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DI-Check Legionella spp. kit

MBK0058 - 100 reactions

INTRODUCTION AND PRODUCT DESCRIPTION

Legionella, which is responsible for Legionellosis, is a gram- bacterium ubiquitously present at low concentration in natural aquatic ecosystems. *Legionella* can easily reach and contaminate man-made aquatic environments, such as buildings water systems, cooling towers, fountains which are important sources of infection for humans.
The reference method for enumeration of *Legionella* spp. in water is culture, however aside from the fact that requires up to 14 days for analysis it often fails to detect legionellae in water samples. In this context, qPCR is an alternative tool for rapid and accurate detection or quantification of *Legionella* in water samples.

The DI-Check Legionella spp. kit is intended for the detection and/or quantification of *Legionella* spp. Dye-labeled probes target unique DNA sequences specific to *Legionella* spp. And synthetic internal amplification control DNA (IAC). Target DNA, if present, is amplified by PCR and detected in real-time PCR instrument and interpreted by the operator.

The kit provides a ready to use PCR mix highly resistant to PCR inhibitors and with an extremely stringent automatic hot-start allowing reaction assembly and temporary storage at room temperature prior to PCR amplification. This robust PCR mix contains an Internal Control to assess the efficiency of amplification reaction revealing the presence of inhibitory factors in the sample. Quantification of the target *Legionella* spp. Is carried out based on a standard curve covering a concentration range of 25000-25 genome units (GU) per PCR reaction.

Procedure overview

The DI-Check Legionella spp. method allows to detect and quantify Legionella spp. In all types of water samples in less that four hours. The method is based on 3 steps:

- Water samples filtration using 0.45 µm polycarbonate membrane filter
- DNA extraction using DNApure Water Isolation kit (code MBK0080)
- PCR amplification for the detection or quantification of *Legionella spp.* DNA using DI-Check Legionella pneumophila kit (code MBK0058)

The performances of the kits are guaranteed when the DNA is extracted using DNApure Water Isolation kit (code MBK0080).

CONTENTS AND STORAGE

The DI-check Legionella spp. kit allows to detect up to 98 samples. For quantification test the kit contains reagent to quantify 45 samples.

Component	Volume
Legionella spp. PCR Mix	2 x 1200 µL
Standard DNA	1 x 10 µL
Dilution Buffer	5 x 1500 µL
Negative PCR Control	1 x 100 µL
Reference material	2 x 50 µL
ROX	1 x 10 µL

Store the kit at -20°C, protect from light. If properly stored, see the expiration date for the stability of the kit.

ADDITIONAL EQUIPMENT AND MATERIAL REQUIRED

Reagents and disposables

- Micropipettes and filter tips,
- Vortex,
- Micro-centrifuge,
- Powder free gloves,
- 1.5 ml tubes,
- PCR tubes or PCR plates, sealing tapes and caps compatible with the PCR cycler to be used.

Instruments

ABI 7500, 7500 Fast,
QuantStudio 3-5,
StepOne,
StepOne Plus,
Rotor-Gene Q,
CFX96 Biorad,
MiniOpticon Biorad.

For use on different PCR cyclers please contact Diatheva

PRECAUTIONS AND RECOMMENDATIONS

- **For the quantification, it is suggested to test Samples, Negative PCR Control (NTC), Standards and Reference Material in duplicates at each amplification series.**
- The test must be performed by specialised, trained and authorised staff,
- Do not use reagents after the expiry date printed on the label,
- Use gloves as well as sterile pipet tips with filters. Change gloves often, especially if you suspect a possible contamination of them,
- It is suggested to provide separate and dedicated spaces, material and equipment for pre- and post-PCR amplification stages,
- Clean working space periodically with at least 5% sodium hypochlorite or another decontaminant agent.

RELEVANT ASSAY CONTROLS

Internal Amplification Control

Each vial of Legionella spp. PCR Mix contain an internal amplification control (IAC) that is co-amplified with the DNA from the sample, allowing to detect the presence of PCR inhibitors in the DNA extract.

