RIDA®GENE STI Mycoplasma Panel

REF PG4945

R-Biopharm AG, An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Phone: +49 (0) 61 51 81 02-0 / Fax: +49 (0) 61 51 81 02-20

1. Intended use

For *in-vitro* diagnostic use. RIDA®GENE STI Mycoplasma Panel is a multiplex 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 multiplex 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 the mycoplasma species mainly existing in the genital area, *Mycoplasma hominis, Mycoplasma genitalium*, *Ureaplasma urealyticum* and *Ureaplasma parvum* are, among others, described as pathogenic.[1,2]

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, 8, 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 multiplex real-time PCR for the direct, qualitative detection of *Mycoplasma hominis, Mycoplasma genitalium*, *Ureaplasma urealyticum*/*parvum* from genital swabs, as well as from urine.
After DNA isolation, amplification of the gene fragment (if present) specific for *Mycoplasma hominis* (16S-rRNA), *Mycoplasma genitalium* (IGS) and *Ureaplasma urealyticum/parvum* (16S-rRNA) occurs. The amplified target is 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 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	1050 µl	yellow
2	Taq-Polymerase	1x	80 µl	red
D	Internal Control DNA	2x	1700 µl	orange
N	No Template Control	1x	450 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 unopened 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

The RIDA®GENE STI Mycoplasma Panel multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2: Necessary equipment

Extraction platforms	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
Real-time PCR instruments	
Roche	LightCycler® 480II, LightCycler® 480 z
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
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 and the LightCycler® 480 z.
- 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
- PCR water (nuclease-free)

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 Safety Data Sheets (SDS) at www.r-biopharm.com.

8. Collection and storage of samples

8.1 DNA preparation from dry genital swabs

For DNA isolation from dry genital swabs, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions. To isolate DNA from dry swabs the following procedure is recommended: Add 400 µl PCR water into a preparation tube. Insert the swab into the water, squeeze it 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 (see also Maxwell® RSC Application ER101).

The RIDA®GENE STI Mycoplasma Panel assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

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.4).

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 must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

8.2 DNA preparation from urine

For DNA isolation from urine, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions (see also Maxwell® RSC Application ER100).

The RIDA®GENE STI Mycoplasma Panel assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

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.4).

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 must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

9. Test procedure

9.1 Master-Mix preparation

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

We recommend calculating an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and briefly centrifuge the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 3: 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	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

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

Tab. 4: 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	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 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 No Template 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 PCR-Mix of the negative control.

Sample: Add 5 µl eluate 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 PCR-Mix of the positive control.

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. 5, Tab. 6, Tab. 7, Tab. 8).

9.3 PCR instrument set-up

9.3.1 DNA real-time PCR profile

Tab. 5: Real-time PCR profile for LightCycler® series and Rotor-Gene Q

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

Note: Annealing and Extension occur in the same step.

Tab. 6: Real-time PCR profile for Mx3005P, ABI 7500 and CFX96™

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

Note: Annealing and Extension occur in the same step.

9.3.2 Universal real-time PCR profile

Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RIDA®GENE RNA real-time PCR assays in one run.

Tab. 7: Universal real-time PCR profile for LightCycler® series

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 8: Universal real-time PCR profile for Mx3005P, ABI7500, Rotor-Gene Q and CFX96™

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 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. 9: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Note
Roche LightCycler® 480II	*M. hominis*	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICD	533/580	
	U. urealyticum/parvum	533/610	
	M. genitalium	618/660	
Roche LightCycler® 480 z	*M. hominis*	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICD	540/580	
	U. urealyticum/parvum	540/610	
	M. genitalium	610/670	
Agilent Techn. Mx3005P	*M. hominis*	FAM	**Check that reference option is none**
	ICD	HEX	
	U. urealyticum/parvum	ROX	
	M. genitalium	Cy5	
ABI 7500	*M. hominis*	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
	U. urealyticum/parvum	ROX	
	M. genitalium	Cy5	
Bio-Rad CFX96™	*M. hominis*	FAM	**-**
	ICD	HEX	
	U. urealyticum/parvum	ROX	
	M. genitalium	Cy5	
Qiagen Rotor-Gene Q	*M. hominis*	Green	**The gain settings have to be set to 5, according to the default settings**
	ICD	Yellow	
	U. urealyticum/parvum	Orange	
	M. genitalium	Red	

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. Negative control and positive control have to show correct results (see Tab. 10, Fig. 1) 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. 10: For a valid run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target Ct
Positive control	Positive	NA *1	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	0

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

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid prepare all new reactions including the controls.

