RUO

For Professional Use Only

Application note

Detection of Chlamydia trachomatis using
eSens Ureaplasma/M.genitalium/M.hominis QL PCR kit, REF ES3048A
for Research Use Only. Not for use in diagnostic procedures.

PURPOSE OF THIS APPLICATION NOTE

The purpose of this application note is to provide an **extended guidance** for utilizing the eSens Ureaplasma/M.genitalium/M.hominis QL PCR kit. This document aims to assist laboratory professionals and researchers in accurately detecting the DNA of **not only** Ureaplasma spp. (U. parvum and U. urealyticum), Mycoplasma genitalium, and Mycoplasma hominis in various clinical samples, but **it also introduces the supplementary feature of the kit that enables the detection of Chlamydia trachomatis DNA for research use only (RUO)**, despite it not being CE IVD validated for this pathogen.

This application note provides specific supplementary data highligted by red color to facilitate the use of this feature.

PRINCIPLE OF PCR DETECTION

Table 1

Channel for fluorophore	FAM	JOE	ROX	Cy5	Cy5.5
DNA-target	*Chlamydia trachomatis (RUO)*	*Ureaplasma* sp.	*Mycoplasma genitalium*	Internal Control-FL	*Mycoplasma hominis*
Target gene	*cryptic plasmid*	*UreC*	*gyrB* gene	genetically engineered construction	*16*s rRNA gene

PROTOCOL

Amplification

Fluorescent signal is detected in the channels for the FAM, JOE, ROX, Cy5 and Cy5.5 fluorophores.

Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5 FI to 10 FI	0.1	On	Off	0%
JOE/Yellow	from 4 FI to 8 FI	0.1	On	Off	5%
ROX/Orange	from 4 FI to 8 FI	0.1	On	Off	5%
Cy5/Red	from 4 FI to 8 FI	0.07	On	On	5%
Cy5.5/Crimson	from 4 FI to 8 FI	0.1	On	On	20%

Test settings for plate-type instruments

Channel	Threshold
FAM, HEX, ROX, Cy5, Cy5.5	For each channel in *Log* Scale set the threshold line at the level of 10-20 % of maximum fluorescence obtained for the Positive Control of Amplification (C+) in the last amplification cycle.

DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in five channels:

- The signal of the **Chlamydia trachomatis DNA** amplification product is detected in the channel for the **FAM** fluorophore

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- **Chlamydia trachomatis DNA is detected** if the Ct value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- ***Chlamydia trachomatis*, *Ureaplasma* sp. (*U.parvum* and *U.urealyticum*), *Mycoplasma genitalium*, and *Mycoplasma hominis* DNA are not detected** in a sample if the *Ct* value is not determined (absent) (fluorescence curve does not cross the threshold line) in the channels for FAM, JOE, ROX and Cy5.5 fluorophores whereas the *Ct* value determined in the channel for the Cy5 fluorophore is less than the boundary *Ct* value.
- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for Cy5 fluorophore or greater than the specified boundary *Ct* value, whereas the *Ct* value in the channel for the FAM, JOE, ROX and Cy5.5 fluorophores is not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the DNA extraction stage

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM, JOE, ROX and Cy5.5	**Cy5**
C–	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
C+	PCR	<boundary value	<boundary value

Table 4

Boundary *Ct* values

Sample	Rotor-type instrument					Plate-type instrument				
	Channel for fluorophore									
	FAM	**JOE**	**ROX**	**Cy5.5**	**Cy5**	**FAM**	**JOE**	**ROX**	**Cy5.5**	**Cy5**
C+	35	35	35	35	33	38	38	38	38	36
C-	*Ct* is absent				33	*Ct* is absent				36
NCA	*Ct* is absent					*Ct* is absent				
Test samples	-	-	-	-	33	-	-	-	-	36

SPECIFICATIONS

Sensitivity

The analytical sensitivity for *Chlamydia trachomatis*, *Ureaplasma* spp., *Mycoplasma genitalium,* and *Mycoplasma hominis* is not less than $5x10^2$ genome equivalents per 1 ml of sample (GE/ml).