Standard DNA

Two vials of Standard DNA are provided to prepare the standard curve. It is provided to allow for 5 independent quantification experiments. The Standard DNA should be diluted to obtain 4 levels of *L. pneumophila* genome unit (GU): 25000, 2500, 250 and 25 GU/5 µL. It is suggested to analyse samples and standards in duplicated.

Positive PCR Control

Positive PCR Control contains purified *L. pneumophila* DNA ATCC33152. The Positive PCR Control should be included in each qualitative amplification series to verify the success of the amplification step ensuring the confidence in your PCR results.

Reference Material

The Reference Material contains purified *L. pneumophila* ATCC33152 DNA connected to the Standard Reference Material. The Reference Material is a ready to use DNA solution corresponding to a concentration of 1200 GU/5 µL, that shall be tested according to ISO/TS 12869 standard. During a quantification experiment the verification of the Reference Material genome units ensures preparation of an accurate standard curve and a correct quantification calculation.

Negative PCR Control

Negative PCR Control should be included in each amplification series to check for possible contamination of the assay during reaction setup. Instead of adding sample DNA to a reaction vial containing L. pneumophila PCR Mix, add the same volume of Negative PCR Control.

PROCEDURE

1. SAMPLE PREPARATION

1.1 WATER SAMPLE FILTRATION AND DNA EXTRACTION

For sample preparation please refer to the product information of DNApure Water Isolation kit (code MBK0080). DNA sample should be mixed and centrifuged briefly prior to amplification.

1.2. BACTERIAL COLONY

Real-time PCR could be used for GVPC or BCYE colony confirmation.
- Dispense 100 µL of sterile water *Legionella*-DNA free in a 1.5 ml tube and dissolve the colony using a sterile loop.
- Boil the sample for 10 minutes.
- Centrifuge at 14 000 rpm for 10 minutes.
- Transfer 50 µL of supernatant in a new 1.5 ml tube taking care to do not disrupt the pellet.
- Vortex, centrifuge briefly and use 1 µL of the DNA in the real-time PCR reaction.

2. PCR MIX PREPARATION

2.1 QUALITATIVE TEST

Samples are tested in single. Include in each amplification series a Negative PCR Control (NTC- No template Control) and a Positive PCR Control (Positive Control).
- Thaw the Legionella spp. PCR Mix, ROX and Negative PCR Control. Vortex 15" and briefly spin vials in a microcentrifuge before opening.
- The Legionella spp. mix is ready to use for instruments that not require ROX (Rotor-Gene Q, CFX96 Biorad, MiniOpticon Biorad) and also for instruments that require a low ROX concentration (ABI 7500, 7500 Fast, QuantStudio 3-5).
- For instruments that require a High concentration of ROX (StepOne, StepOne Plus), upon first use the Legionella spp. PCR mix should be completed immediately before the use with the addition of 3.75 µL of ROX. Vortex 15 seconds and centrifuge briefly.

- Aliquot 20 µL of Legionella spp. PCR mix in the PCR tubes or in the plate prepared for the experiment.

Note: once aliquoted the Legionella spp. PCR mix may be stored for 3 h at 4°C.

- Add 5 µL of Negative PCR Control in the corresponding tube.
- In a separate area, add 5 µL of DNA samples to be tested, into the corresponding PCR tubes or wells containing amplification mixes.
- Add 5 µL of Positive PCR Control (prepared as indicated in section 3) into the corresponding PCR tubes or wells containing amplification mixes.
- Seal hermetically the PCR tubes or plate and load into the real-time PCR instrument, following the manufacturer's instructions.

2.2 QUANTITATIVE TEST

It is suggested to test each Samples, Standards, Negative PCR Control (NTC) and Reference Material (Positive Control) in duplicates.
- Thaw the Legionella spp. PCR Mix, ROX and Negative PCR Control. Vortex 15" and briefly spin vials in a microcentrifuge before opening.
- The Legionella spp. mix is ready to use for instruments that not require ROX (Rotor-Gene Q, CFX96 Biorad, MiniOpticon Biorad) and also for instruments that require a low ROX concentration (ABI 7500, 7500 Fast, QuantStudio 3-5).
- For instruments that require a High concentration of ROX (StepOne, StepOne Plus), upon first use the Legionella spp. PCR Mix should be completed immediately before the use with the addition of 3.75 µL of ROX. Vortex 15 seconds and centrifuge briefly.
- Aliquot 20 µL of Legionella spp. PCR Mix in the PCR tubes or in the plate prepared for the experiment.