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

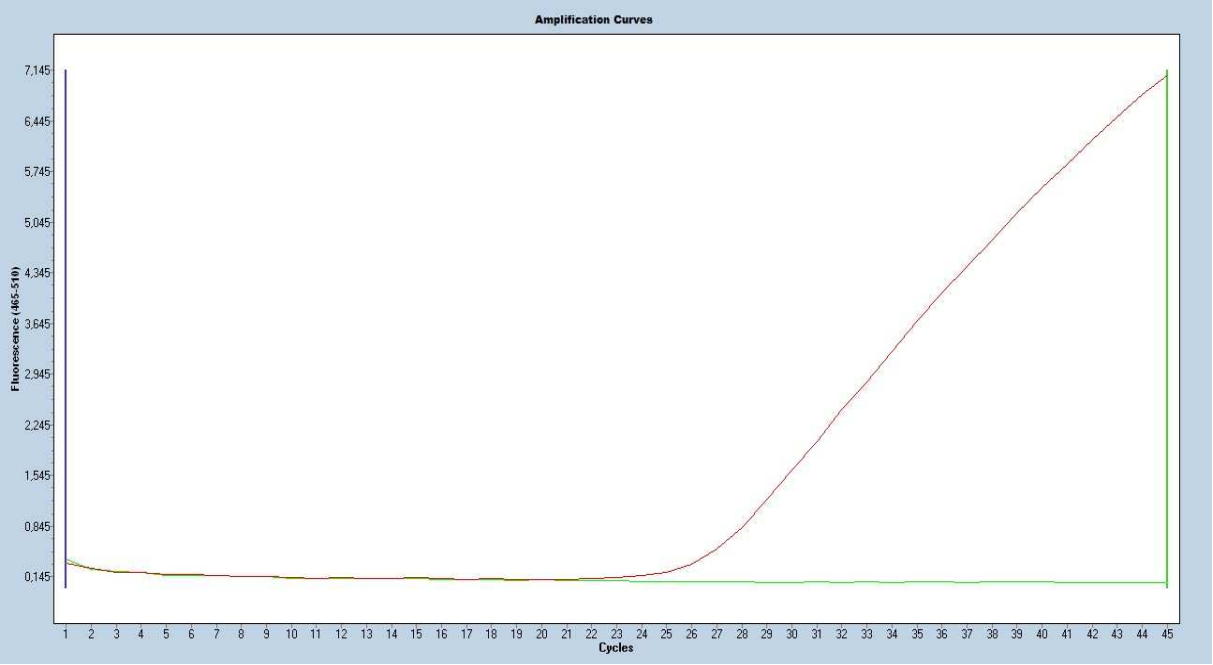

Fig. 1: Correct run of the positive control (red) and negative control (green) (*Mycoplasma hominis*) on the LightCycler® 480II

