Iron (III) citrate ($C_6H_5O_7Fe$), 7% (m/m) solution*	Equivalent - -
6.3 Other selective medium [4]	- *Tryptose-sulfite agar* **TSC (base) agar** 50 x 20 mL tubes - BM03908 10 x 200 mL vials - BM07708 500 g vial - BK031HA	Total

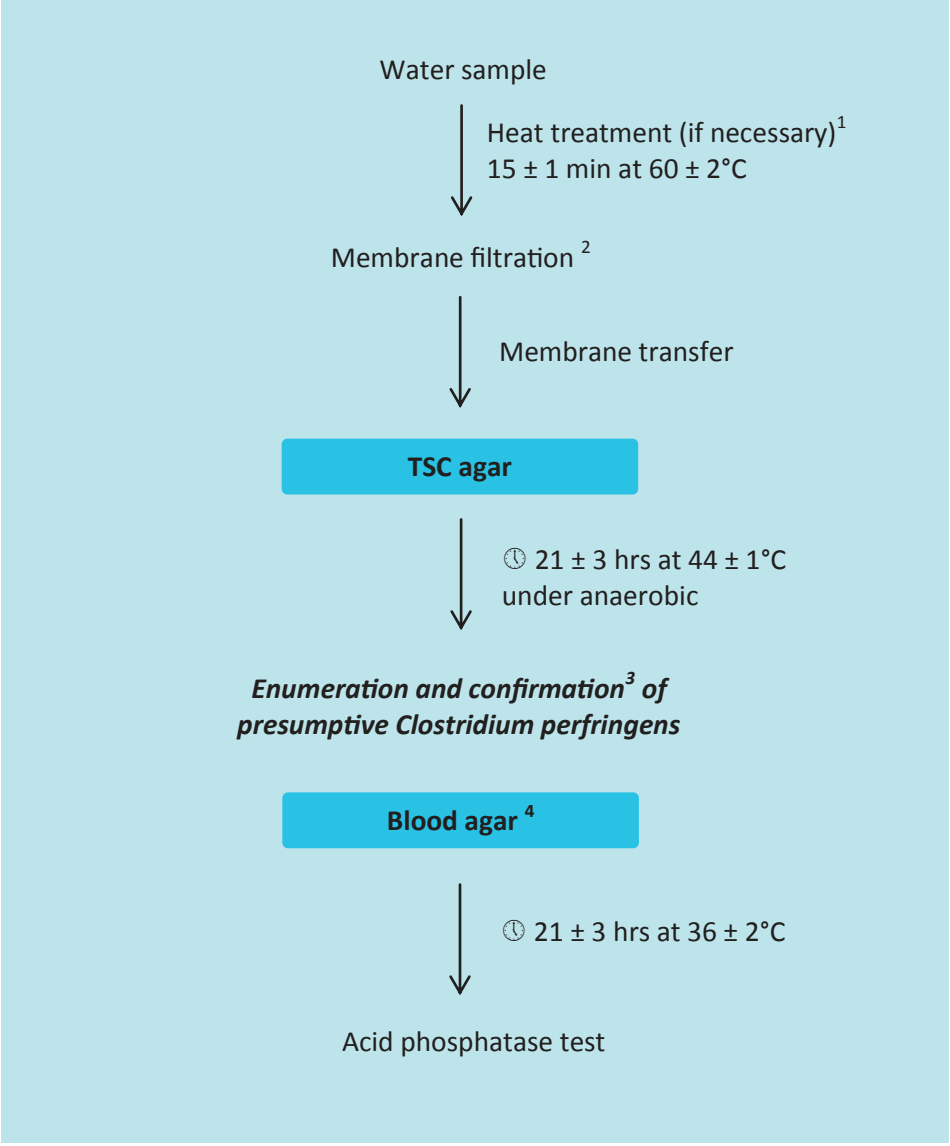
[4] At the operator's discretion.

