Instruction for Use

diarellaSTI-1

real time PCR Kit

For *in vitro* detection of the DNA of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium extracted from biological specimens.

REF **G01144-96**

96

- 18°C

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1 Intended Purpose

The Kit is designed for the qualitative detection of the nucleic acid of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in eluates from biological specimens.
For research use only. Not for use in diagnostic procedures.

2 Pathogen Information

Chlamydia trachomatis are gram-negative bacteria which can only replicate in host cells. C. trachomatis are grouped in different serovars with the serovars A - C including ocular strains, serovars D - K gential strains and L1 – L3 strains that causes lymphogranuloma venereum. While most infections with C. trachomatis are asymptomatic, it can still lead to symptoms like pelvic inflammatory disease [1].
Neisseria gonorrhoeae are gram-negative diplococci bacteria. They cause the genitourinary infection gonorrhea and may infect throat and eyes. Untreated, the infection may cause pelvic inflammatory disease in women along with possible infertility or may spread to the rest of the body and lead to a disseminated gonorrhea infection [2].
Mycoplasma genitalium are small pathogenic bacteria, living in the human urinary and genital tracts. They are known to cause negative health effects in men and women and are suspected to increase the risk for an HIV infection [3].

3 Principle of the Test

The diarellaSTI-1 real time PCR Kit contains specific primers and dual-labeled probes for the amplification of the DNA of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium extracted from biological specimens.
The presence of nucleic acid is detected by an increase in fluorescence due to hydrolysis of the probes during amplification. The fluorescence of the Chlamydia trachomatis specific probes is measured in the FAM channel. The fluorescence of the Mycoplasma genitalium specific probes is measured in the ROX channel. The fluorescence of the Neisseria gonorrhoeae specific probes is measured in the Cy5 channel.
Furthermore, diarellaSTI-1 real time PCR Kit contains a Control DNA (Internal Process Control, IPC), which is added during DNA extraction and detected in the same reaction by a HEX-labeled probe.

The Control DNA allows the detection of PCR inhibition and acts as control, that the nucleic acid was isolated from the biological specimen.

4 Package Contents

The reagents supplied are sufficient for 96 reactions.

Table 1: Components of the diarellaSTI-1 real time PCR Kit

Label	Lid Colour	Content 96
Reaction Mix	yellow	1 x 1344 µl
Positive Control	red	1 x 150 µl
Negative Control	green	1 x 150 µl
Control DNA	colourless	1 x 480 µl

5 Equipment and Reagents to be Supplied by User

- DNA isolation kit (e.g. NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

6 Transport, Storage and Stability

The diarellaSTI-1 real time PCR Kit is shipped on dry ice. All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. Up to 20 freeze and thaw cycles are possible.
Protect kit components from direct sunlight during the complete test run.

7 Warnings and Precautions

Read the Instructions for Use carefully before using the product.
Before first use check the product and its components for:

- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.
- Do not mix components from different lots

8 Sample Material

Starting material for diarellaSTI-1 real time PCR is DNA isolated from biological specimens. By the nature of the pathogens, sample material like vaginal swabs or urine are commonly used.

9 Sample Preparation

Commercial kits for DNA isolation such as the following are recommended:

- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

Please follow the instructions for use of the respective extraction kit.

Important:

In addition to the samples always run a ‚water control' in your extraction. Treat this water control analogous to a sample.

Comparing the amplification of the Control DNA in the samples to the amplification of the internal control in the water control will give insights on possible inhibitions of the real time PCR. Furthermore, possible contaminations during DNA extraction will be detectable.

Please note the chapter ‚Control DNA'.

If the real time PCR is not performed immediately, store extracted DNA according to the instructions given by the manufacturer.

10 Control DNA

A Control DNA is supplied as extraction control. This allows the user to control the DNA isolation procedure and to check for possible real time PCR inhibition.

Add 5 µl Control DNA per extraction (5 µl x (N+1)). Mix well. Perform the DNA isolation according to the manufacturer's instructions.

The Control DNA must be added to the Lysis Buffer of the extraction kit.