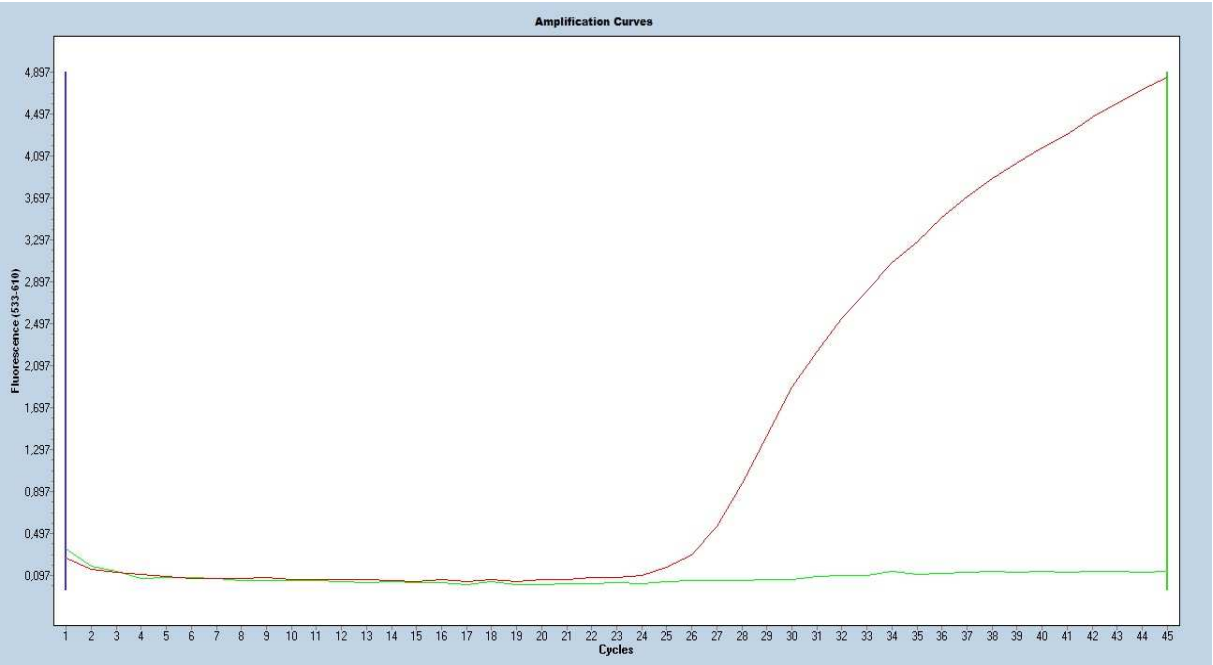

Fig. 2: Correct run of the positive control (red) and negative control (green) (*Ureaplasma urealyticum/parvum*) on the LightCycler® 480II