Enumeration of *Clostridium* perfringens

Method by membrane filtration

NF EN ISO 14189: 05-2017
T 90-189

1. Protocol



[1] Heat pre-treatment is necessary if enumeration of the spores is only required.

[2] A volume of water suited to the type of water to be analysed should be filtered. For water intended for human consumption, the volume of water filtered is generally 100 mL. Record the volume of water filtered.

[3] Select at least 1 to 10 colonies and at least 10 colonies randomly selected for counts greater than 10 colonies, and reinoculate on blood agar.

[4] Another suitable non-selective agar may be used for the subcultures, such as Columbia base agar or TSA.

2. Media and reagents

Section	Media and reagents	Compliance
A.1 Culture media	- *Tryptose-Sulfite-Cycloserine (TSC) agar* **TSC (base) agar** 50 x 20 mL tubes - BM03908 10 x 200 mL vials - BM07708 500 g vial - BK031HA **D-cycloserine 200 mg selective supplement** 10 vials q.s. 200 mL - BS00608 **D-cycloserine liquid selective supplement** 10 vials q.s. 9 litres - BS0908 1 vial q.s. 5 litres - BS09408	Total [5]
A.2 Subculture media	- *Blood agar* **Columbia (base) agar** 500 g vial - BK019HA - *Sterile sheep or horse blood*	Total -
A.3 Confirmation reagents	- *Acid phosphatase reagent* -	-

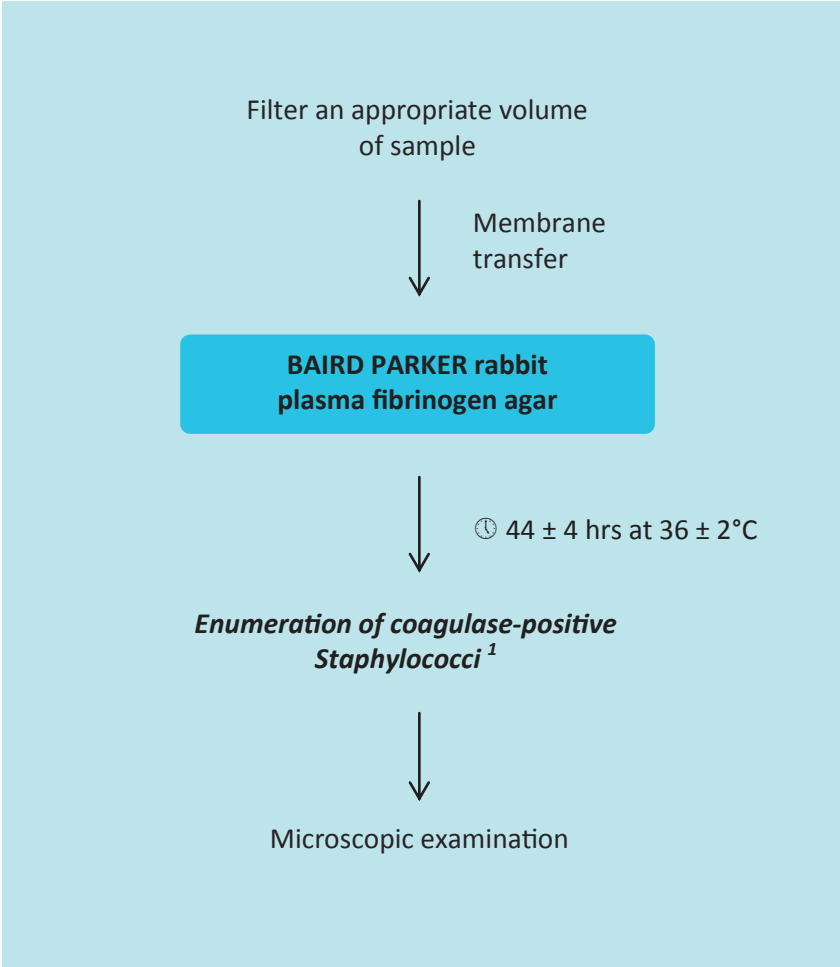
[5] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

Enumeration of pathogenic (coagulase-positive) Staphylococci

Method by membrane filtration

NF T 90-412: 06-2016
T 90-412

1. Protocol



[1] Randomly select 5 characteristic colonies and examine under a microscope.