Note: once aliquoted the Legionella spp. PCR Mix may be stored for 3 h at 4°C.

- Add 5 µL of Negative PCR Control in the corresponding tubes.
- In a separate area, add 5 µL of DNA samples to be tested, into the corresponding PCR tubes or wells containing amplification mixes.
- Add 5 µL of standard curve dilutions (prepared as indicated in section 4) into the corresponding PCR tubes or wells containing amplification mixes.
- Seal hermetically the PCR tubes or plate and load into the real-time PCR instrument, following the manufacturer's instructions.

Note: for PCR instrument with 96 wells block check that the reagents are at the bottom of each well, if not, briefly centrifuge at 800 x g for 1 minute.

3. POSITIVE PCR CONTROL PREPARATION (only for qualitative test)

The Standard DNA could be used for the preparation of Positive PCR Control:
- Thaw one vial of Dilution Buffer and Standard DNA,
- Vortex 10" and briefly spin the vial in a microcentrifuge before opening Standard DNA,
- Pipette 998.7 µL of Dilution Buffer into a 1.5 ml tube,
- Pipette 1.3 µL of Standard DNA into the STD1 tube,
- Vortex 10" and centrifuge briefly.

Note: Once prepared the Positive PCR Control could be stored at -20°C for 3 months.

4. STANDARD CURVE PREPARATION (only for quantitative test)

The Standard DNA and Dilution Buffer reagents are provided to prepare the standard curve. In a separate area proceed with the preparation of serial dilutions to create the standard curve.

Standard	Concentration to be entered as standard [GU/5 µL]
25000	25000 GU/5 µL
2500	2500 GU/5 µL
250	250 GU/5 µL
25	25 GU/5 µL

- Thaw one vial of Dilution Buffer and Standard DNA,
- Vortex 10" and briefly spin the vial in a microcentrifuge before opening Standard DNA,
- Prepare four 1.5 ml tubes in series named from *25000* to *25*,
- Pipette 998.7 µL of Dilution Buffer into tube *25000* and 90 µL into the three tubes remained (*2500*, *250*, *25*),
- Pipette 1.3 µL of Standard DNA into the *25000* tube,
- Vortex 10" and centrifuge briefly,
- Pipette 10 µL from tube *25000* into tube *2500*,
- Vortex 10" and centrifuge briefly,
- Repeat steps, to complete the dilution series for *250* and *25*.

Note: Once prepared the standard curve solutions could be stored at 4°C for 3 hours.

5. PROGRAM SETUP

Program PCR instrument before preparing the reaction mix.
Use the following real-time PCR-protocol for the DI-Check Legionella spp. kit. For details on how to program the experimental protocol, see the Instrument Operator's Manual of your real-time PCR cycler:

Step	Temperature and times	Cycles
Initial denaturation	95°C 3 min	1 X
Denaturation	95°C 15 sec	45 X
Annealing-extension	60°C 90 sec	

Fluorescence is detected during annealing-extension step on green channel (FAM dye) for *Legionella* spp. and yellow channel (VIC dye) for Internal Amplification Control.

If required, select ROX as passive reference dye and specify the type of probes quencher. The DI-Check legionella spp. kit contains probes with a non-fluorescent quencher (NFQ).

6. PLATE SETUP

Define the plate set-up.

Qualitative Test: Fill in information in each well according to the table below

Controls and Samples	**Task**	**Sample Name**
Negative PCR Control	NTC	NTC
Positive PCR Control	Unknown	Positive Control
Sample	Unknown	Assign different names to different samples

Quantitative Test: Fill in information in each well according to the table below

Controls, Standards and Samples	**Task**	**Sample Name**

Negative PCR Control	NTC	NTC
Reference Material	Unknown	Reference Material
Standard	Standard	25000, 2500, 250, 25
Sample	Unknown	Assign different names to different samples

Check the instrument is ready and start the run.