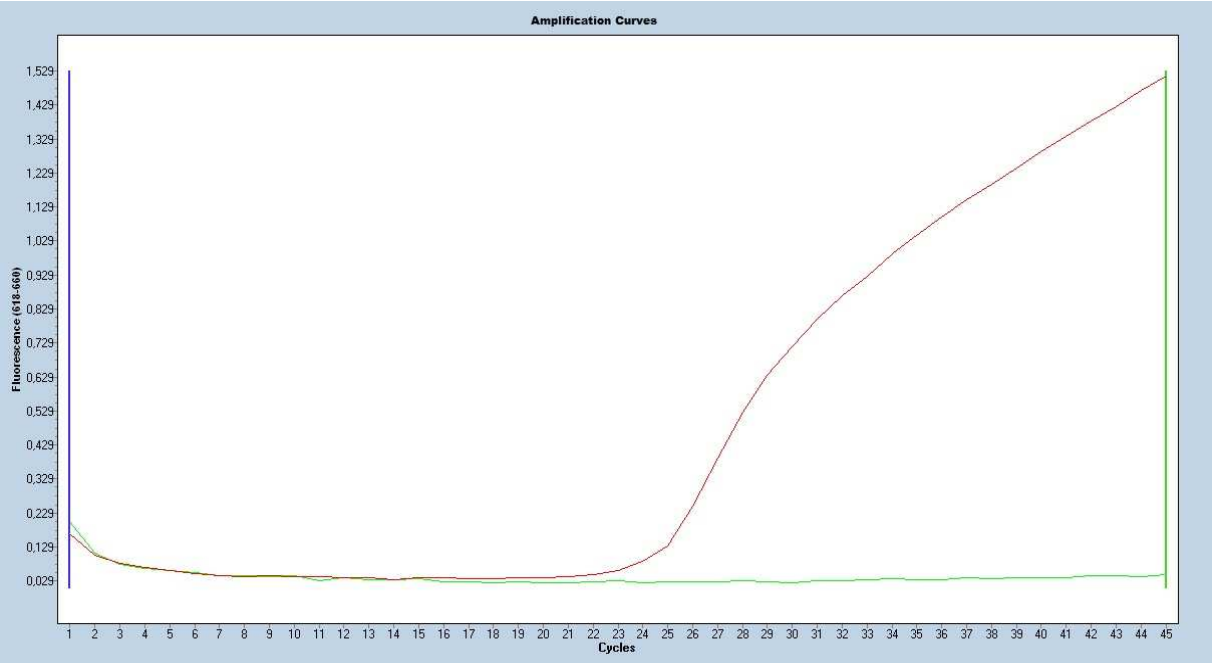

Fig. 3: Correct run of the positive control (red) and negative control (green) (*Mycoplasma genitalium*) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Tab. 11.

Tab. 11: Sample interpretation

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

A sample is evaluated positive, if the sample DNA and the Internal Control DNA show an amplification signal in the detection system.

A sample is also evaluated positive, if the sample DNA shows an amplification signal but none for the Internal Control DNA 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.

A sample is evaluated negative, if the sample DNA shows no amplification signal, but an amplification signal for the Internal Control DNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA.

A sample is invalid, if the sample DNA and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and amplified again, 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 human genital swabs and urine samples.
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. 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 gene (16S-rRNA, IGS).
8. In case genital swabs are used, Mucin may show an interfering effect already in small quantities (validated in U. urealyticum/parvum channel).

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE STI Mycoplasma Panel multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction.

The following figures 4, 5, and 6 show a dilution series of *M. hominis*, *U. urealyticum/parvum* and *M. genitalium* (each 10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II.

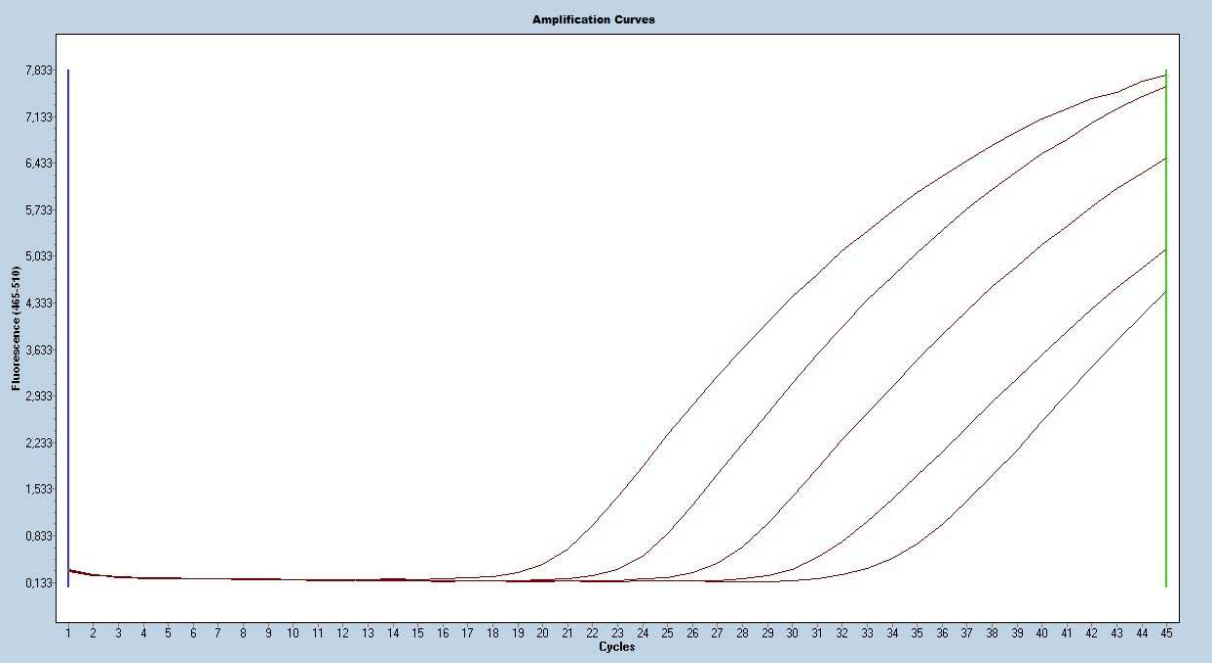

Fig. 4: Dilution series *Mycoplasma hominis* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