2. Media and reagents

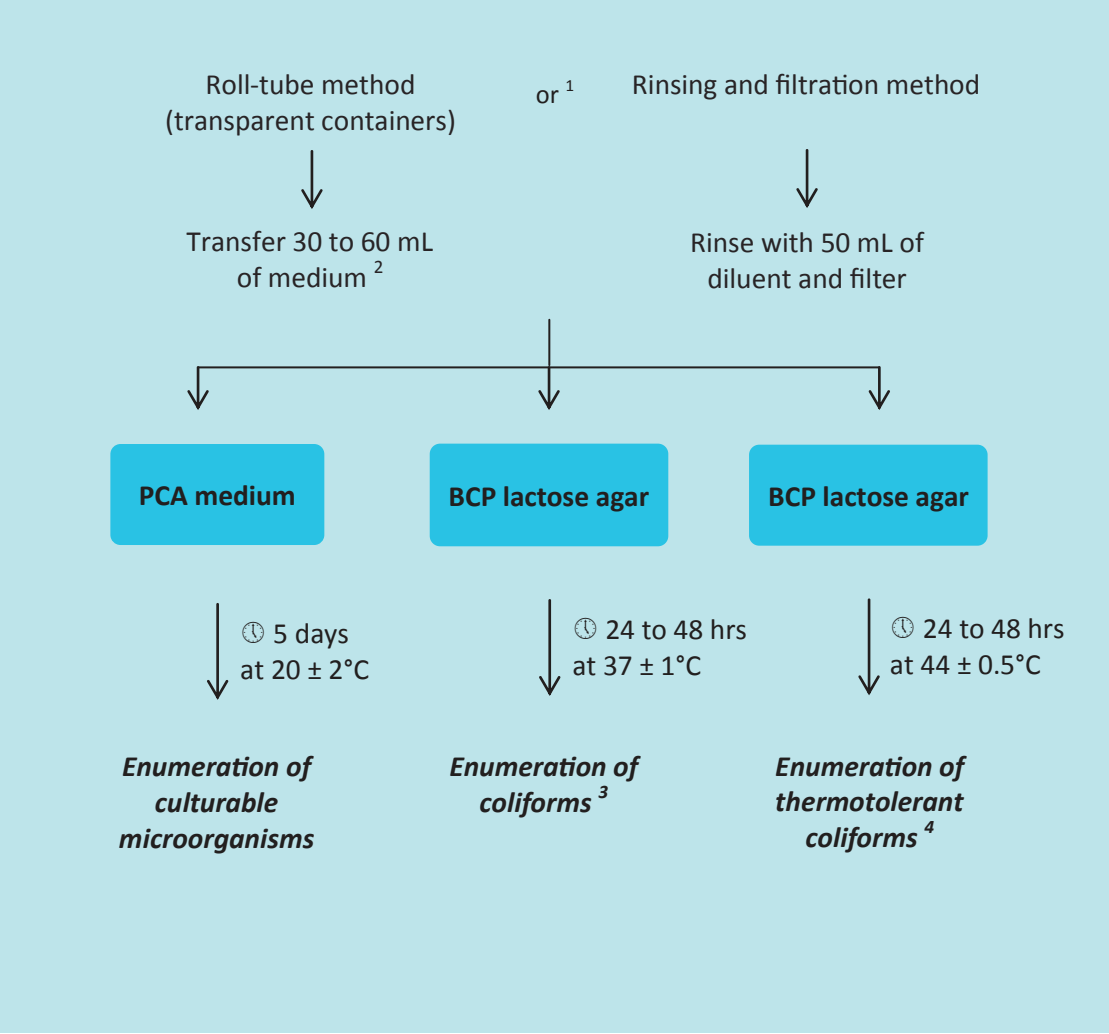
Section	Media and reagents	Compliance
7.3 Culture medium	*- Rabbit plasma fibrinogen (RPF) agar* **BAIRD-PARKER RPF agar** 20 Petri dishes Ø 90 mm - BM06708 20 Petri dishes Ø 55 mm - BM15908 Kit 6 x 90-mL base medium vials + 6 vials of supplement - BT00508 Kit 6 x 190-mL base medium vials + 6 vials of supplement - BT01008 **BAIRD-PARKER (base) agar** 500 g vial - BK055HA 5 kg drum - BK055GC **Rabbit plasma fibrinogen selective supplement** 8 vials q.s. 100 mL - BS03408 Vial q.s. 500 mL - BS03808	Total [2]