11 Real time PCR

11.1 Important Points Before Starting:

- Please pay attention to the chapter 7 ‚Warnings and Precautions'.
- Before setting up the real time PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.

- In every PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed, and centrifuged very briefly.

11.2 Procedure

The Master Mix contains all of the components needed for PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the Master Mix

Volume per Reaction	Volume Master Mix
14.0 µl Reaction Mix	14.0 µl x (N+1)

Real time PCR set-up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument / take an optical PCR reaction plate.
- Pipet **14 µl** of the Master Mix into each optical PCR reaction tube / the optical PCR reaction plate.
- Add **6 µl** of the eluates from the DNA isolation (including the eluate of the water control), the Positive Control and the Negative Control to the corresponding optical PCR reaction tube / the optical PCR reaction plate (Table 3).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 3: Preparation of the real time PCR

Component	Volume
Master Mix	14.0 µl
Sample	6.0 µl
Total Volume	20.0 µl

11.3 Instrument Settings

For the real time PCR use one of the thermal profiles shown in Table 4 and Table 5.

Table 4: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	
Annealing and Extension	40 sec	60°C	45
	Acquisition at the end of this step		

If in the same run samples should be tested for pathogens with RNA genome, use the thermal profile shown in Table 5.

Table 5: real time RT-PCR thermal profile

Description	Time	Temperature	Number of Cycles
Reverse Transcription	10 min	45°C	1
Initial Denaturation	5 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	
Annealing and Extension	40 sec	60°C	45
	Acquisition at the end of this step		

Dependent on the real time instrument used, further instrument settings have to be adjusted according to Table 6.

Table 6: Overview of the instrument settings required for the diarellaSTI-1 real time PCR.

Real time PCR Instrument	**Parameter**	**Detection Channel**	**Notes**		
LightCycler 480II			Color Compensation Kit Multiplex 1 (G070MP1-CC) required		
			Melt Factor	Quant Factor	Max Integration Time (sec)
	C. trachomatis	465-510	1	10	1
	Control DNA (IPC)	533-580	1	10	2
	M. genitalium	533-610	1	10	2
	N. gonorrhoeae	618-660	1	10	3
Stratagene Mx3000P / Mx3005P	C. trachomatis	FAM	Gain 8	Reference Dye: None	
	Control DNA (IPC)	HEX	Gain 1		
	M. genitalium	ROX	Gain 1		
	N. gonorrhoeae	Cy5	Gain 4		
QuantStudio 5 CFX96 CFX Opus 96 Aria Mx qTower³ G	C. trachomatis	FAM	Option Reference Dye ROX: NO		
	Control DNA (IPC)	HEX			
	M. genitalium	ROX			
	N. gonorrhoeae	Cy5			
Mic qPCR Cycler	C. trachomatis	Green	Gain 8		
	Control DNA (IPC)	Yellow	Gain 10		
	M. genitalium	Orange	Gain 10		
	N. gonorrhoeae	Red	Gain 10		

12 Data Analysis

Following results can occur:

Signal/C_T Values				**Interpretation**
FAM Channel Chlamydia trachomatis	**Cy5 Channel** Neisseria gonorrhoeae	**ROX Channel** Mycoplasma genitalium	**HEX Channel** IPC	
positive[1]	negative	negative	positive or negative[2]	**Positive result,** the sample contains *Chlamydia trachomatis* DNA.
negative	**positive**[1]	negative	positive or negative[2]	**Positive result,** the sample contains Neisseria gonorrhoeae DNA.
negative	negative	**positive**[1]	positive or negative[2]	**Positive result,** the sample contains Mycoplasma genitalium DNA.
negative	negative	negative	≤ 34 [3]	**Negative result,** the sample contains no *Chlamydia trachomatis, Neisseria gonorrhoeae or Mycoplasma genitalium* DNA.
negative	negative	negative	negative or > 34 [3]	**No diagnostic statement can be made.** The real time PCR is either inhibited or errors occurred while DNA extraction.

[1] Positive signals in multiple channels can occur. In this case, multiple targets are detected.

[2] A strong positive signal in the FAM, ROX or the Cy5 can inhibit the IPC. In such cases the result for the Control DNA can be neglected.

[3] In case of high C_T values, the IPC should be compared to the water extraction control as described in the chapter 'Assay validation'.

13 Assay Validation

Negative Controls

The Negative Control must show no C_T in the FAM, Cy5, ROX and HEX channel.

Positive Controls

The Positive Control must show a positive (i.e. exponential) amplification curve in the different channels FAM, Cy5 and ROX. The Positive Control must fall below C_T 30.

Internal Controls

The following values for the amplification of the internal controls are valid using gerbion nucleic acid extraction kit NukEx Mag RNA/DNA. The Control DNA (IPC) must show a positive (i.e. exponential) amplification curve.

The IPC must fall below a C_T of 34. If the IPC is above C_T 34 this points to a purification problem or a strong positive sample that can inhibit the IPC. In the latter case, the assay is valid. It is recommended to perform the extraction of a water control in each run. The IPC in the water control must fall below a C_T of 34.

If other nucleic acid extraction kits are used, the customer must define own cutoffs. In this case the C_T value of the Control DNA (IPC) in an eluate from a sample should not be delayed for more than 4 C_T in comparison to an eluate from an extracted water control.

14 Limitations of the Method

- Strict compliance with the Instruction for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods must be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.

15 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

No fluorescence signal in the FAM, Cy5, ROX channel of the Positive Control	
The selected channel for analysis does not comply with the protocol	Select the FAM channel for analysis of the *C. trachomatis* specific amplification, the Cy5 channel for the N. gonorrhoeae specific amplification, the ROX channel for the amplification of the M. genitalium and the HEX channel for the amplification of the Control DNA (IPC).
Incorrect configuration of the real time PCR	Check your work steps and compare with chapter 'Procedure'.
The programming of the thermal profile is incorrect	Compare the thermal profile with the protocol in chapter 'Instrument Settings'.
Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability'.
Weak or no signal of the Control DNA (IPC) and simultaneous absence of a signal in the bacteria specific FAM and/or Cy5 and/or ROX channel.	
real time PCR conditions do not comply with the protocol	Check the real time PCR conditions (chapter 'Real time PCR').
real time PCR inhibited	Make sure that you use an appropriate isolation method (see chapter 'Sample Preparation') and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed.
sample material not sufficient	Make sure that enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter 'Sample Preparation') and follow the manufacturer's instructions.
DNA loss during isolation process	In case the Control DNA was added before extraction, the lack of an amplification signal can indicate that the DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability'.

Detection of a fluorescence signal in the FAM and/or Cy5 and/or ROX channel of the Negative Control	
Contamination during preparation of the PCR	Repeat the real time PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time PCR.

16 Kit Performance

16.1 Analytical Sensitivity

For the FAM, ROX and Cy5 channels, the limits of detection (LoD) of diarellaSTI-1 real time PCR Kit were determined using serial dilutions of the of synthetic DNA-fragments containing the specific gene target sequence. The determination of the LoD was done on a CFX Opus 96 Instrument (Bio-Rad).

The LoD of diarellaSTI-1 real time PCR Kit is $\leq$ 0.25 genome copies per µl for the FAM, Cy5 and ROX channel.

16.2 Analytical Specificity

The specificity of the diarellaSTI-1 real time PCR was evaluated with different ring trial samples of known status and different other relevant viruses and bacteria found in biological samples and basing on in silico analyses. Additionally, 324 qualified field samples were tested with the diarellaSTI-1 real time PCR.
All ring trial samples and other eluates with known status were detected correctly. Results are shown in tables 7 - 10.
The results for the field samples are summarized for each target in tables 11 – 13.

Table 7: Ring trial samples tested for the validation of the sensitivity and specificity of the diarellaSTI-1 real time PCR Kit.

sample	C. trachomatis	N. gonorrhoeae	M. genitalium
	FAM channel	Cy5 channel	ROX channel
QCMD 2020 Sexually Transmitted Infections I			
STI_I101S-01 Trichomonas vaginalis *	negative	negative	negative
STI_I101S-02 Mycoplasma hominis	negative	negative	negative
STI_I101S-03 G. vaginalis + T. vaginalis *	negative	negative	negative
STI_I101S-04 M. genitalium (drug resistant)	negative	negative	**positive**
STI_I101S-05 M. genitalium (wilt type)	negative	negative	**positive**
STI_I101S-06 negative	negative	negative	negative
STI_I101S-07 Gardnerella vaginalis *	negative	negative	negative
STI_I101S-08 Trichomonas vaginalis	negative	negative	negative
STI_I101S-09 M. hominis + C. trachomatis *	**positive**	negative	negative
STI_I101S-10 Trichomonas vaginalis	negative	negative	negative
QCMD 2020 Sexually Transmitted Infections II			
STI_II101S-01 Herpes simplex virus 2	negative	negative	negative
STI_II101S-02 Treponema pallidum *	negative	negative	negative
STI_II101S-03 Herpes simplex virus 1	negative	negative	negative
STI_II101S-04 C. trachomatis + M. hominis	**positive**	negative	negative
STI_II101S-05 Neisseria gonorrhoeae	negative	**positive**	negative
STI_II101S-06 C. trachomatis + N. gonorrhoeae +M. hominis *	**positive**	**positive**	negative
STI_II101S-07 C. trachomatis + M. hominis	**positive**	negative	negative
STI_II101S-08 Neisseria gonorrhoeae	negative	**positive**	negative
STI_II101S-09 Chlamydia trachomatis	**positive**	negative	negative
STI_II101S-10 Neisseria gonorrhoeae	negative	**positive**	negative

* Educational sample, very low amount of target copies

Table 8: Ring trial samples tested for the validation of the sensitivity and specificity of the diarellaSTI-1 real time PCR Kit.

sample	C. trachomatis	N. gonorrhoeae	M. genitalium
	FAM channel	Cy5 channel	ROX channel
QCMD 2020 Chlamydia trachomatis DNA			
CTDNA101S-01 C. trachomatis (LGV)	**positive**	negative	negative
CTDNA101S-02 negative	negative	negative	negative
CTDNA101S-03 C. trachomatis (LGV)	**positive**	negative	negative
CTDNA101S-04 C. trachomatis (LGV)	**positive**	negative	negative
CTDNA101S-05 C. trachomatis (LGV)	**positive**	negative	negative
CTDNA101S-06 C. trachomatis (LGV)	**positive**	negative	negative
CTDNA101S-07 C. trachomatis (Genovar F)	**positive**	negative	negative
CTDNA101S-08 C. trachomatis (LGV)	**positive**	negative	negative
CTDNA101S-09 Negative	negative	negative	negative
CTDNA101S-10 C. trachomatis (LGV) + N. gonorrhoeae (St 49226)	**positive**	**positive**	negative
QCMD 2020 Chlamydia trachomatis and Neisseria gonorrhoeae DNA			
CTNg101S-01 C. trachomatis (LGV)	**positive**	negative	negative
CTNg101S-02 Negative	negative	negative	negative
CTNg101S-03 C. trachomatis (LGV)	**positive**	negative	negative
CTNg101S-04 C. trachomatis (Genovar F)	**positive**	negative	negative
CTNg101S-05 N. gonorrhoeae (St 49226)	negative	**positive**	negative
CTNg101S-06 C. trachomatis (LGV)	**positive**	negative	negative
CTNg101S-07 N. gonorrhoeae (St 49226)	negative	**positive**	negative
CTNg101S-08 Negative	negative	negative	negative
CTNg101S-09 N. gonorrhoeae (St 49226)	negative	**positive**	negative
CTNg101S-10 N. gonorrhoeae (St 49226) + C. trachomatis (LGV)	**positive**	**positive**	negative

Table 9: Ring trial samples tested for the validation of the sensitivity and specificity of the diarellaSTI-1 real time PCR Kit.

sample	C. trachomatis	N. gonorrhoeae	M. genitalium
	FAM channel	Cy5 channel	ROX channel
QCMD 2020 Neisseria gonorrhoeae DNA			
NgDNA101S-01 N. gonorrhoeae (LvlNg PorA)	negative	**positive**	negative
NgDNA101S-02 N. gonorrhoeae (St 49226)	negative	**positive**	negative
NgDNA101S-03 N. gonorrhoeae (St 49226)	negative	**positive**	negative
NgDNA101S-04 Negative	negative	negative	negative
NgDNA101S-05 N. gonorrhoeae (St 49226)	negative	**positive**	negative
NgDNA101S-06 N. gonorrhoeae (St 49226)	negative	**positive**	negative
NgDNA101S-07 N. gonorrhoeae (St 49226)	negative	**positive**	negative
NgDNA101S-08 Negative	negative	negative	negative
NgDNA101S-09 N. gonorrhoeae (St 49226)	negative	**positive**	negative
NgDNA101S-10 N. gonorrhoeae (St 49226)	negative	**positive**	negative
QCMD 2018 Mycoplasma genitalium			
MG18S-01 Negative	negative	negative	negative
MG18S-02 M. genitalium G37	negative	negative	**positive**
MG18S-03 M. genitalium G37 *	negative	negative	**positive**
MG18S-04 M. genitalium G37	negative	negative	**positive**
MG18S-05 M. genitalium G37 *	negative	negative	negative
MG18S-06 M. genitalium G37	negative	negative	**positive**
MG18S-07 M. genitalium G37	negative	negative	**positive**
MG18S-08 M. genitalium M6306	negative	negative	**positive**
MG18S-09 M. genitalium M6306	negative	negative	**positive**
MG18S-10 M. genitalium M6306	negative	negative	**positive**

* Educational sample, very low amount of target copies

Table 10: Eluted DNA/RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of diarellaSTI-1 real time PCR Kit.

Eluates with known status	**diarellaSTI-1** C. trachomatis **FAM channel**	**diarellaSTI-1** N. gonorrhoeae **Cy5 channel**	**diarellaSTI-1** M. genitalium **ROX channel**
Cytomegalievirus	negative	negative	negative
Chlamydia pneumoniae	negative	negative	negative
Herpes Simplex Virus Type 1	negative	negative	negative
Herpes Simplex Virus Type 2	negative	negative	negative
Mycoplasma pneumoniae	negative	negative	negative
Mycoplasma hyopneumoniae	negative	negative	negative
Treponema phagadenis	negative	negative	negative
Varizella Zoster Virus Genotype 3	negative	negative	negative
Varizella Zoster Virus Genotype 5	negative	negative	negative
Chlamydia trachomatis	**positive**	negative	negative
Mycoplasma genitalium	negative	negative	**positive**
Neisseria gonorrhoeae	negative	**positive**	negative
Gardnerella vaginalis	negative	negative	negative
Trichomonas vaginalis	negative	negative	negative
Mycoplasma hominis	negative	negative	negative
Ureaplasma parvum	negative	negative	negative
Ureaplasma urealyticum	negative	negative	negative

Table 11: Qualified field samples tested for C. trachomatis using diarellaSTI-1 real time PCR.

		Chlamydia trachomatis	
		positive	negative
diarellaSTI-1	positive	12	0
	negative	0	312
		Sensitivity (%)	Specificity (%)
		100	100

Table 12: Qualified field samples tested for N. gonorrhoeae using diarellaSTI-1 real time PCR.

		Neisseria gonorrhoeae	
		positive	negative
diarellaSTI-1	positive	5	0
	negative	0	319
		Sensitivity (%)	Specificity (%)
		100	100

Table 13: Qualified field samples tested for M. genitalum using diarellaSTI-1 real time PCR.

		Mycoplasma genitalium	
		positive	negative
diarellaSTI-1	positive	2	0
	negative	0	322
		Sensitivity (%)	Specificity (%)
		100	100

16.3 Linear Range

The linear range of the diarellaSTI-1 real time PCR Kit was evaluated by analysing logarithmic dilution series of quantified synthetic DNAs of the target sequences.

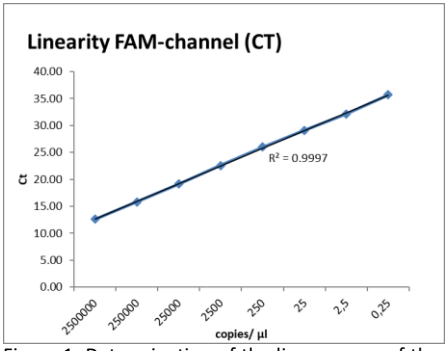

Figure 1: Determination of the linear range of the diarellaSTI-1 real time PCR Kit for Chlamydia trachomatis (CT) in the FAM channel.

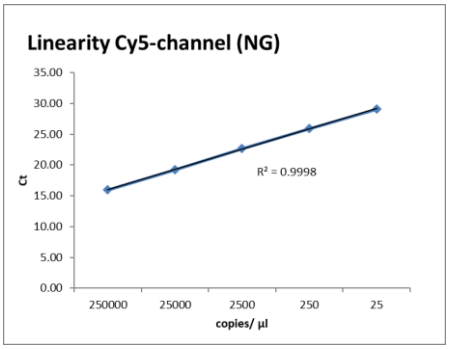

Figure 2: Determination of the linear range of the diarellaSTI-1 real time PCR Kit for Neisseria gonorrhoeae (NG) in the Cy5 channel.

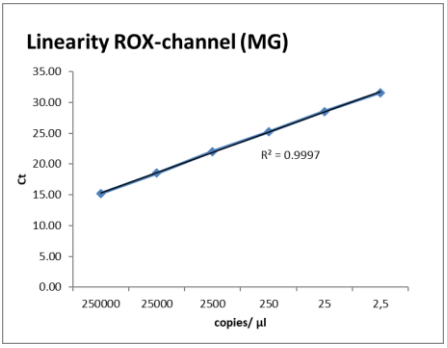

Figure 3: Determination of the linear range of the diarellaSTI-1 real time PCR Kit for the Mycoplasma genitalium (MG) in the ROX channel.

16.4 Precision

The precision of the diarellaSTI-1 real time PCR Kit was determined as intra-assay variability, inter-assay variability and inter-lot variability.
Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of C. trachomatis specific synthetic DNA, N. gonorrhoeae specific synthetic DNA, M. genitalium specific synthetic DNA and on the threshold cycle of the Control DNA (IPC). The results are shown in Table 14.

Table 14: Precision of the diarellaSTI-1 real time PCR Kit

C. trachomatis (FAM)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	2.5	0.31	0.97
Inter-Assay-Variability	2.5	0.14	0.42
Inter-Lot-Variability	2.5	0.19	0.59
N. gonorrhoeae (Cy5)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	2.5	0.44	1.37
Inter-Assay-Variability	2.5	0.25	0.79
Inter-Lot-Variability	2.5	0.13	0.40
M. genitalium (ROX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	2.5	0.30	0.95
Inter-Assay-Variability	2.5	0.53	1.67
Inter-Lot-Variability	2.5	0.20	0.62
IPC (HEX)	copies/µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	250	0.22	0.75
Inter-Assay-Variability	250	0.32	1.07
Inter-Lot-Variability	250	0.61	2.01

17 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid	REF	Catalog number
PCR	Polymerase Chain Reaction	Σ	Contains sufficient for <n> test
REACTION MIX	Reaction Mix	-18°C	Upper limit of temperature
CONTROL +	Positive Control		Manufacturer
CONTROL −	Negative Control		Use by YYYY-MM-DD
CONTROL DNA IPC	Control DNA (IPC)	LOT	Batch code
CONT	Content	RUO	Research use only
			Consult Instruction for Use

18 Literature

[1] Bébéar and de Barbeyrac. 2009. Genital Chlamydia trachomatis infections. Clin Microbiol Infect 2009; 15: 4 - 10

[2] Ng and Martin. 2005. The laboratory diagnosis of Neisseria gonorrhoeae. Can J Infect Dis Med Microbiol. 16(1) 15 – 25

[3] Gaydos. 2017. Mycoplasma genitalium: Accurate Diagnosis is necessary for adequate treatment. Journal of Infectious Diseases. 216(S2): S406 - 411