



RUO

For Professional Use Only

Application note

Detection of Chlamydia trachomatis using eSens N.gonorrhoeae/ M.genitalium/T.vaginalis QL PCR kit, **REF ES3050A**

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/T.vaginalis QL PCR kit. This document aims to assist laboratory professionals and researchers in accurately detecting the DNA of **not only** *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* 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	Cy5.5
DNA-target	<i>N.gonorrhoeae</i>	<i>C.trachomatis</i>	<i>M.genitalium</i>	Internal Control-FL	<i>T. vaginalis</i>
Target gene	<i>16s rRNA</i> gene	<i>cryptic plasmid</i>	<i>gyrB</i> gene	Genetically engineered construction	DNA repeats for PCR identification

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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	10%

Test settings for plate-type instruments

Channel	Threshold
FAM, HEX , ROX, Cy5, Cy5.5	The threshold line for each channel is to be set at the level corresponding to 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 **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, Mycoplasma genitalium** and **Trichomonas vaginalis** DNA is **not detected** in a sample if the Ct values are not determined (absent) in the channels for the 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 in the channel for the Cy5 fluorophore is not determined (absent) or greater than the specified boundary Ct value, and the Ct value in the channels 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 should be repeated. If the same result is obtained in the second run, the analysis should be repeated starting from the DNA extraction stage.

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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	Cy5.5	FAM	JOE	ROX	Cy5	Cy5.5
C-	Ct is absent			33	Ct is absent	Ct is absent			36	Ct is absent
NCA	Ct is absent					Ct is absent				
C+	33	30	33	33	33	36	33	36	36	36
Test samples	-	-	-	33	-	-	-	-	36	-

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SPECIFICATIONS

Sensitivity

Clinical material	Nucleic acid extraction kit	PCR kit	Microorganism	Sensitivity, GE/ml*
Cervical or urethral swabs** Urine***	DNA-sorb-AM	ES3050A	<i>Neisseria gonorrhoeae</i>	5x10 ²
			<i>Chlamydia trachomatis</i>	5x10 ²
			<i>Mycoplasma genitalium</i>	1x10 ³
			<i>Trichomonas vaginalis</i>	5x10 ²
Cervical or urethral swabs** Urine***	ePure STD DNA Extraction Kit M	ES3050A	<i>Neisseria gonorrhoeae</i>	1x10 ³
			<i>Chlamydia trachomatis</i>	1x10 ³
			<i>Mycoplasma genitalium</i>	2x10 ³
			<i>Trichomonas vaginalis</i>	1x10 ³

* The quantity of genome equivalents of microorganism per 1 ml of the sample placed in a transport medium specified.

** Cervical and urethral swabs are to be placed in the transport medium for swabs or Transport Medium with Mucolytic Agent (REF 952-CE).

*** Pretreatment is required for urine samples.

NOTE: The analytical sensitivity of each microorganism does not change even in the case of high concentration of the other analyzed microorganisms – up to 10⁹ GE/ml.

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