[2] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

Bacteriological examinations of the containers and sealing systems intended for conditioned water

NF T 90-425: 02-1992
T 90-425

1. Protocol



[1] The roll-tube method produces a greater yield and should be preferable to the rinsing and filtration method. However, it only applies to transparent vials.

[2] Under aseptic conditions, transfer a sufficient quantity of agar medium to each vial under supercooling, so as to coat the entire rotated inner surface with an agar film; the film solidifies as the vial is rotated in the air or under a stream of cold water.

[3] Refer to standard NF T 90-414.

[4] Refer to standard NF T 90-413.

2. Media and reagents

Section	Media and reagents	Compliance
5.2.1 Diluent [5]	- *Ringer's solution* **Ringer's solution (1/4 strength)** 100 tablets - BR00108 **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408	Total
5.2.2 Culture media	- *Agar for enumeration of culturable microorganisms* [6] **Plate count agar (PCA)** 500 g vial - BK144HA 5 kg drum - BK144GC 10 x 100 mL vials - BM01508 10 x 200 mL vials - BM03308 - *BCP lactose agar* **BCP lactose agar** 500 g vial - BK023HA	Total [7] Total [7]

[5] When analysing washed or rinsed bottles, the potential presence of halogenated disinfectant will be inhibited by adding 0.16 g of sodium thiosulfate per litre of diluent.

[6] When analysing washed or rinsed bottles, the potential presence of halogenated disinfectant will be inhibited by adding 1 g of sodium thiosulfate per litre of agar.

[7] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

General guidance on the enumeration of micro-organisms by culture

NF EN ISO 8199: 11-2018
T 90-400

1. Use

- Inoculate a known volume of a water sample, according to the protocol of the chosen method, based on the origin of the water and the test microorganism.

2. Media and reagents

Section	Media and reagents	Compliance
5.2 Diluents	*- Saline solution* -	-
	- Peptone water **0.1% peptone water** 2 x 5 L flexible bags - BM16408	Total [1]
	- Peptone saline solution **Tryptone-salt broth** 50 x 9 mL tubes - BM00808 10 x 90 mL vials - BM11408 3 x 3 L flexible bags - BM13508 500 g vial - BK014HA	Total [1]
	- Quarter-strength Ringer's solution **Ringer's solution (1/4 strength)** 100 tablets - BR00108 **Sterile distilled water** 50 x 18 mL tubes - BM11508 10 x 90 mL vials - BM19408	Total
	- Phosphate buffer solution -	-

[1] "Tryptone" is a peptone obtained by pancreatic digestion of casein.