7. DATA ANALYSIS

The analysis of the results must be done with the program of the PCR instrument, please refer to the manual for detailed information.
Set the baseline and threshold values. Some software performs the data analysis automatically in this case it is advisable to check these settings. For a manual data analysis, analyse the PCR file for the two fluorophores separately. For a correct definition of the threshold it is necessary to select a value distinction from the background after the linear phase growth.

8. INTERPRETATION AND EXPRESSION OF RESULTS

Continue with Section:

A. if you work with real-time PCR instrument with Peltier thermal block i.e. QuantStudio 96-wells

B. if you work with real-time PCR instrument with Rotor i.e. Rotor-Gene Q

Section A: real-time PCR instrument with Peltier thermal block

8.1 QUALITATIVE TEST

a. Quality Control

Before proceeding with the analysis of samples, check the validity of controls. If both controls are "OK", the operator can proceed with the result interpretation of samples. Otherwise, if controls are "KO", the PCR is invalid and must be repeated (see table below).

Control type	**Control validity**	***Legionella* spp. result**	**Internal Amplification Control result**
Negative PCR Control	OK	N/A*	27≤Ct≤37
	OK	Ct>Intercept value**	27≤Ct≤37
	KO	Ct<Intercept value**	Non-significant
Positive PCR Control	OK	18≤Ct≤28	Non-significant
	KO	18>Ct>28	Non-significant

* N/A means "Not Applicable". The software indicates N/A when the fluorescence curve doesn't rise above the threshold.
**The intercept value to be considered in the contest of Qualitative analysis is Ct=40
This value is used to give an indication of the intercept value chosen during the validation.

b. Samples

Sample results shall be interpreted as shown in the table below

Legionella* spp. *result	***Internal Amplification Control result***	***Results and interpretation***
Ct≤Intercept value*	Non-significant	*Legionella* spp. detected
Ct>Intercept value* Ct>Intercept value*	27≤Ct≤37	*Legionella* spp. not detected
	Ct>37	Inhibition. Dilute the extracted DNA using Elution Buffer (MBK0080) or PCR grade water and repeat PCR amplification

* The intercept value to be considered in the contest of Qualitative analysis is Ct=40
This value is used to give an indication of the intercept value chosen during the validation.

c. Detection limit of the molecular method (LOD_{meth})

The limit of detection of the PCR step corresponds to the smallest number of genome units that provides a positive PCR result at the 90% threshold (ISO/TS 12869:2012). The LOD of the PCR step is 5 GU per 5 µL of extracted DNA. The LOD of the method (LOD_{meth}) depends on the volume of water sample filtered according to the following formula:

$$LOD_{meth} = \frac{5 \times F \times D}{V}$$

Where:
F = conversion factor GU/5 µL to GU/L. The F conversion factor of DNApure Water Isolation kit (MBK0080) is 64. For more information refer to the manual of the kit.
V= volume of water sample filtered expressed in litres.
D= DNA dilution factor (if the DNA has been diluted before PCR run).

8.2 QUANTITATIVE TEST

a. Controls and Standard

Before proceeding with the analysis of the samples, check the validity of controls and Standard curve. If the parameters are "OK", the operator can proceed with the result interpretation of samples. Otherwise, if parameters are "KO", the PCR is invalid and must be repeated (see table below).

