



RUO

For Professional Use Only

## Application note

Detection of Chlamydia trachomatis using **eSens N.gonorrhoeae /M.genitalium QL PCR kit, REF ES3049A** 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 N.gonorrhoeae /M.genitalium QL PCR kit. This document aims to assist laboratory professionals and researchers in accurately detecting the DNA of **not only** *Neisseria gonorrhoeae* and *Mycoplasma genitalium* 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 **highlighted by red color** to facilitate the use of this feature.

### PRINCIPLE OF PCR DETECTION

Table 1

Channel for fluorophore	FAM	JOE	ROX	Cy5
DNA-target	<i>N.gonorrhoeae</i>	<i>C.trachomatis</i>	<i>M.genitalium</i>	Internal Control-FL
Target gene	16s rRNA gene	<i>cryptic plasmid</i>	<i>gyrB</i> gene	genetically engineered construction

### PROTOCOL

#### Amplification

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

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## 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	5-10%

### Test settings for plate-type instruments

Channel	Threshold
FAM, HEX, ROX, Cy5	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 four channels:

- The signal of the **Chlamydia trachomatis DNA** amplification product is detected in the channel for the **JOE** 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 **JOE** fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- **Neisseria gonorrhoeae, Chlamydia trachomatis and Mycoplasma genitalium DNA are not detected** in a sample if the Ct value is not determined (absent) in the channels for FAM, **JOE** and ROX 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 channels for the FAM, **JOE**, ROX and Cy5 fluorophores. In such cases, the PCR analysis should be repeated.

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Table 3

## Results for controls

Control	Stage for control	Ct value in the channel for fluorophore	
		FAM, JOE, ROX	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	FAM	JOE	ROX	Cy5
C-	Ct is absent			33	Ct is absent			36
NCA	Ct is absent				Ct is absent			
C+	33	30	33	33	36	33	36	36
Test samples	-	-	-	33	-	-	-	36

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## SPECIFICATIONS

### Sensitivity

Clinical material	Nucleic acid extraction kit	PCR kit	Microorganism	Sensitivity, GE/ml*
Urogenital swabs	<b>DNA-sorb-AM ePure STD DNA Extraction Kit</b>	ES3049A	<i>Neisseria gonorrhoeae</i>	5x10 <sup>2</sup>
			<i>Chlamydia trachomatis</i>	5x10 <sup>2</sup>
			<i>Mycoplasma genitalium</i>	10 <sup>3</sup>
Urine**	<b>DNA-sorb-AM ePure STD DNA Extraction Kit</b>	ES3049A	<i>Neisseria gonorrhoeae</i>	10 <sup>3</sup>
			<i>Chlamydia trachomatis</i>	10 <sup>3</sup>
			<i>Mycoplasma genitalium</i>	2x10 <sup>3</sup>

\*The quantity of genome equivalents of microorganism per 1 ml of the sample from transport medium.

\*\* Pretreatment is required.

The analytical sensitivity for each microorganism is preserved in the presence of high DNA concentrations of other analyte microorganisms (up to 10<sup>9</sup> GE/ml).

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