APPENDIX A: Other reference standards relating to the microbiology of water

Microbiology of food, animal feed and water Preparation, production, storage and performance testing of culture media Modified by the Amendment A1	NF EN ISO 11133/ A1	V 08-104/A1	March 2018
Requirements for establishing performance characteristics of quantitative micro-biological methods	NF EN ISO 13843	T 90-460	July 2017
Quality control of culture media	FD T 90-461	T 90-461	August 2016
Microbiology of food, animal feed and water Preparation, production, storage and performance testing of culture media	NF EN ISO 11133	V 08-104	July 2014
Criteria for establishing equivalence between microbiological methods	NF EN ISO 17994	T 90-462	April 2014
Determination of pH	NF EN ISO 10523	T 90-418	May 2012
Sampling guide for monitoring quality of water in the environment Part 3: Sampling of ground water	FD T 90-523-3	T 90-523-3	January 2009
Sampling guide for monitoring quality of water in the environment Part 2: Sampling of wastewater	FD T 90-523-2	T 90-523-2	February 2008
Sampling guide for monitoring quality of water in the environment Part 1: Sampling of surface water	FD T 90-523-1	T 90-523-1	February 2008
Sampling for microbiological analysis	NF EN ISO 19458	T 90-480	November 2006
Bacteriological examinations of swimming pool water	NF T 90-421	T 90-421	August 2006
Determination of the methylene blue active substances (MBAS) index Method using continuous flow analysis (CFA)	NF EN ISO 16265	T 90-410	May 2012

APPENDIX B: List of products

Product	Presentation	Standard
Acetamide broth	7 x 5 mL tubes - BM09508	NF EN ISO 16266: 08-2008 (pages 28-29)
BAIRD-PARKER (base) agar	500 g vial - BK055HA	NF T 90-412: 06-2016 (pages 40-41)
	5 kg drum - BK055GC	
BAIRD-PARKER RPF agar	20 plates Ø 90 mm - BM06708	NF T 90-412: 06-2016 (pages 40-41)
	20 plates Ø 55 mm - BM15908	
	KIT 6 x 90 mL vials of base + 6 vials of suppl. - BT00508	
	KIT 6 x 190 mL vials of base + 6 vials of suppl. - BT01008	
BEA agar	500 g vial - BK158HA	NF EN ISO 7899-2: 08-2000 (pages 16-17)
	10 x 100 mL vials - BM10408	
BCP lactose broth	500 g vial - BK119HA	NF T 90-413: 10-1985 (pages 4-5)
BCP lactose agar	500 g vial - BK023HA	NF T 90-425: 02-1992 (pages 42-43)
BCYEα agar with cysteine	20 plates Ø 90 mm - BM07208	NF T 90-431: 08-2017 (pages 20-21)
		NF EN ISO 11731: 07-2017 (pages 22-25)
BCYEα agar without cysteine	20 plates Ø 90 mm - BM07308	NF T 90-431: 08-2017 (pages 20-21)
		NF EN ISO 11731: 07-2017 (pages 22-25)
Brilliant green bile lactose broth (BGBLB)	500 g vial - BK002HA	NF T 90-413: 10-1985 (pages 4-5)
	50 x 10 mL tubes, with Durham tubes - BM01108	
Buffered peptone water (20 g/L)	500 g vial - BK131HA	NF EN ISO 19250: 06-2013 (pages 30-31)
	5 kg drum - BK131GC	
Buffered peptone water (25.5 g/L)	500 g vial - BK018HA	NF EN ISO 19250: 06-2013 (pages 30-31)
	5 kg drum - BK018GC	
	10 x 225 mL vials - BM01008	
	50 x 9 mL tubes - BM05608	
	10 x 90 mL vials - BM05708	
	3 x 3 L flexible bags - BM13108	
	2 x 5 L flexible bags - BM13208	
CCA agar	20 plates Ø 90 mm - BM18208	ISO 9308-1: 09-2014 (pages 8-9)
	500 g vial - BK204HA	
CN agar for *Pseudomonas*	500 g vial - BK165HA	NF EN ISO 16266: 08-2008 (pages 28-29)
	20 plates Ø 55 mm - BM14508	
	120 plates Ø 55 mm - BM19608	
Columbia agar	500 g vial - BK019HA	NF EN ISO 14189: 05-2017 (pages 38-39)
COMPASS® cc Agar	20 plates Ø 55 mm - BM15308	Alternative method (pages 12-13)
	500 g vial - BK210HA	
	Suppl. 10 vials q.s. 500 mL - BS08408	
COMPASS® *Enterococcus* Agar	20 plates Ø 55 mm - BM15708	Alternative method (pages 18-19)
	10 x 100 mL vials - BM11608	
	500 g vial - BK183HA	

