RIDA®GENE STI Mycoplasma Panel

real-time PCR

Art. Nr.: PG4945

100 reactions

For *in vitro* diagnostic use.

-20 °C

R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany

Tel.: +49 (0) 61 51 81 02-0 / Telefax: +49 (0) 61 51 81 02-20

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1. Intended use

For *in-vitro* diagnostic use. RIDA®GENE STI Mycoplasma Panel is a real-time PCR for the direct, qualitative detection of *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum/parvum* from human genital swabs and urine.

The RIDA®GENE STI Mycoplasma Panel real-time PCR is intended for use as an aid in diagnosis of urinary tract infections or infections of the genital area caused by *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum/parvum*.

2. Summary and Explanation of the test

Mycoplasma species may persist as part of the normal human flora of the respiratory system or the genital area[1]. Of seven known mycoplasma species from the genital area, so far only four have been described as pathogenic: *Mycoplasma hominis, Mycoplasma genitalium*, *Ureaplasma urealyticum* und *Ureaplasma parvum.*

Mycoplasma hominis (*M. hominis*) mainly colonizes the genital tract of sexually active men and women, however most of the *M. hominis* described infections have been diagnosed in woman[2]. *M. hominis* is associated with pelvic inflammatory disease (PID) and may cause infections during or after pregnancy, such as endometritis or neonatal pneumonia[1]. Common symptoms for infections with *M. hominis* include e.g. frequent urination, yellow discharge or dysuria[1,3].

Globally, the prevalence of *Mycoplasma genitalium* (*M. genitalium*) ranges between 1- 4 % for men and 1 – 6.4 % for women. In men, *M. genitalium* may result in non-specific urethritis and is the second most common cause after *Chlamydia trachomatis*. About 30 % of persistent urethritis is linked to *M. genitalium*. In women, *M. genitalium* infections may lead to cervicitis, endometritis, urethritis or pelvic inflammatory disease (PID)[1,4].

Ureaplasma urealyticum (*U. urealyticum*) and *Ureaplasma parvum* (*U. parvum*) are parasitic, gram-negative bacteria which can be part of the urogenitalflora of men and women.

In 2002, the earlier existing nomenclature of 14 *U. urealyticum* serovars has been updated, so that serovar 1, 3, 6 and 14, which groups to Parvo Biovar (Biovar 1), are now listed as separate species (*U. parvum*). Serovars 2, 4, 5, 7, 8T, 9, 10, 11, 12 and 13 belong to T960 Biovar (Biovar 2) and therefore are listed as *U. urealyticum*[5].

In women, *U. urealyticum* prevalently causes pelvic inflammatory disease (PID) and *Ureaplasma* colonizes the vaginal flora of up to 50 % of pregnant women. During pregnancy *Ureaplasma* may be transmitted to the child, which can lead to pneumonia or diseases of the central nervous system[1].

3. Test principle

The RIDA®GENE STI Mycoplasma Panel is a real-time PCR for the direct, qualitative detection of *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum/parvum.*

After DNA-isolation, amplification of gene fragments (if present) specific for *Mycoplasma hominis* (16S rRNA), *Mycoplasma genitalium* (IGS) and *Ureaplasma urealyticum/parvum* (16S rRNA) occurs.

The amplified targets for *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum/parvum* are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE STI Mycoplasma Panel assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and/or to determine possible PCR inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount	Lid Color
1	Reaction Mix	2x 1100 µl	yellow
2	Taq-Polymerase	1x 11 µl	red
D	Internal Control DNA	2x 1800 µl	orange
N	PCR Water	1x 500 µl	white
P	Positive Control	1x 200 µl	blue

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw and fully defrost reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all reagents should be stored cold in an appropriate way (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

- Sterile, media-free Rayon or Nylon flocked swabs (e.g. Copan Diagnostic Inc., catalogue no. 155C or 552C)
- The RIDA®GENE STI Mycoplasma Panel real-time PCR Assay is suitable for use with following extraction platforms and real-time PCR instruments:
  - Extraction platform:
    RIDA® Xtract (R-Biopharm)
    Maxwell® 16 (Promega)
    NucliSENS® easyMAG™ (BioMérieux)
  - Real-time PCR instrument:

Roche:	LightCycler® 480II
Agilent Technologies:	Mx3005P
Applied Biosystems:	ABI 7500
Abbott:	m2000rt
Bio-Rad:	CFX96™
QIAGEN:	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves

7. Precautions for users

For *in-vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com

8. Collection and Storage of Samples

8.1 Sample preparation

8.1.1 DNA Isolation from dry swabs

For DNA isolation from swabs the following procedure is recommended: Add 400 µl water (RNase-free) into a preparation tube. Insert the swab into the water and cut or break the swab stem. Cap the preparation tube tightly, vortex shortly and continue according to manufacturer's instruction of the DNA extraction kit or DNA extraction system.

The RIDA®GENE STI Mycoplasma Panel real-time PCR kit contains an Internal Control DNA (ICD) that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.
If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab. 3).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and **not** directly to the specimen.

8.1.2 DNA Isolation from urine

To isolate DNA from urine, we recommend using a commercially available DNA extraction system. Isolate DNA according to manufacturer's instructions.

The RIDA®GENE STI Mycoplasma Panel real-time PCR kit contains an Internal Control DNA (ICD) that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.
If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab. 3).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and **not** directly to the specimen.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and negative control must be included in each assay run.

We recommend calculating an additional volume of 10% to compensate imprecise pipetting (see Tab. 2, Tab. 3). Thaw, mix gently and briefly centrifuge the Reaction Mix, the Taq-Polymerase, the Positive Control, the PCR Water and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 2: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 µl
	Total	**20 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit Code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl PCR Water as negative control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the negative control PCR-Mix.

Sample: Add 5 µl DNA extract to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the positive control PCR-Mix.

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument Set-up (see Tab.4, Tab. 5).

9.3 PCR Instrument Set-up

9.3.1 DNA real-time PCR profile

Tab. 4: DNA real-time PCR profile for LightCycler® 480II, Rotor-Gene Q

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	10 sec, 95 °C
Annealing/Extension	15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 5: DNA real-time PCR profile for Mx3005P, ABI7500, CFX96™ and m2000rt

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	15 sec, 95 °C
Annealing/Extension	30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.4 Detection Channel Set-up

Tab. 6: Selection of appropriate detection channels

Real-time PCR Instrument	Detection	Detection Channel	Note
Roche LightCycler® 480II	*Mycoplasma hominis*	465/510	**RIDA®GENE Color Compensation Kit I (PG0001) is required**
	ICD	533/580	
	U. urealyticum/parvum	533/610	
	M. genitalium	618/660	
ABI 7500	*Mycoplasma hominis*	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
	U. urealyticum/parvum	ROX	
	M. genitalium	Cy5	
Abbott m2000rt	*Mycoplasma hominis*	FAM	**-**
	ICD	VIC	
	U. urealyticum/parvum	ROX	
	M. genitalium	Cy5	
Agilent Techn. Mx3005P	*Mycoplasma hominis*	FAM	**Check that passive reference option ROX is none**
	ICD	HEX	
	U. urealyticum/parvum	ROX	
	M. genitalium	Cy5	
Qiagen Rotor-Gene Q	*Mycoplasma hominis*	Green	**The gain settings have to be set to 5**
	ICD	Yellow	
	U. urealyticum/parvum	Orange	
	M. genitalium	Red	
Bio-Rad CFX96™	*Mycoplasma hominis*	FAM	**-**
	ICD	VIC	
	U. urealyticum/parvum	ROX	
	M. genitalium	Cy5	

10. Quality Control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instructions. Positive and negative controls have to show correct results (see Table 7, Fig. 1, Fig. 2, Fig. 3) in order to determine a VALID run.
The positive control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Tab. 7: For a VALID run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target Ct
PTC	Positive	NA *[1]	See Quality Assurance Certificate
NTC	Negative	Ct > 20	0

*[1] *No Ct value is required for the ICD to make a positive call for the positive control.*

For the Internal Control DNA (ICD) the range of validity is at Ct 20 – 35.