Parameter	**Quality Control validity**	***Legionella* spp. result**	**Internal Amplification Control result**
Negative PCR Control	OK	N/A[a]	27≤Ct≤37
	OK	Ct>Intercept value[b]	27≤Ct≤37
	KO	Ct<Intercept value[b]	Non-significant
Slope	-3.75≤ Slope ≤-3.00	-3.75≤ Slope ≤-3.00	Non-significant
Correlation coefficient (R^2)	≥0.95	≥0.95	
Reference Material	OK	601≤ GU/well ≤2398[c]	Non-significant
	KO	601≥ GU/well ≥2398[c]	Non-significant
LOQ	OK	Compliant to ISO-TS 12869 standard	Non-significant
	KO	Compliant to ISO-TS 12869 standard	Non-significant

[a] N/A means "Not Applicable". The software indicates N/A when the fluorescence curve doesn't rise above the threshold
[b] The intercept value to be considered in the contest of Quantitative analysis is systematically calculated in the calibration function of the amplification run.
[c] The Reference Material shall be tested according to ISO-TS 12869 standard

The above values were determined during the method validation.

b. Samples

Detection of *Legionella* spp. N GU/5 µl	***Internal Amplification Control (VIC)* - Inhibition**	***Legionella* spp. detection Results and expression per liter (GU/L)[a]**	**Note**
N < 5	*27≤ Ct ≤37* No Inhibition	*Legionella* spp. not detected	Refer to paragraph 8.2.d for LOD_{meth} calculation
	Ct ≥37 Inhibition	The sample DNA shall be diluted and PCR run repeated	
5 ≤ N < 25	*27≤ Ct ≤37* No Inhibition	*Legionella* spp. detected below the limit of quantification	Refer to paragraph 8.2.d for LOD_{meth} calculation
	Ct ≥37 Inhibition	The sample DNA shall be diluted and PCR run repeated	
25000 ≥ N ≥ 25	*27≤ Ct ≤37* No Inhibition	*Legionella* spp. quantitatively detected	Refer to paragraph 8.2.c
	Ct ≥37 Inhibition	The sample DNA shall be diluted and PCR run repeated	
N > 25000	*27≤ Ct ≤37* No Inhibition	*Legionella* spp. detected above the upper limit of quantification	Refer to paragraph 8.2.d for UQL_{meth} calculation. The quantification can be obtained after DNA dilution
	Ct ≥37 Inhibition	The sample DNA shall be diluted and PCR run repeated	

[a] Please if the sample has been diluted the Result GU/L must be multiplied by the dilution factor

c. Calculation of *Legionella spp.* concentration in water samples

To calculate the amount of *Legionella spp.* in the water sample analysed it is necessary to consider (1) the volume of sample filtered in litres and (2) the *F* conversion factor. The F conversion factor of DNApure Water Isolation kit (MBK0080) is 64. For more information refer to the manual of the kit.
The instrument software automatically calculates the amount of GU of *Legionella spp.* per reaction (in 5 µL), this value corresponds to **N** parameter. To obtain the *Legionella spp.* GU contained in 1 Liter please use the following formula:

$$\textit{Legionella spp.}\ \text{Genome Units per liter} = \frac{N \times F \times D}{V}$$

Where:
N= number GU of *Legionella spp.*/5 µL (PCR result)
F = conversion factor GU/5 µL to GU/L.
V= volume of water sample filtered expressed in litres
D**=** DNA dilution factor (if the DNA has been diluted before PCR run)

Express the results in number of Genome Units of *Legionella spp.* per litre of water sample, considering two significant figures: ex. 12 312 GU/L of *Legionella spp.* is expressed as "12 000 GU/L of *Legionella spp*".

d. Theoretical detection limit (LODmeth, Quantification limit (LOQ_{meth}), and Upper quantification limit (UQL_{meth}) of the molecular method

The detection limit of the PCR step is 5 GU/5 µL. The LOD of the method (LOQ_{meth}) is 320 GU/L when 1 Liter is filtered. LOD_{meth} depends on the volume of water sample filtered and can be calculated according to the following formula:

$$LOD_{meth} = \frac{5 \times F}{V}$$

The limit of quantification of the PCR step is 25 GU/5 µL. The LOQ of the method (LOQ_{meth}) is 1600 GU/L when 1 Liter is filtered. The LOQ_{meth} depends on the volume of water sample filtered and can be calculated according to the following formula:

$$LOQ_{meth} = \frac{25 \times F}{V}$$

The upper quantification limit of the method (UQL_{meth}) is 1.6×10^6 GU/L when 1 Liter is filtered.
The UQL_{meth} can be obtained with the following formula:

$$UQL_{meth} = \frac{25\,000 \times F}{V}$$

Where:
F = conversion factor GU/5 µL to GU/L.
V= volume of water sample filtered expressed in litres