Product	Presentation	Standard
D-cycloserine (200 mg) selective supplement	10 vials q.s. 500 mL - BS00608	NF T 90-415: 10-1985 (pages 32-33)
D-cycloserine liquid supplement	10 vials q.s. 9 L - BS09208	NF EN ISO 14189: 05-2017 (pages 38-39)
	1 vial q.s. 5 L - BS09408	
Distilled water (Sterile)	50 x 18 mL tubes - BM11508	NF T 90-413: 10-1985 (pages 4-5)
	10 x 90 mL vials - BM19408	NF EN ISO 7899-1: 03-1999 (pages 14-15)
		NF EN ISO 7899-2: 08-2000 (pages 16-17)
		NF EN ISO 9308-3: 03-1999 (pages 10-11)
		NF T 90-431: 08-2017 (pages 20-21)
		NF T 90-415: 10-1985 (pages 32-33)
		NF EN ISO 8199: 01-2008 (page 44)
		NF T90-425: 02-1992 (pages 42-43)
GVPC agar for *Legionella*	20 plates Ø 90 mm - BM07108	NF T 90-431: 08-2017 (pages 20-21)
		NF EN ISO 11731: 07-2017 (pages 22-25)
KING B agar	7 x 7 mL tubes - BM10508	NF EN ISO 16266: 08-2008 (pages 28-29)
Lactose TTC agar with Tergitol-7	500 g vial (base) - BK123HA	NF EN ISO 9308-1: 09-2000 (pages 6-7)
	20 plates Ø 55 mm - BM14708	
	120 plates Ø 55 mm - BM09308	
Meat-liver glucose agar	500 g vial - BK157HA	NF T 90-415: 10-1985 (pages 32-33)
MUG/EC microplate	25 opaque plates - BT00108	NF EN ISO 9308-3: 03-1999 (pages 10-11)
MUD/SF microplate	25 opaque plates - BT00308	NF EN ISO 7899-1: 03-1999 (pages 14-15)
Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth	500 g vial (MKTTn base without iodine, novobiocin) - BK169HA	NF EN ISO 19250: 06-2013 (pages 30-31)
	500 g vial (MKTTn base without iodine) - BK208HA	
	50 x 10 mL tubes - BM07808	
Novobiocin selective supplement	10 vials (10 mg) - BS03308	NF EN ISO 19250: 06-2013 (pages 30-31)
Nutrient agar 2%	500 g vial - BK185HA	NF EN ISO 19250: 06-2013 (pages 30-31)
	50 x 18 mL tubes - BM11808	NF EN 26461-2: 07-1993 (pages 36-37)
Nutrient agar 2.5%	50 x 18 mL tubes - BM12508	NF EN ISO 11731: 07-2017 (pages 22-25)
		NF EN ISO 16266: 08-2008 (pages 28-29)
		NF EN ISO 19250: 06-2013 (pages 30-31)
		NF EN 26461-2: 07-1993 (pages 36-37)
Peptone water 0.1%	2 x 5 L flexible bags	NF EN ISO 8199: 01-2008 (page 44)
Plate count agar (PCA)	500 g vial - BK144HA	NF T90-425: 02-1992 (pages 42-43)
	5 kg drum - BK144GC	10 x 200 mL vials - BM03308
	10 x 100 mL vials - BM01508	
Rabbit plasma fibrinogen supplement	8 vials q.s. 100 mL - BS03408	NF T 90-412: 06-2016 (pages 40-41)
	Vial q.s. 500 mL - BS03808	
RAPPAPORT-VASSILIADIS broth with soya (RVS)	500 g vial - BK148HA	NF EN ISO 19250: 06-2013 (pages 30-31)
	50 x 10 mL tubes - BM07408	
Ringer's solution (1/4 strength)	100 tablets - BR00108	NF T 90-413: 10-1985 (pages 4-5)
		NF T 90-415: 10-1985 (pages 32-33)
		NF EN ISO 8199: 01-2008 (page 44)
		NF T 90-425: 02-1992 (pages 42-43)