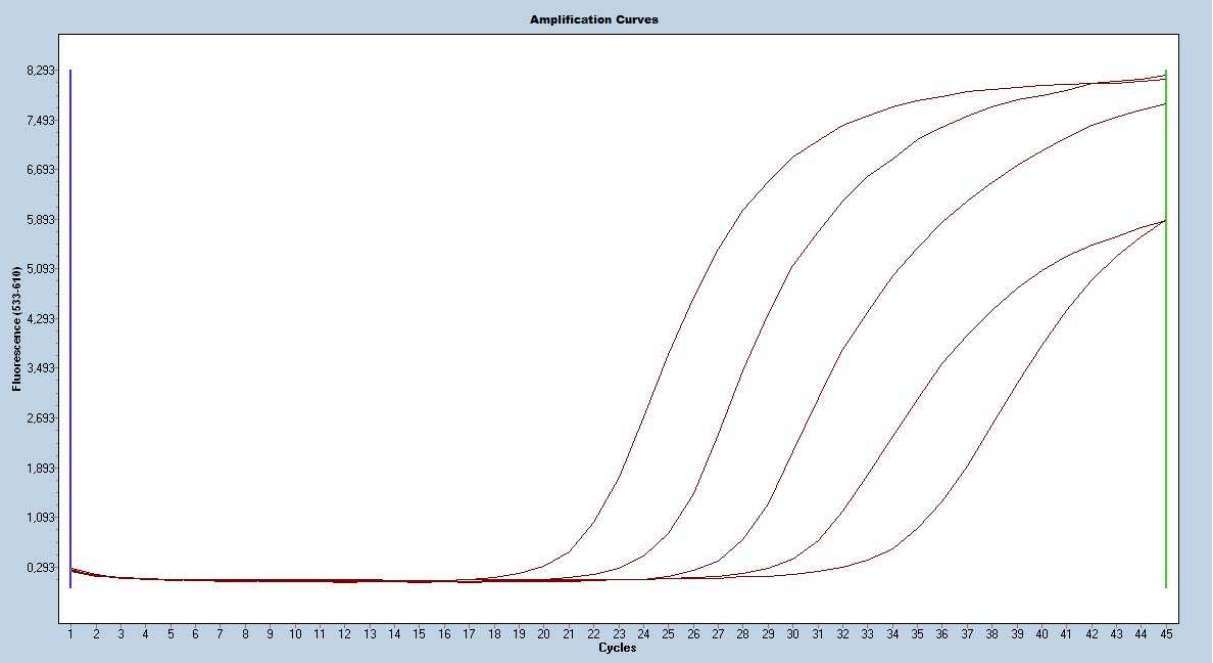

Fig. 5: Dilution series *Ureaplasma urealyticum/parvum* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

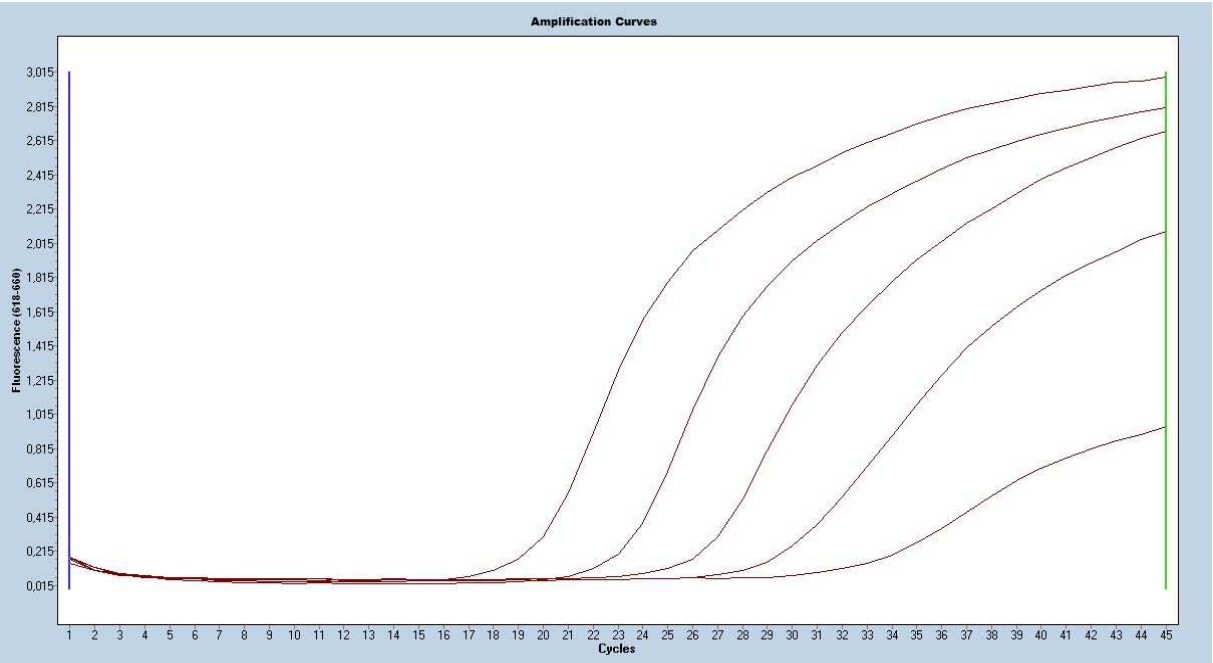

Fig. 5: Dilution series *Mycoplasma genitalium* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

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 multiplex real-time PCR is specific for *M. hominis*, *U. urealyticum/parvum* and *M. genitalium*. No cross-reaction could be detected for the following species (see Tab. 12):

Tab. 12: Cross-reactivity testing

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

13.3 Analytical reactivity

The reactivity of the RIDA®GENE STI Mycoplasma multiplex real-time PCR was evaluated against multiple *Mycoplasma* and *Ureaplasma* subtypes (see Tab. 13). The subtypes listed below were detected by the RIDA®GENE STI Mycoplasma Panel multiplex real-time PCR assay.

Tab. 13: Analytical reactivity testing

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

14. Version history

Version number	Chapter and designation
2019-07-23	General revision 4. Reagents provided 5. Storage instructions 6. Additional necessary reagents and necessary equipment 7. Precautions for users 8. Collection and storage 9. Test procedure 10. Quality control 12. Limitations of the method 13. Performance characteristics 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

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

Testspecific symbols

Not applicable

16. Literature

1. Waites *et al.* Mycoplasmas and ureaplasmas as neonatal pathogens. Clin Microbiol Rev. 2005. 18(4): 757–789.
2. Ljubin-Sternak *et al.* Chlamydia trachomatis and Genital Mycoplasmas: Pathogens with an Impact on Human Reproductive Health. Journal of Pathogens, 2014. 2014:183167.
3. Advameg, Inc. Human Diseases and Conditions. 2019. http://www.humanillnesses.com/Infectious-Diseases-He-My/Mycoplasma-Infections.html (accessed 31.07.2019)
4. McGowin *et al.* Mycoplasma genitalium: an emerging cause of sexually transmitted disease in women. PLoS Pathog. 2011. 7(5).
5. Robertson *et al.* Proposal of Ureaplasma parvum sp. nov. and emended description of Ureaplasma urealyticum (Shepard et al. 1974) Robertson et al. 2001. International Journal of Systematic and Evolutionary Microbiology 2002, 52, 587–597.