Section B: real-time PCR instrument with Rotor

8.1 QUALITATIVE TEST

a. Quality Control

Before proceeding with the analysis of samples, check the validity of controls. If both controls are "OK", the operator can proceed with the result interpretation of samples. Otherwise, if controls are "KO", the PCR is invalid and must be repeated (see table below).

Control type	**Control validity**	**Legionella *spp.* result**	**Internal Amplification Control result**
Negative PCR Control	OK	N/A*	23≤Ct≤33
	OK	Ct>Intercept value**	23≤Ct≤33
	KO	Ct<Intercept value**	Non-significant
Positive PCR Control	OK	18≤Ct≤28	Non-significant
	KO	18>Ct>28	Non-significant

* N/A means "Not Applicable". The software indicates N/A when the fluorescence curve doesn't rise above the threshold.
**The intercept value to be considered in the contest of Qualitative analysis is Ct=37
This value is used to give an indication of the intercept value chosen during the validation.

b. Samples

Sample results shall be interpreted as shown in the table below

Legionella *spp. result*	***Internal Amplification Control result***	***Results and interpretation***
Ct≤Intercept value*	Non-significant	**Legionella *spp.*** detected
Ct>Intercept value* Ct>Intercept value*	23≤Ct≤33	**Legionella *spp.*** not detected
	Ct>33	Inhibition. Dilute the extracted DNA using Elution Buffer (MBK0080) or PCR grade water and repeat PCR amplification

* The intercept value to be considered in the contest of Qualitative analysis is Ct=37
This value is used to give an indication of the intercept value chosen during the validation.

c. Detection limit of the molecular method (LOD_{meth})

The limit of detection of the PCR step corresponds to the smallest number of genome units that provides a positive PCR result at the 90% threshold (ISO/TS 12869:2012). The LOD of the PCR step is 5 GU per 5 µL of extracted DNA. The LOD of the method (LOD_{meth}) depends on the volume of water sample filtered according to the following formula:

$$LOD_{meth} = \frac{5 \times F \times D}{V}$$

Where:
F = conversion factor GU/5 µL to GU/L. The F conversion factor of DNApure Water Isolation kit (MBK0080) is 64. For more information refer to the manual of the kit.
V= volume of water sample filtered expressed in litres.
D= DNA dilution factor (if the DNA has been diluted before PCR run).

8.2 QUANTITATIVE TEST

a. Controls and Standard

Before proceeding with the analysis of the samples, check the validity of controls and Standard curve. If the parameters are "OK", the operator can proceed with the result interpretation of samples. Otherwise, if parameters are "KO", the PCR is invalid and must be repeated (see table below).

Parameter	**Quality Control validity**	**Legionella *spp.* result**	**Internal Amplification Control result**
Negative PCR Control	OK	N/A[a]	23≤Ct≤33
	OK	Ct>Intercept value[b]	23≤Ct≤33
	KO	Ct<Intercept value[b]	Non-significant
Slope	-3.75≤ Slope ≤-3.00	-3.75≤ Slope ≤-3.00	Non-significant
Correlation coefficient (R^2)	≥0.95	≥0.95	
Reference Material	OK	601≤ GU/well ≤2398[c]	Non-significant
	KO	601≥ GU/well ≥2398[c]	Non-significant
LOQ	OK	Compliant to ISO-TS 12869 standard	Non-significant
	KO	Compliant to ISO-TS 12869 standard	Non-significant

[a] N/A means "Not Applicable". The software indicates N/A when the fluorescence curve doesn't rise above the threshold

[b] The intercept value to be considered in the contest of Quantitative analysis is systematically calculated in the calibration function of the amplification run.