Product	Presentation	Standard
Synthetic sea salt	100 g vial - BR00308	NF EN ISO 7899-1: 03-1999 (pages 14-15)
	50 x 18 mL tubes - BM08808	NF EN ISO 9308-3: 03-1999 (pages 10-11)
SLANETZ and BARTLEY agar	500 g vial (base) - BK129HA	NF EN ISO 7899-2: 08-2000 (pages 16-17)
	500 g vial (complete) - BK037HA	
	20 plates Ø 55 mm - BM14608	
	120 plates Ø 55 mm - BM09408	
Sulfamethazine 25 mg selective supplement	10 vials q.s. 500 mL - BS02808	NF T 90-412: 06-2016 (pages 40-41)
Trypto-casein Soy Agar (TSA)	500 g vial - BK047HA	NF EN ISO 9308-1: 09-2000 (pages 6-7)
	20 plates Ø 90 mm - BM05008	ISO 9308-1: 09-2014 (pages 8-9)
	10 x 100 mL vials - BM01708	NF EN ISO 11731: 07-2017 (pages 22-25)
	10 x 200 mL vials - BM04908	
Tryptone-salt	500 g vial - BK014HA	NF EN ISO 8199: 01-2008 (page 44)
	50 x 9 mL tubes - BM00808	
	10 x 90 mL vials - BM11408	
	3 x 3-L flexible bags - BM13508	
Tryptophan broth	500 g vial - BK163HA	NF EN ISO 9308-1: 09-2000 (pages 6-7)
	50 x 3 mL tubes - BM07608	
Tryptone soy (blood agar base)	500 g vial - BK028HA	NF EN ISO 11731: 07-2017 (pages 22-25)
Tryptose lauryl sulfate broth	500 g vial - BK010HA	NF T 90-413: 10-1985 (pages 4-5)
	50 x 10 mL tubes, with Durham tubes (single strength) - BM09708	
	50 x 10 mL tubes, with Durham tubes (double strength) - BM09808	
TSC agar	500 g vial (base) - BK031HA	NF T 90-415: 10-1985 (pages 32-33)
	50 x 20 mL tubes (base) - BM03908	NF EN 26461-2: 07-1993 (pages 36-37)
	10 x 200 mL vials (base) - BM07708	
TSI agar	500 g vial - BK221HA	NF EN ISO 19250: 06-2013 (pages 30-31)
TTC supplement	10 vials (12.5 mg) - BS02608	NF EN ISO 7899-2: 08-2000 (pages 16-17)
	10 vials (50 mg) - BS02708	NF EN ISO 9308-1: 09-2000 (pages 6-7)
Yeast extract agar (PCA without glucose)	500 g vial - BK153HA	NF EN ISO 6222: 07-1999 (pages 26-27)
	10 x 200 mL vials - BM06808	
XLD agar	500 g vial - BK168HA	NF EN ISO 19250: 06-2013 (pages 30-31)
	20 plates Ø 90 mm - BM08708	

BIOKAR Diagnostics - Support and services

Online information: www.biokar-diagnostics.com

Direct access to our product technical data sheets (**TDS**), material safety data sheets (**MSDS**), and quality control certificates (**QCC**) on our website.

BIOKAR Diagnostics contact

✆ +33 (0)1 48 10 19 40
🖷 +33 (0)3 44 14 33 34

Europe, Africa, North America
Athanassios GIANNAKOPOULOS
@ athanassios.giannakopoulos@solabia.fr

Asia, South America
Anthony FELIN
@ anthony.felin@solabia.fr

Distributors information

List of our distributors on our website section «contact»
Local contact :

BIOKAR Diagnostics
Rue des Quarante Mines
ZAC de Ther-Allonne - B.P. 10245
60002 Beauvais Cedex - France
www.biokar-diagnostics.com