Instruction Manual to application of program for quantitative detection of *Legionella pneumophila* DNA using AmpliSens® *Legionella pneumophila*-FRT PCR kit

Quantitative characterized calibrators are used for quantification of *Legionella pneumophila* DNA and Internal Control STI-338 DNA copies in a test tube.

To perform the calculation one should take account of Internal Control DNA losses in order to calculate the real concentration of *Legionella pneumophila* DNA in each test sample of water.

To perform the calculation one should take account of **water concentration level. Therefore the water pretreatment should be strictly performed in accordance with the *Instruction Manual* to the reagent kit.**

Calculation of concentration values of *Legionella pneumophila* DNA ($C_{DNA\ Lp}$) in 1 l of water is performed in accordance with the following formula:

$$C_{DNA\ Lp}\ (copies/l) = Q_{DNA\ Lp} / Q_{IC\ STI\text{-}338} * C_{IC\ STI\text{-}338} * 2,$$ **where:**

$C_{DNA\ Lp}$ (copies/l) is the quantity of *Legionella pneumophila* DNA copies in 1 l of water sample,

$Q_{DNA\ Lp}$ (copies/ml) is the calculated quantity of *Legionella pneumophila* DNA copies in 1 ml of a test sample,

$Q_{IC\ STI\text{-}338}$ (copies/ml) is the calculated quantity of Internal Control STI-338 DNA copies in 1 ml of the Internal Control in a test sample,

$C_{IC\ STI\text{-}338}$ (copies/ml) is the number copies of Internal Control STI-338 DNA in 1 ml of Internal Control (specified in the *Important Product Information Bulletin*,

2 is the recalculation coefficient which take account of the volume changes during filtration.

NOTE: For quantitation each water sample should be tested in two runs (beginning with DNA extraction stage). In such case the result is given as an average value of two obtained values.

The *Program for Calculation of Legionella DNA Quantity.xls* can be used to calculate the concentration of ***Legionella pneumophila DNA*.**

Procedure

1. Open ***Program for Calculation of Legionella DNA Quantity.xls***
2. Save file with ***Book Microsoft Excel*** extension, specify the date of analysis.
3. In the ***Quant. results – Cycling A.FAM / Cycling A. Green*** window of **Rotor-Gene** program copy ***Name, Type, Ct, Given Conc (copies/ml), Calc Conc (copies/ml)***

columns and paste them into similar columns of **FAM / Green** field (**Excel** program).

4. In the ***Quant. results – Cycling A.JOE / Cycling A. Yellow*** window of **Rotor-Gene** program copy ***Name, Type, Ct, Given Conc (copies/ml), Calc Conc (copies/ml)*** columns and paste them into similar columns of **JOE / Yellow** field (**Excel** program).
5. Enter the concentration of value Internal Control STI-338 specified in the *Important Product Information Bulletin* into all rows of ***C Internal Control STI-338*** column (**Excel** program).
6. **Concentrations of *Legionella pneumophila* DNA (copies/l of tested water sample) calculated automatically in accordance with the given formula will be displayed in the *C DNA Lp* column of the program.**