[c] The Reference Material shall be tested according to ISO-TS 12869 standard

The above values were determined during the method validation.

b. Samples

Detection of Legionella spp. **N GU/5 µl**	***Internal Amplification Control (VIC)* - Inhibition**	***Legionella* spp. detection Results and expression per liter (GU/L)[a]**	**Note**
N < 5	23≤Ct≤33 No Inhibition	*Legionella* spp. not detected	Refer to paragraph 8.2.d for LOD_{meth} calculation
	Ct ≥33 Inhibition	The sample DNA shall be diluted and PCR run repeated	
5 ≤ N < 25	*27≤ Ct ≤33* No Inhibition	*Legionella* spp. detected below the limit of quantification	Refer to paragraph 8.2.d for LOD_{meth} calculation
	Ct ≥33 Inhibition	The sample DNA shall be diluted and PCR run repeated	
25000 ≥ N ≥ 25	23≤Ct≤33 No Inhibition	*Legionella* spp. quantitatively detected	Refer to paragraph 8.2.c
	Ct ≥33 Inhibition	The sample DNA shall be diluted and PCR run repeated	
N > 25000	23≤Ct≤33 No Inhibition	*Legionella* spp. detected above the upper limit of quantification	Refer to paragraph 8.2.d for UQL_{meth} calculation. The quantification can be obtained after DNA dilution
	Ct ≥33 Inhibition	The sample DNA shall be diluted and PCR run repeated	

[a] Please if the sample has been diluted the Result GU/L must be multiplied by the dilution factor

c. Calculation of *Legionella spp.* concentration in water samples

To calculate the amount of *Legionella spp.* in the water sample analysed it is necessary to consider (1) the volume of sample filtered in litres and (2) the *F* conversion factor. The F conversion factor of DNApure Water Isolation kit (MBK0080) is 64. For more information refer to the manual of the kit.

The instrument software automatically calculates the amount of GU of *Legionella spp.* per reaction (in 5 µL), this value corresponds to **N** parameter. To obtain the *Legionella spp.* GU contained in 1 Liter please use the following formula:

$$\textit{Legionella spp.}\ \text{Genome Units per liter} = \frac{N \times F \times D}{V}$$

Where:
N= number GU of *Legionella spp.*/5 µL (PCR result)
F = conversion factor GU/5 µL to GU/L.
V= volume of water sample filtered expressed in litres
D**=** DNA dilution factor (if the DNA has been diluted before PCR run)

Express the results in number of Genome Units of *Legionella spp.* per litre of water sample, considering two significant figures: ex. 12 312 GU/L of *Legionella spp.* is expressed as "12 000 GU/L of *Legionella spp*".

d. Theoretical detection limit (LODmeth, Quantification limit (LOQ_{meth}), and Upper quantification limit (UQL_{meth}) of the molecular method

The detection limit of the PCR step is 5 GU/5 µL. The LOD of the method (LOQ_{meth}) is 320 GU/L when 1 Liter is filtered. LOD_{meth} depends on the volume of water sample filtered and can be calculated according to the following formula:

$$LOD_{meth} = \frac{5 \times F}{V}$$

The limit of quantification of the PCR step is 25 GU/5 µL. The LOQ of the method (LOQ_{meth}) is 1600 GU/L when 1 Liter is filtered. The LOQ_{meth} depends on the volume of water sample filtered and can be calculated according to the following formula:

$$LOQ_{meth} = \frac{25 \times F}{V}$$

The upper quantification limit of the method (UQL_{meth}) is 1.6×10^6 GU/L when 1 Liter is filtered.
The UQL_{meth} can be obtained with the following formula:

$$UQL_{meth} = \frac{25\,000 \times F}{V}$$

Where:
F = conversion factor GU/5 µL to GU/L.
V= volume of water sample filtered expressed in litres

9. REFERENCES

Ballard, A. L., Fry, N. K., Chan, L., Surman, S. B., Lee, J. V., Harrison, T. G. & Towner, K. J. (2000). Detection of Legionella pneumophila using a real-time PCR hybridization assay. J Clin Microbiol 38, 4215–4218.

ISO/TS 12869:2012 Water quality — Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR).