Instruction for Use

diarellaSTI-2

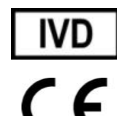
real time PCR Kit

For *in vitro* detection of the DNA of Trichomonas vaginalis, Ureaplasma parvum, Ureaplasma urealyticum and Mycoplasma hominis extracted from biological specimens.

G01145-96

96

gerbion GmbH & Co. KG
Remsstr. 1
70806 Kornwestheim, DE
phone: +49 7154 806 20 0
fax: +49 7154 806 20 29
e-mail: info@gerbion.com
www.gerbion.com



Index

1 Intended Purpose ... 3

2 Pathogen Information ... 3

3 Principle of the Test ... 3

4 Package Contents ... 4

5 Equipment and Reagents to be Supplied by User ... 4

6 Transport, Storage and Stability ... 5

7 Warnings and Precautions ... 5

8 Sample Material ... 6

9 Sample Preparation ... 6

10 Control DNA ... 6

11 Real time PCR ... 7

11.1 Important Points Before Starting: ... 7

11.2 Procedure ... 7

11.3 Instrument Settings ... 8

12 Data Analysis ... 10

13 Assay Validation ... 11

14 Limitations of the Method ... 11

15 Troubleshooting ... 12

16 Kit Performance ... 13

16.1 Analytical Sensitivity ... 13

16.2 Analytical Specificity ... 13

16.3 Linear Range ... 17

16.4 Precision ... 19

16.5 Diagnostic Sensitivity ... 20

17 Abbreviations and Symbols ... 21

18 Literature ... 21

1 Intended Purpose

The Kit is designed for the qualitative detection of the nucleic acid of Trichomonas vaginalis, Ureaplasma parvum, Ureaplasma urealyticum, and Mycoplasma hominis in eluates from biological specimens. The assay is an in vitro diagnostic medical device and intended to be used by professional users in a laboratory environment. It can be performed manually or using an automated platform. The assay serves as an aid in the diagnosis, screening and monitoring of Trichomonas vaginalis, Ureaplasma parvum, Ureaplasma urealyticum, and Mycoplasma hominis.

2 Pathogen Information

Trichomonas vaginalis is an anaerobic, flagellated protozoan parasite. It is the causative agent of the sexual transmitted dissease trichomonasis. While infections in men are usually asymptomatic, infections in women usually comes along with symptoms of vaginitis [1], [2].

Ureaplasma parvum, Ureaplasma urealyticum and Mycoplasma hominis belong to the family of Mycoplasmatales. The Mycoplasmatales are small bacteria that lack a cell wall and possess a three-layered cellular membrane. Therefore, these bacteria are resistant to a lot of different antibiotics targeting the cell wall synthesis.

Both Ureaplasma species are known to cause inflammatory vulvovaginitis, urethritis, bacterial vaginosis and infertility. The ability to perform urea hydrolysis which creates ammonia as a product which comes along with a characteristic smell.

Mycoplasma hominis is associated with pelvic inflammatory disease, vaginal bacteriosis and male infertility [3].

3 Principle of the Test

The diarellaSTI-2 real time PCR Kit contains specific primers and dual-labeled probes for the amplification of the DNA of Trichomonas vaginalis, Ureaplasma parvum, Ureaplasma urealyticum and Mycoplasma hominis 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 Trichomonas vaginalis specific probes is measured in the FAM channel. The fluorescence of the Ureaplasma specific probes is measured in the ROX

channel. The fluorescence of the Mycoplasma hominis specific probes is measured in the Cy5 channel.
Furthermore, diarellaSTI-2 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-2 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-2 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-2 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 of the respective nucleic acid extraction kit.

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 the thermal profile shown in Table 4.

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	45
Annealing and Extension	40 sec	60°C	
	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	45
Annealing and Extension	40 sec	60°C	
	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-2 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)
	T. vaginalis	465-510	1	10	1
	Control DNA (IPC)	533-580	1	10	2
	U. urealyticum, U. parvum	533-610	1	10	2
	M. hominis	618-660	1	10	3
Stratagene Mx3000P / Mx3005P	T. vaginalis	FAM	Gain 8	Reference Dye: None	
	Control DNA (IPC)	HEX	Gain 1		
	U. urealyticum, U. parvum	ROX	Gain 1		
	M. hominis	Cy5	Gain 4		
QuantStudio 5 CFX96 CFX Opus 96 Aria Mx qTower³ G	T. vaginalis	FAM	Option Reference Dye ROX: NO		
	Control DNA (IPC)	HEX			
	U. urealyticum, U. parvum	ROX			
	M. hominis	Cy5			
Mic qPCR Cycler	T. vaginalis	Green	Gain 8		
	Control DNA (IPC)	Yellow	Gain 10		
	U. urealyticum, U. parvum	Orange	Gain 10		
	M. hominis	Red	Gain 10		

12 Data Analysis

Following results can occur:

Signal/C_T Values				Interpretation
FAM Channel Trichomonas vaginalis	**Cy5 Channel** Mycoplasma hominis	**ROX Channel** U. parvum / U. urealyticum	**HEX Channel** IPC	
positive	negative	negative	positive or negative[1]	**Positive result,** the sample contains Trichomonas vaginalis DNA.
negative	**positive**	negative	positive or negative[1]	**Positive result,** the sample contains Mycoplasma hominis DNA.
negative	negative	**positive**	positive or negative[1]	**Positive result,** the sample contains Ureaplasma parvum or Ureaplasma urealyticum DNA.
negative	negative	negative	≤ 34 [2]	**Negative result**, the sample contains no Trichomonas vaginals, Mycoplasma hominis, Ureaplasma parvum or Ureaplasma urealyticum DNA.
negative	negative	negative	negative or > 34 [2]	**No diagnostic statement can be made.** The real time PCR is either inhibited or errors occurred while DNA extraction.

[1] A strong positive signal in the FAM, CY5 or the ROX channel can inhibit the IPC. In such cases the result for the Control DNA can be neglected.

[2] 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 have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the diarellaSTI-2 real time PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

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 T. vaginalis specific amplification, the Cy5 channel for the M. hominis specific amplification, the ROX channel for the amplification of U. parvum and U. urealyticum 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 described 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 chapter ‘Transport, Storage and Stability’.
Weak or no signal of the Control DNA (IPC) and simultaneous absence of a signal in the 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 chapter ‘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 occurred 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-2 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-2 real time PCR Kit is $\leq$ 2.5 genome copies per µl for the FAM, Cy5 and ROX channel.

16.2 Analytical Specificity

The specificity of the diarellaSTI-2 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, 321 qualified field samples were tested with the diarellaSTI-2 real time PCR.

All ring trial samples and other eluates with known status were detected correctly. Results are shown in Table 7 and Table 8.

The results for the field samples are summarized for each target in Table 9, Table 10 and Table 11.

Table 7: Ring trial samples tested for the validation of the sensitivity and specificity of the diarellaSTI-2 real time PCR Kit.

sample	T. vaginalis	M. hominis	U. parvum U. urealyticum
	FAM channel	Cy5 channel	ROX channel
QCMD 2020 Sexually Transmitted Infections I			
STI_I101S-01 Trichomonas vaginalis	**positive**	negative	negative
STI_I101S-02 Mycoplasma hominis	negative	**positive**	negative
STI_I101S-03 G. vaginalis + T. vaginalis	**positive**	negative	negative
STI_I101S-04 M. genitalium (drug resistant)	negative	negative	negative
STI_I101S-05 M. genitalium (wilt type)	negative	negative	negative
STI_I101S-06 negative	negative	negative	negative
STI_I101S-07 Gardnerella vaginalis	negative	negative	negative
STI_I101S-08 Trichomonas vaginalis	**positive**	negative	negative
STI_I101S-09 M. hominis + C. trachomatis	negative	**positive**	negative
STI_I101S-10 Trichomonas vaginalis	**positive**	negative	negative
QCMD 2020 Sexually Transmitted Infections II			
STI_II101S-01 Herpes simplex virus (type 2)	negative	negative	negative
STI_II101S-02 Treponema pallidum	negative	negative	negative
STI_II101S-03 Herpes simplex virus (type 1)	negative	negative	negative
STI_II101S-04 C. trachomatis + M. hominis	negative	**positive**	negative
STI_II101S-05 Neisseria gonorrhoeae	negative	negative	negative
STI_II101S-06 C. trachomatis + N. gonorrhoeae +M. hominis	negative	**positive**	negative
STI_II101S-07 C. trachomatis + M. hominis	negative	**positive**	negative
STI_II101S-08 Neisseria gonorrhoeae	negative	negative	negative
STI_II101S-09 Chlamydia trachomatis	negative	negative	negative
STI_II101S-10 Neisseria gonorrhoeae	negative	negative	negative
QCMD 2020 Trichomonas vaginalis			
TV101S-01 Trichomonas vaginalis	**positive**	negative	negative
TV101S-02 Trichomonas vaginalis	**positive**	negative	negative
TV101S-03 T. vaginalis + C. trachomatis + M. hominis	**positive**	**positive**	negative
TV101S-04 T. vaginalis + G. vaginalis	**positive**	negative	negative
TV101S-05 Trichomonas negative	negative	negative	negative
TV101S-06 Trichomonas vaginalis	**positive**	negative	negative
TV101S-07 Trichomonas vaginalis	**positive**	negative	negative

Table 8: Eluted DNA/RNA from bacterial and viral pathogens tested for the determination of the analytical specificity of diarellaSTI-2 real time PCR Kit.

Eluates with known status	**diarellaSTI-2** T. vaginalis **FAM channel**	**diarellaSTI-2** M. hominis **Cy5 channel**	**diarellaSTI-2** U. parvum U. urealyticum **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	negative	negative	negative
Mycoplasma genitalium	negative	negative	negative
Neisseria gonorrhoeae	negative	negative	negative
Gardnerella vaginalis	negative	negative	negative
Trichomonas vaginalis	**positive**	negative	negative
Mycoplasma hominis	negative	**positive**	negative
Ureaplasma parvum	negative	negative	**positive**
Ureaplasma urealyticum	negative	negative	**positive**

Table 9: Qualified field samples tested for T. vaginalis using diarellaSTI-2 real time PCR.

		Trichomonas vaginalis	
		positive	negative
diarellaSTI-2	positive	2	0
	negative	0	319
		Sensitivity (%)	Specificity (%)
		100	100

Table 10: Qualified field samples tested for M. hominis using diarellaSTI-2 real time PCR.

		Mycoplasma hominis	
		positive	negative
diarellaSTI-2	positive	26	0
	negative	0	295
		Sensitivity (%)	Specificity (%)
		100	100

Table 11: Qualified field samples tested for U. parvum and U. urealyticum using diarellaSTI-2 real time PCR.

		U. parvum / U. urealyticum	
		positive	negative
diarellaSTI-2	positive	140	0
	negative	0	181
		Sensitivity (%)	Specificity (%)
		100	100

16.3 Linear Range

The linear range of the diarellaSTI-2 real time PCR Kit was evaluated by analysing logarithmic dilution series of in quantified synthetic DNAs of the target sequences.

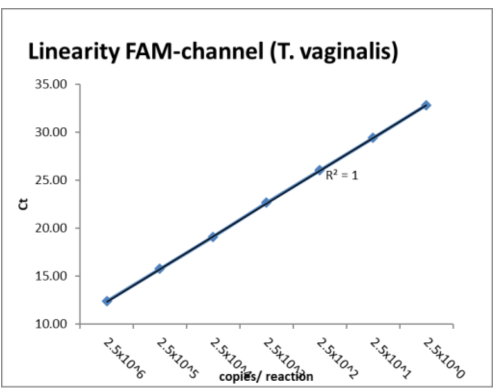


Figure 1: Determination of the linear range of the diarellaSTI-2 real time PCR Kit for T. vaginalis in the FAM channel:

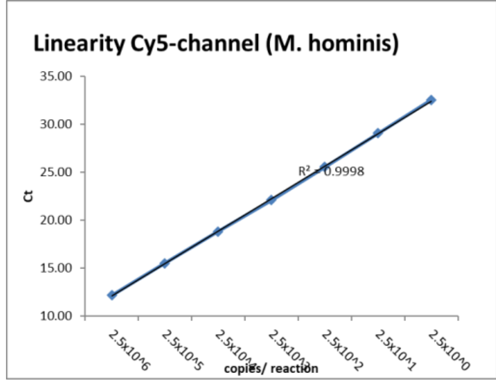


Figure 2: Determination of the linear range of the diarellaSTI-2 real time PCR Kit for M. hominis in the Cy5 channel:

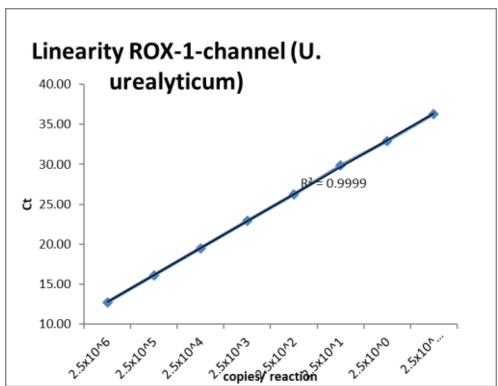


Figure 3: Determination of the linear range of the diarellaSTI-2 real time PCR Kit for the U. urealyticum in the ROX channel:

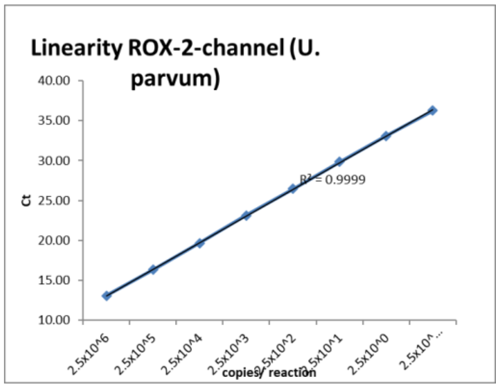


Figure 4: Determination of the linear range of the diarellaSTI-2 real time PCR Kit for the U. parvum in the ROX channel:

16.4 Precision

The precision of the diarellaSTI-2 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 T. vaginalis specific synthetic DNA, M. hominis specific synthetic DNA, U. parvum and U. urealyticum specific synthetic DNA and on the threshold cycle of the Control DNA (IPC). The results are shown in Table 12.

Table 12: Precision of the diarellaSTI-2 real time PCR Kit

T. vaginalis (FAM)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2.5	0.37	1.13
Inter-Assay-Variability	2.5	0.61	1.85
Inter-Lot-Variability	2.5	0.28	0.86
M. hominis (Cy5)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2.5	0.42	1.30
Inter-Assay-Variability	2.5	0.61	1.88
Inter-Lot-Variability	2.5	0.07	0.23
U. urealyticum (ROX)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2.5	0.25	0.76
Inter-Assay-Variability	2.5	0.27	0.84
Inter-Lot-Variability	2.5	0.21	0.65
U. parvum (ROX)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	2.5	0.33	1.00
Inter-Assay-Variability	2.5	0.53	1.61
Inter-Lot-Variability	2.5	0.17	0.52
IPC (HEX)	copies/µl	Standard Deviation	Coefficient of Variation (%)
Intra-Assay Variability	5000	0.17	0.58
Inter-Assay-Variability	5000	0.22	0.75
Inter-Lot-Variability	5000	0.27	0.90

16.5 Diagnostic Sensitivity

The diagnostic sensitivity of real time (RT-) PCR assays is mainly dependent on the DNA/RNA extraction method used to isolate DNA and RNA from various biological specimens. DNA/RNA extraction reagents are not part of the gerbion real time (RT-) PCR kits. gerbion real time (RT-) PCR kits include an extraction control and guidelines for the validation criteria of the extraction control in each reaction. The extraction control indicates inhibition of the real time (RT-) PCR and/or inefficient nucleic acid extraction. It cannot be used as a calibrator.

Therefore, gerbion guarantees the analytical sensitivities and specificities of the real time (RT-) PCR kits, performed with eluted DNA and RNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. gerbion does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of gerbion real time (RT-) PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods. It is the responsibility of the user to qualify the extraction methods used for DNA/RNA isolation from biological samples.

17 Abbreviations and Symbols

Symbol	Meaning	Symbol	Meaning
DNA	Deoxyribonucleid 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	IVD	*In vitro* diagnostic medical device
i	Consult instructions for use	CE	European Conformity
UDI	Unique Device Identification		

18 Literature

[1] Herath et al. (2021) Comparison of diagnostic methods and analysis of socio-demographic factors associated with Trichomonas vaginalis infection in Sri Lanka. Plos One 16(10)

[2] Frolund et al. (2014) Comparison between Culture and Multiplex Quantitative Real-Time Polymerase Chain Reaction Assay Detecting Ureaplasma urealyticum and U. parvim. Plos One 9(7)

[3] Campos et al. (2015) Prevalence of Mycoplasma genitalium and Mycoplasma hominis in urogenital tract of Brazilian women. BMC Infectious Disease 15:60