If the Positive Control (PTC) is not positive within the specified Ct range but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and a new Positive Control.

If the Negative Control (NTC) is not negative but the Positive control is valid prepare all new reactions using remaining purified nucleic acids and a new Negative Control.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

Fig. 1: Correct run of the positive and negative control (*M. hominis*) on the LightCycler® 480II

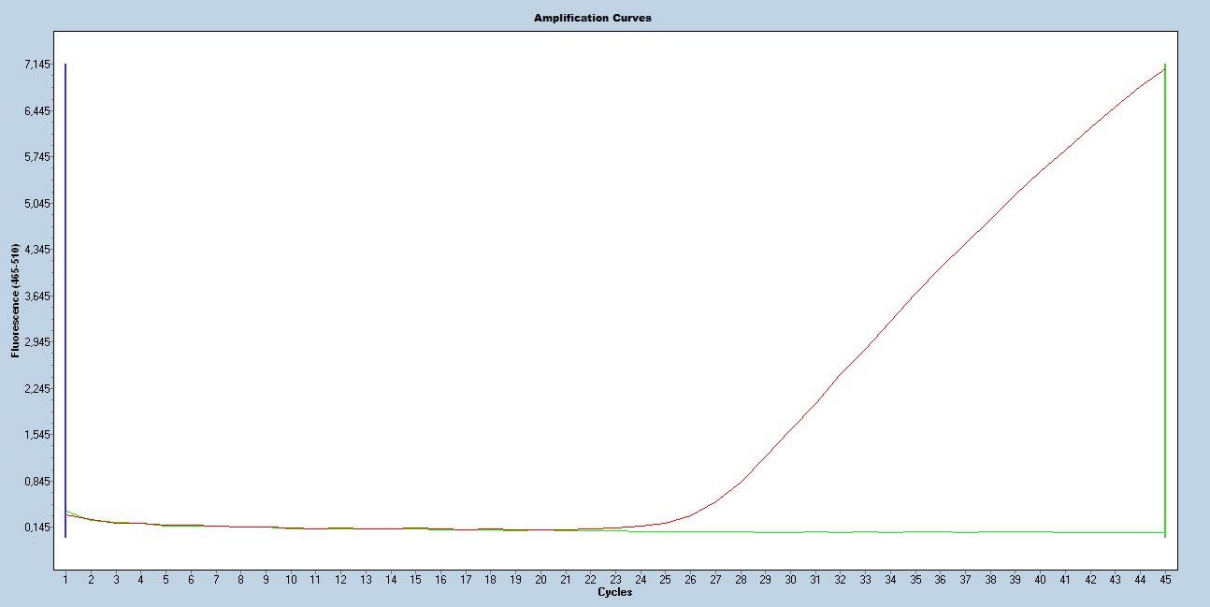


Fig. 2: Correct run of the positive and negative control (*U. urealyticum/parvum*) on the LightCycler® 480II

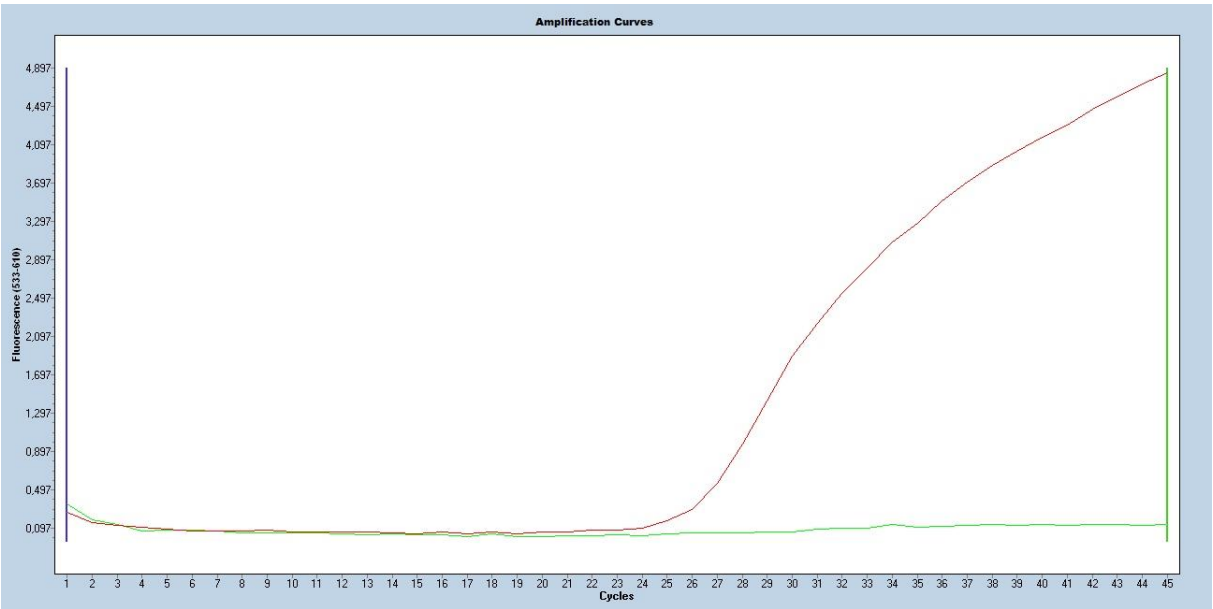
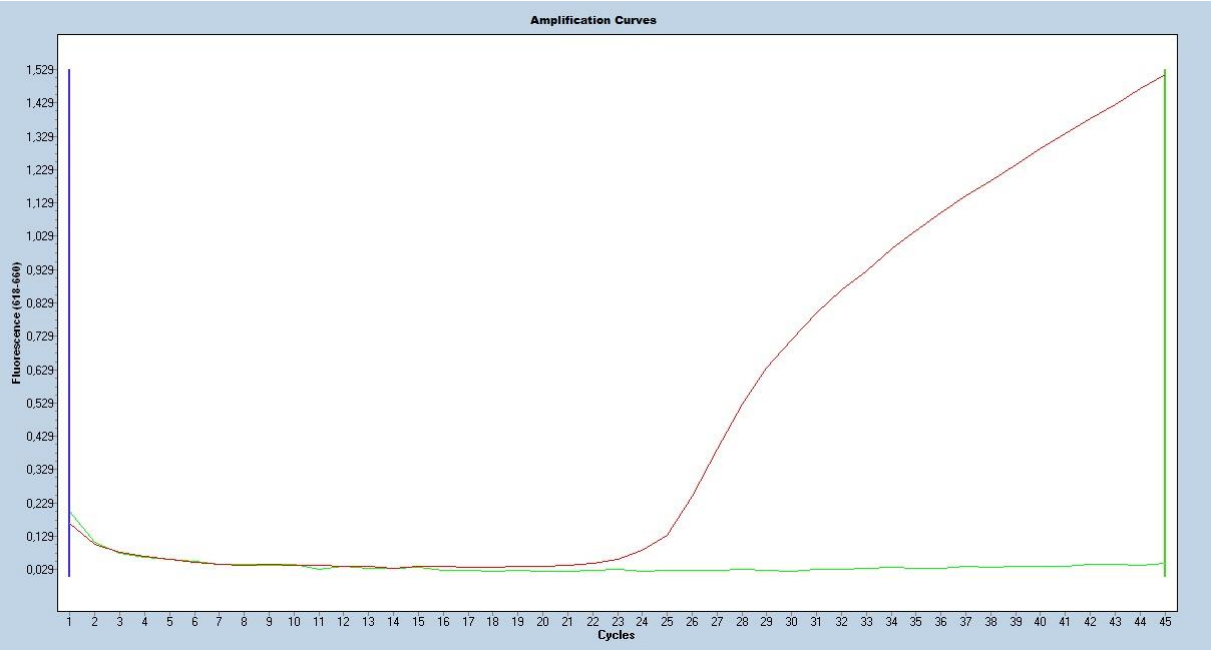


Fig. 3: Correct run of the positive and negative control (*M. genitalium*) on the LightCycler® 480II



11. Result interpretation

The result interpretation is done according to Table 8.

Tab. 8: Sample interpretation

Detection				
M. hominis	**U. urealyticum/ parvum**	**M. genitalium**	**ICD**	**Result**
positive	negative	negative	**positive/negative**	***M. hominis* detected**
negative	**positive**	negative	**positive/negative**	***U. urealyticum/parvum* detected**
negative	negative	**positive**	**positive/negative**	***M. genitalium* detected**
positive	**positive**	negative	**positive/negative**	***M. hominis*, *U. urealyticum/parvum* detected**
negative	**positive**	**positive**	**positive/negative**	***U. urealyticum/parvum*, *M. genitalium* detected**
positive	negative	**positive**	**positive/negative**	***M. hominis*, *M. genitalium* detected**
positive	**positive**	**positive**	**positive/negative**	***M. hominis*, *U. urealyticum/parvum*, *M. genitalium* detected**
negative	negative	negative	**positive**	**Target genes not detected**
negative	negative	negative	negative	**Invalid**

Mycoplasma hominis, *Mycoplasma genitalium* or *Ureaplasma urealyticum/parvum* are detected, if the sample DNA and the Internal Control DNA (ICD) show an amplification signal in the detection system.

Mycoplasma hominis, Mycoplasma genitalium or *Ureaplasma urealyticum/parvum* are also detected, if the sample DNA shows an amplification signal but none for the Internal Control DNA (ICD) in the detection system. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA (ICD).

Mycoplasma hominis, *Mycoplasma genitalium* or *Ureaplasma urealyticum/parvum* is not detected, if the sample DNA shows no amplification signal, but an amplification signal for the Internal Control DNA (ICD) in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA (ICD).

A sample is invalid, if the sample DNA and Internal Control DNA (ICD) show no amplification signal in the detection system. The sample contains a PCR inhibitor. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for genital swabs and urine.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE STI Mycoplasma Panel assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. Mucin may exhibit an interfering effect even if present in low concentrations.
8. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target genes for *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum/parvum*.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE STI Mycoplasma Panel real-time PCR has a detection limit of ≥ 10 DNA copies per reaction (see Fig. 4, Fig.5, Fig. 6).

Fig. 4: Dilution series *M. hominis* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

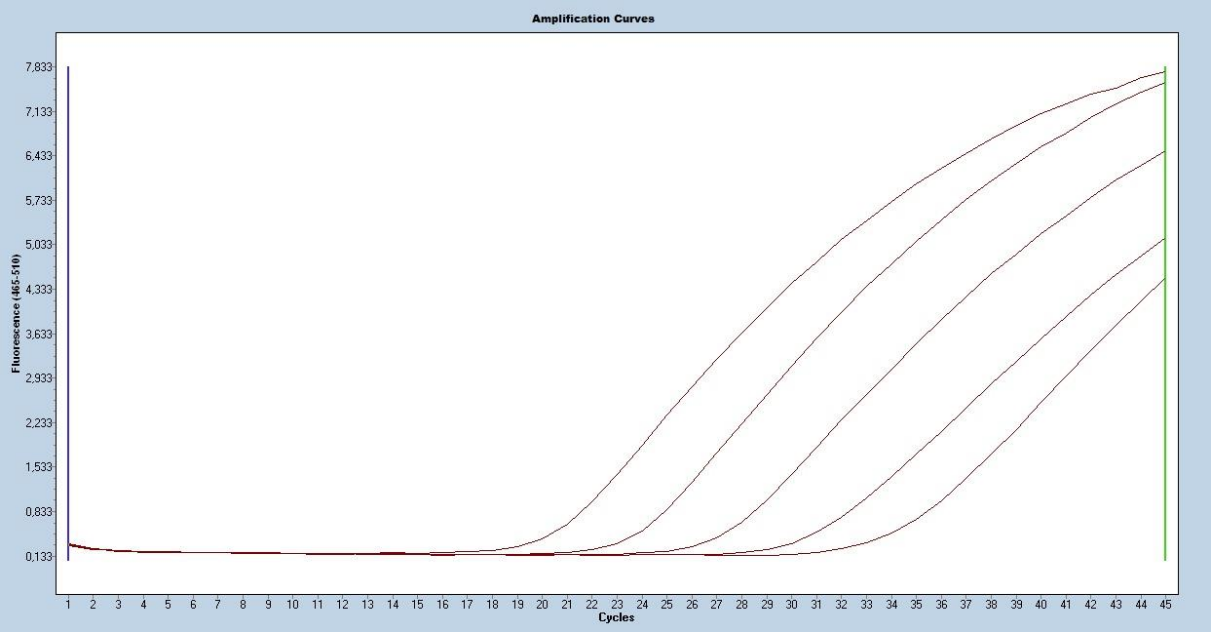


Fig. 5: Dilution series *U. urealyticum/parvum* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

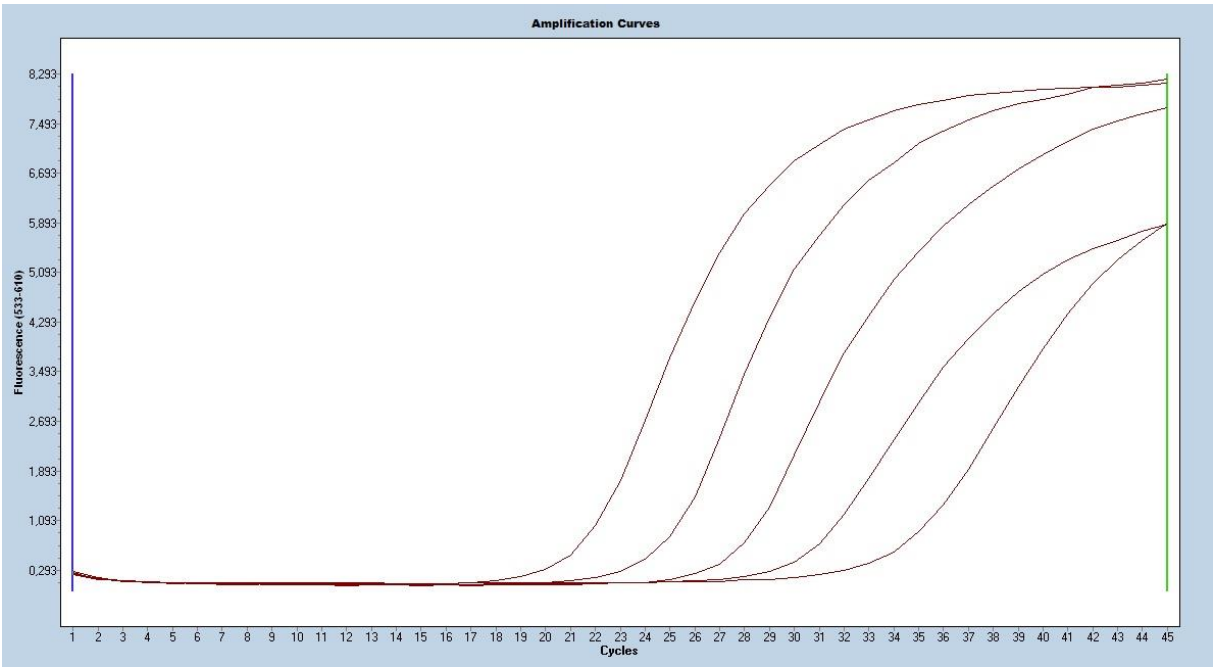
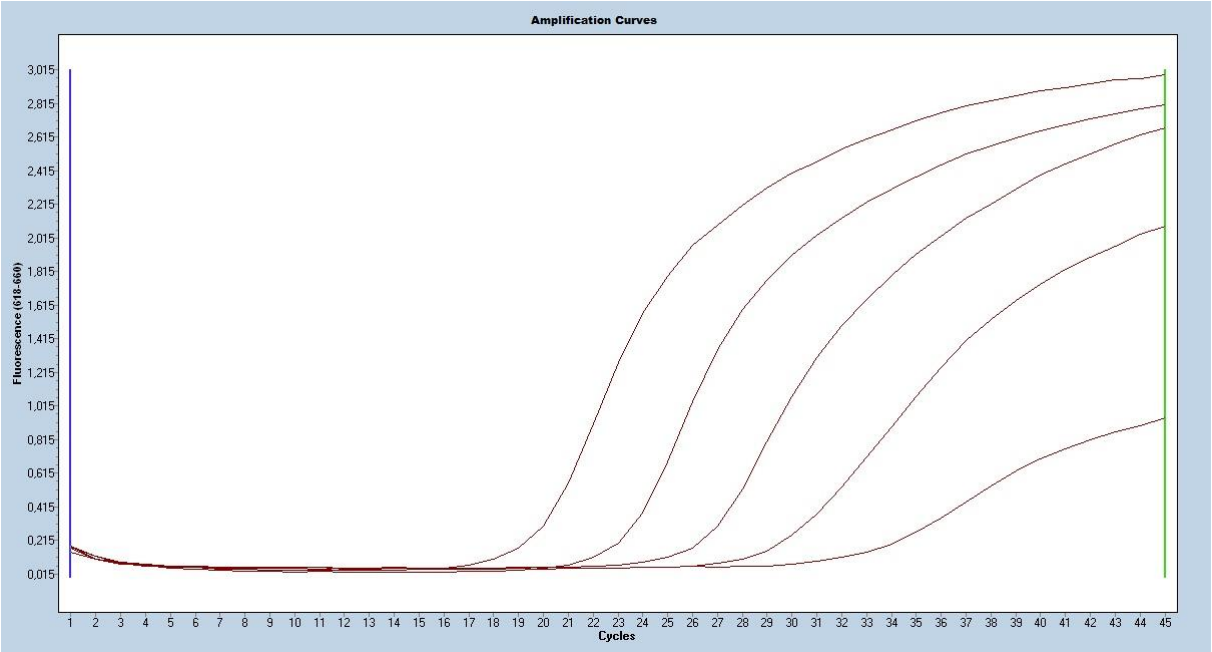


Fig. 6: Dilution series *M. genitalium* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II



The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

13.2 Analytical specificity

The analytical specificity of the RIDA®GENE STI Mycoplasma Panel real-time PCR is specific for *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum* from human genital swabs and urine. No cross-reaction could be detected for the following species (see Tab. 9):

Tab. 9 Cross-reactivity testing

Candida albicans	-	*Klebsiella pneumoniae*	-	*Staphylococcus aureus*	-	HSV 2	-	*Mycoplasma pneumoniae*	-
Candida glabrata	-	*Proteus mirabilis*	-	*Staphylococcus epidermidis*	-	*Trichomonas vaginalis*	-	*Mycoplasma fermentans*	-
Citrobacter freundii	-	*Proteus vulgaris*	-	*Staphylococcus saprophyticus*	-	*Neisseria gonorrhoeae*	-		
E. coli	-	*Pseudomonas aeruginosa*	-	*Streptococcus agalactiae*	-	HPV 16	-		
Enterobacter cloacae	-	*Serratia liquefaciens*	-	*Chlamydia trachomatis*	-	HPV 18	-		
Enterococcus faecalis	-	*Serratia marcescens*	-	HSV 1	-	HPV 6b	-		

13.3 Analytical Reactivity

The reactivity of the RIDA®GENE STI Mycoplasma Panel real-time PCR was evaluated against multiple *Mycoplasma* and *Ureaplasma* subtypes (see Tab. 10). Subtypes listed below were detected by the RIDA®GENE STI Mycoplasma Panel real-time PCR:

Tab. 10: Analytical reactivity testing

Mycoplasma			
M.hominis (Serotype 3)	+	*M.genitalium*	+
M.hominis (Serotype 5)	+		
Ureaplasma			
U. urealyticum (Serovar 8)	+	*U. parvum* (Serovar 1)	+

Explanation of Symbols

IVD	For *in vitro* diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
	Number of test
	Date of manufacture
	Manufacturer

Literature

1. Waites et al., Clin Microbiol Rev. 2005 Oct; 18(4): 757–789.
2. Ljubin-Sternak et al., Journal of Pathogens, 2014
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4. McGowin et al., PLoS Pathog. 2011 May; 7(5)
5. Robertson et al., International Journal of Systematic and Evolutionary Microbiology 2002, 52, 587–597