



MH/UU/UP REALTIME PCR KIT

REF	RTPCR026-LP		48
 0123	For in vitro diagnostic use		

INTENDED PURPOSE

Real Time RT-PCR kit to detect nucleic acids from *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP) in urogenital swabs and urine samples.

The device is intended to be used with general population with suspected infection by the microorganism.

The test is a qualitative/quantitative and automated assay, intended to be used as an aid to diagnosis.

INTRODUCTION

Mycoplasma hominis (MH) was the first *Mycoplasma* species isolated from humans in 1937. MH belongs to the class *Mollicutes*, that comprises fastidious small bacteria lacking a cell wall. MH is an opportunistic mycoplasma which may form part of the commensal urogenital flora of the adult. It has been implicated in genital infections in women such as urinary tract infections, bacterial vaginosis, pelvic inflammatory disease and cervicitis. MH is also associated with pregnancy complications and disease in neonates besides having a pathogenic role in infertility.

Ureaplasma urealyticum (UU, serovars 2, 4, 5, 7-13) and the separate specie *Ureaplasma parvum* (UP, serovars 1, 3, 6, 14) are tiny bacteria belonging to the *Mollicutes* class too. Both are generally regarded as commensal bacteria being detected in the lower genital tract of 40-80% of women of reproductive age that can be sexually transmitted, with the potential to cause non-gonococcal urethritis, pelvic inflammatory disease and infertility. Studies confirm UU causes non-gonococcal urethritis in males. In pregnant women, upper genital tract infection with *Ureaplasma* species has been associated with adverse pregnancy outcome and preterm birth.

TEST PRINCIPLE

It is based on the amplification and detection of a specific fragment of *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP) by real time PCR.

One lyophilized master mix (RT-PCR MIX) is provided for screening and confirmation using one independent target for each pathogen.

The PCR mix targets specific fragment of the *ftsY* gene for MH, the *ureC* gene for both UU and UP and the human *RNAse P* encoded gene.

An amplification control is included to check the absence of carry-over of amplification inhibitors and the correct amplification set-up. This control consists of an artificial vector and a specific oligo pair/probe for its amplification.

4 quantification standards (QS1, QS2, QS3 and QS4) containing MH, UU, UP and human *RNAse P* encoded gene are included. Quantification standards can be used as a positive control for qualitative detection or to generate a standard curve and determine the concentration of MH, UU, UP and human DNA in the sample.

The technique is divided into 2 main steps: DNA extraction and amplification/detection with specific oligo pairs and probes. UU DNA is detected in the FAM channel, UP DNA is detected in the HEX/VIC channel, MH is detected in the Texas/ROX channel and human *RNAse P* is detected in the Q705 channel. The internal control (IC) is detected in Cy5 channel.

Absolute concentration values of MH, UP and UU DNA indicate the total content of these microorganisms in the clinical sample.

KIT FEATURES

VIRCELL RT-PCR MIX and VIRCELL QUANTIFICATION STANDARD SET are lyophilized. It is necessary to reconstitute them before use (see «Preparatory treatment of the device» section). The rest of the reagents are ready to use.

This kit is based on amplification and detection using real time PCR.

MATERIALS PROVIDED

[1] VIRCELL MUR RT-PCR MIX LP: 6 strips with 8 tubes containing Taq polymerase, buffer and specific primers/probe for MH (*ftsY* gene), for both UU and UP (*ureC* gene) and for human DNA (*RNAse P*). Also, primers/probe for internal control (IC). 1 reaction per tube. Lyophilized.

[4] VIRCELL NEGATIVE CONTROL: 200 µl of deionized water to be used as negative control. Green cap.

[5] VIRCELL PCR MIX RECONSTITUTION SOLUTION: 1 ml of aqueous solution to reconstitute the PCR mix. Yellow cap.

[6] VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION: 500 µl of aqueous solution to reconstitute the positive control. Brown cap.

[7] VIRCELL RT-PCR MIX CAPS 6: 6 strips of 8 caps RT-PCR compatible.

[8] VIRCELL MUR QUANTIFICATION STANDARD SET: 1 set of 4 vials containing a mixture of lyophilized non-infectious nucleic acids to be used as positive control and as quantification standards QS1-4.

Special materials required but not provided:

- Microbiological safety cabinet.
- DNA/RNA extraction kit (see recommendations in "Assay procedure").
- Real Time PCR thermocycler (compatible with low profile white tubes with FAM, HEX/VIC, Texas/ROX, Cy5 and Q705/Cy5.5 detection).
- Precision micropipettes.
- Sterile tips with aerosol barrier.
- Microcentrifuge.
- PCR cabinet (recommended).
- Vortexer.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCELL QUANTIFICATION STANDARD SET reconstituted: store it between -25°C and -15°C and use until expiration date. Avoid more than 10 freeze-thaw cycles during this time period.

VIRCELL RT-PCR MIX reconstituted: store it between 2°C and 8°C and use before 60 minutes.

Rest of reagents: Refer to package label for expiration date (at 2-8°C).

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
11. Keep containers for samples and reagents closed while they are not being handled.
12. Avoid using samples subjected to repeated freeze-thaw cycles.
13. Handle in aseptic conditions to avoid microbial contaminations.
14. Reagents in this kit could include nucleic acids. Observe the local regulations for waste disposal.
15. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only VIRCELL NEGATIVE CONTROL, VIRCELL PCR MIX RECONSTITUTION SOLUTION and VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION are compatible with the equivalents in other RTPCR VIRCELL references and lots.
17. Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

18. Testing of all the samples at the earliest interval following collection will help ensure the most accurate test results. Variation in storage times during specimen shipment has not been assessed.

19. It is recommended to have two different areas to perform the test: Pre-Amplification and Amplification areas.

20. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored to purity.

21. It is recommended to use conventional DNA/RNA purification kits.

22. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

The kit has been validated for urine and urogenital samples.

It is recommended to avoid delay on transport and laboratory investigations. If immediate delivery to the laboratory is not possible, store specimens in a refrigerator (2 to 8°C). Store urogenital specimens for which testing will be delayed beyond 10 days after collection at -70°C or lower; avoid freezing at higher temperatures and freeze-thaw cycles.

Recommended guidance: Cervical and Urethral Swabs. p.6.4.15. and Urine. p.6.4.16. MM13-A_Collection, Transport, Preparation and Storage of Specimens for Molecular Methods, 1st ed. CLSI.

PREPARATORY TREATMENT OF THE DEVICE

All reagents supplied are ready to use, except for the VIRCELL RT-PCR MIX [1] and VIRCELL QUANTIFICATION STANDARD SET [8].

[1] VIRCELL RT-PCR MIX. For reconstitution add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube.

⚠ The reconstituted VIRCELL RT-PCR MIX must be used within 60 minutes of adding the reconstitution solution stored at 2-8°C if the start of the test is delayed. In this case, a freeze rack is recommended.

[8] VIRCELL QUANTIFICATION STANDARD SET. Follow the next steps to reconstitute it:

- Centrifuge the corresponding tubes for 5 seconds at 5000 g.
- Add 100 µl of VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION [6]
- Mix with vortex for 1-2 seconds.
- Centrifuge the tubes for 5 seconds at 5000 g.

After reconstitution, the VIRCELL QUANTIFICATION STANDARD SET [8] must be frozen at temperature between -25°C and -15°C to be used in subsequent reactions.

ASSAY PROCEDURE

- DNA/RNA extraction (performed in the Pre-Amplification area):
 - It is recommended to use a commercial extraction kit for DNA/RNA extraction. In order to use commercial extraction kits, follow the manufacturer instructions. Consult with Customer Service.
- Amplification using RT-PCR (performed in the Amplification area):
 - Preparation of the VIRCELL RT-PCR MIX tubes: Add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube. Maintain cold after reconstitution.
 - Addition of the sample: Add 5 µl of each extracted DNA/RNA sample to each tube.
 - In case of qualitative analysis, add 5 µl of at least one vial of the VIRCELL QUANTIFICATION STANDARD SET [8] and the VIRCELL NEGATIVE CONTROL [4] to the corresponding VIRCELL RT-PCR MIX [1] tubes. The negative control is water.
 - In case of quantitative analysis, add 5 µl of each vial of the VIRCELL QUANTIFICATION STANDARD SET [8] to the corresponding VIRCELL RT-PCR MIX [1] tubes.
 - Secure caps VIRCELL RT-PCR MIX CAPS [7] on the tubes.
 - It is recommended to briefly centrifuge the plate/strips of tubes with the purpose of ensuring vial content is at the bottom of the tube.
 - RT-PCR program: Insert the PCR tubes in the real time thermocycler and run the following program*:

1 cycle	95 °C	3 minutes
45 cycles	95 °C	15 seconds
	58 °C	45 seconds*

* Fluorescence data (FAM, HEX/VIC, Texas/ROX, Cy5 and Q705/Cy5.5) should be collected.

QUANTITATIVE ANALYSIS

The kit includes 4 quantification standards QS1-QS4 that can be used to generate standard curves and determine the concentration of MH, UU, UP and human DNA in the sample. The standards are prepared to be directly analyzed by RT-PCR (do not require DNA extraction).

VIRCELL QUANTIFICATION STANDARD	Concentration (copies/µl)
QS1	500000
QS2	50000
QS3	5000
QS4	500

Standard curve specification: PCR efficiency >90%; correlation coefficient R²>0.980.

QUALITATIVE ANALYSIS

VIRCELL QUANTIFICATION STANDARD SET included in this kit can be used as a POSITIVE CONTROL for a qualitative analysis. This control allows to check the efficiency of the test. (See «Validation protocol for users»).

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control testing before releasing, complying with strict specifications.

VALIDATION PROTOCOL FOR USERS

It is recommended to include one negative control in each run performed. The negative control will monitor reagent or environmental contamination.

For a qualitative analysis, a positive control is recommended to be included on each run. A positive control monitors for reagent failures and for correct operation of essential procedure.

For a quantitative analysis, it is necessary to generate a standard curve analyzing the VIRCELL QUANTIFICATION STANDARD SET in the first use of the kit. In subsequent runs of the same batch, only one standard should be included as point of control. The generated standard curve is specific of each batch, it should be repeated if a different batch is used. The use of Vircom PCR LIS Connection (Vircell) software will facilitate sample quantification and assay validation.

Absolute quantification indicates the number of genome copies of MH, UP and UU per 1 ml of clinical sample (copies/ml). To perform this absolute calculation, apply the following formula:

$$\text{Sample concentration (copies/ml)} = \frac{\text{Elution volumen (}\mu\text{l)} \times \text{Elution concentration (copies}/\mu\text{l)}}{\text{Sample volumen (ml)}}$$

The relative concentration of MH, UP and UU DNA to a certain number of human cells could also be calculated. Consult with Customer Service.

The thermocycler software is likely to automatically calculate the baseline fluorescence value (threshold) based on the amplification curve for each target (fluorescence detection). Nevertheless, it is recommended to set the thresholds for the different detection channels individually. In order to set a threshold for each target, it is recommended to use as a reference the amplification curves of the positive and negative controls. The threshold should be fixed at the beginning of the exponential reading of fluorescence and above the background signal.

The controls result interpretation is as follows:

CONTROL	UU (FAM)	UP (HEX/VIC)	MH (Texas/ROX)	<i>RNase P</i> (Q705)	IC (Cy5)	Interpretation
VIRCELL MUR QUANTIFICATION STANDARD SET	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 38)	Amplification (Ct < 40)	Correct
	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >38	No amplification or Ct >40	Invalid
VIRCELL NEGATIVE CONTROL	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >38	Amplification (Ct < 40)	Correct
	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 38)	No amplification or Ct >40	Invalid

To validate VIRCELL MUR QUANTIFICATION STANDARD SET qualitatively, compare the result obtained for this control with the result obtained during the generation of the standard curve, admitting a deviation of Ct±2 between both.

INTERPRETATION OF RESULTS

The result interpretation is described in the tables below:

RESULT	UU (FAM)	UP (HEX/VIC)	MH (Texas/ROX)	<i>RNase P</i> (Q705)	IC (Cy5) ¹	Interpretation
1	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >38	No amplification or Ct >40	Invalid (sample/kit/setup related)
2	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40)	Negative
3	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 38)	Amplification (Ct < 40) or no amplification	Negative
4	Amplification (Ct < 40)	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40) or no amplification	UU
5	No amplification or Ct >40	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40) or no amplification	UP
6	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40) or no amplification	MH
7	Amplification (Ct < 40)	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40) or no amplification	UU, UP
8	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40) or no amplification	UU, MH
9	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40) or no amplification	UP, MH
10	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 38) or no amplification	Amplification (Ct < 40) or no amplification	UU, UP, MH

¹ In case of a high copy number of the target nucleic acid, the amplification of the internal control (IC) in results 3 to 10 may be affected. The late amplification or absence of IC amplification does not change the interpretation of the result.

In case of invalid or inconclusive result, it is recommended to re-extract DNA/RNA from original specimen and re-test it. In the case of failure of amplification of internal control, improper extraction of nucleic acids or inhibition of amplification could be assumed. Testing a new sample is recommended.

LIMITATIONS OF USE

- The performance with other types of specimens different to urine and urogenital swabs has not been evaluated.
- The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures.
- Detection of the pathogens nucleic acids depends on the organism load present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and/or strain. False negative results may also occur if amplification inhibitors are present in the specimen. The kit was validated with a specific nucleic acid extraction method. Alternative extraction procedures might be also appropriate but require user validation. A 260/280 purity ratio among 1.8-2.0 is acceptable.
- The test provides qualitative and quantitative results. The test results are quantitative if the VIRCELL QUANTIFICATION STANDARD SET [8] is used and, in that case, there is a correlation between the magnitude of the positive result and the number of microorganisms in the sample.
- The test only works within the limits of the genomic regions from which the primers and probes have been chosen. The test targets highly conserved regions, however due to the high variability of DNA genomes it is possible that certain subtypes might not be detected. At design time, mutations of the target regions were not detected.
- A negative test result does not exclude the presence of the target organism at levels below the detection limit of the assay.
- A positive test does not rule out the possibility that other pathogens may be present.

8. The values obtained in the sensitivity and specificity performance study correspond to the total number of samples tested and may vary depending on the type of sample.

9. The performance results showed were generated using the thermocycler CFX96 (Bio-Rad).

10. The performance results showed correspond to comparative studies with commercial predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.

11. A summary of safety and performance is available on EUDAMED or it can be requested at email address customerservice@vircell.com.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Mycoplasma hominis

Positive human urogenital swabs samples (n=44), positive human urine samples (n=14) and previously confirmed negative human urogenital swabs samples (n=24) and negative human urine samples (n=26) were analysed. Samples were tested against a commercial Real-time PCR kit.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.	108	
Sensitivity (%)	98	
	95% CI	91-100
Specificity (%)	100	

	95% CI	93-100
PPV (%)		100
NPV (%)		98
LR+/LR-		-0.99/-0.97
True Positive		57
True Negative		50
False Positive		0
False Negative		1
Borderline		0

CI: Confidence intervals
 PPV: Positive predictive value
 NPV: Negative predictive value
 LR+: Positive likelihood ratio
 LR-: Negative likelihood ratio

Ureaplasma parvum

Positive human urogenital swabs samples (n=54), positive human urine samples (n=16) and previously confirmed negative human urogenital swabs samples (n=24) and negative human urine samples (n=26) were analysed. Samples were tested against a commercial Real-time PCR kit.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.	120	
Sensitivity (%)	97	
	95% CI	90-99
Specificity (%)	100	
	95% CI	93-100
PPV (%)	100	
NPV (%)	96	
LR+/LR-	-0.98/-0.96	
True Positive	68	
True Negative	50	
False Positive	0	
False Negative	2	
Borderline	0	

CI: Confidence intervals
 PPV: Positive predictive value
 NPV: Negative predictive value
 LR+: Positive likelihood ratio
 LR-: Negative likelihood ratio

Ureaplasma urealyticum

Positive human urogenital swabs samples (n=34), positive human urine samples (n=16) and previously confirmed negative human urogenital swabs samples (n=24) and negative human urine samples (n=26) were analysed. Samples were tested against a commercial Real-time PCR kit.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.	100	
Sensitivity (%)	96	
	95% CI	86-99
Specificity (%)	100	
	95% CI	93-100
PPV (%)	100	
NPV (%)	96	
LR+/LR-	-0.97/-0.95	
True Positive	48	
True Negative	50	
False Positive	0	
False Negative	2	
Borderline	0	

CI: Confidence intervals
 PPV: Positive predictive value
 NPV: Negative predictive value
 LR+: Positive likelihood ratio
 LR-: Negative likelihood ratio

PRECISION

9 samples (4 positive, standards and negative control) were amplified twice in 2 runs per day in 2 different qRT-PCR thermocyclers on 20 consecutive days. Samples were run in CFX96 (Bio-Rad). Within-run precision, between-run precision, between-day precision and within-laboratory precision were determined.

The results were as follows:

Mycoplasma hominis

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
QS1	0.6	1.1	1.0	1.6
QS2	0.6	0.9	1.3	1.7
QS3	1.1	1.1	1.0	1.8
QS4	0.6	0.6	0.5	1.0
Positive sample 1	0.9	0.8	1.1	1.6
Positive sample 2	0.8	0.6	0.7	1.2
Positive sample 3	1.4	2.5	0.8	2.9
Positive sample 4	1.8	0.3	1.7	2.5
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

Ureaplasma parvum

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
QS1	0.4	0.7	1.1	1.4
QS2	0.5	0.6	1.3	1.5
QS3	1.4	0.6	1.1	1.9
QS4	0.7	0.5	0.6	1.1
Positive sample 1	0.9	1.0	0.9	1.6
Positive sample 2	0.9	0.3	0.9	1.3
Positive sample 3	0.8	2.3	0.7	2.6
Positive sample 4	1.9	1.1	1.6	2.7
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

Ureaplasma urealyticum

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
QS1	0.5	1.0	0.8	1.4
QS2	0.6	0.8	1.2	1.5
QS3	1.1	0.9	1.0	1.8
QS4	0.6	0.5	0.7	1.1
Positive sample 1	1.1	1.1	1.1	1.9
Positive sample 2	0.7	0.4	0.8	1.2
Positive sample 3	1.2	2.3	1.0	2.8
Positive sample 4	1.7	0.3	1.7	2.4
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

INTERFERENCES

A study has been performed to evaluate the effect of potentially interfering substances.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Urine

Interfering substances	Samples No.	Maximum added concentration without interference
Human whole blood	1	2.5 % (V/V)
Ascorbic acid	1	342 µmol/L
Acetil salicilic	1	3.62 µmol/L
Amoxicilina	1	206 µmol/L
Azathioprine	1	10.8 µmol/L

Interfering substances	Samples No.	Maximum added concentration without interference
Cyclosporine	1	5 mg/L
Ibuprofen	1	2425 µmol/L
Tetracycline	1	34 µmol/L
Human serum albumin	1	60 g/L
Bilirubin	1	342 µmol/L
Glucose	1	55 mmol/L

Urogenital swab

Interfering substances	Samples No.	Maximum added concentration without interference
Human whole blood	1	2.5 % (V/V)
Acyclovir	1	5% (w/v)
Clotrimazol	1	2% (w/v)
Estradiol	1	4.41 nmol/L
Metronidazole	1	701 µmol/L
Progesterone	1	5% (w/v)
Vagisil Cream	1	5% (w/v)
Human serum albumin	1	60 g/L
Bilirubin	1	342 µmol/L

CROSS REACTIVITY

A study has been performed to evaluate the effect of potentially cross-reactive microorganisms. Samples were run in CFX96 (Bio-Rad).

The results were as follows:

Microorganism	Samples No.	Positives No.
<i>Atopobium vaginae</i>	1	0
<i>Candida albicans</i>	1	0
<i>Candida auris</i>	1	0
<i>Candida dubliniensis</i>	1	0
<i>Candida glabrata</i>	1	0
<i>Candida guilliermondii</i> (Meyerozyma guilliermondii)	1	0
<i>Candida krusei</i>	1	0
<i>Candida parapsilosis</i>	1	0
<i>Candida tropicalis</i>	1	0
<i>Chlamydia trachomatis</i> serovar B	1	0
<i>Chlamydia trachomatis</i> serovar D	1	0
<i>Chlamydia trachomatis</i> serovar E	1	0
<i>Chlamydia trachomatis</i> serovar J	1	0
<i>Chlamydia pneumoniae</i>	1	0
<i>Chlamydia psittaci</i>	1	0
Cytomegalovirus	1	0
Enterovirus 68	1	0
Epstein-Barr virus	1	0
<i>Escherichia coli</i> (EAEC)	1	0
<i>Gardnerella vaginalis</i>	1	0
HCoV-229E	1	0
<i>Helicobacter pylori</i>	1	0
Herpes simplex virus type 1	1	0
Herpes simplex virus type 2	1	0
<i>Lactobacillus jensenii</i>	1	0
<i>Metamycoplasma arthritidis</i>	1	0
<i>Metamycoplasma gateae</i>	1	0
<i>Mycobacterium tuberculosis</i>	1	0
<i>Mycoplasma fermentans</i>	1	0
<i>Mycoplasma genitalium</i>	1	0
<i>Mycoplasma imitans</i>	1	0
<i>Mycoplasma leachii</i>	1	0
<i>Mycoplasma mycoides</i>	1	0
<i>Mycoplasma pneumoniae</i>	1	0
<i>Neisseria gonorrhoeae</i>	1	0
<i>Neisseria lactamica</i>	1	0
<i>Neisseria meningitidis</i> serogroup A	1	0
<i>Neisseria mucosa</i>	1	0
<i>Neisseria perflava</i>	1	0
<i>Neisseria polysaccharea</i>	1	0
<i>Neisseria sicca</i>	1	0

Microorganism	Samples No.	Positives No.
Papillomavirus type 16	1	0
Papillomavirus type 18	1	0
<i>Pseudomonas aeruginosa</i>	1	0
<i>Saccharomyces boulardii</i>	1	0
<i>Shigella flexneri</i>	1	0
<i>Staphylococcus aureus</i> (mecA-)	1	0
<i>Staphylococcus epidermidis</i>	1	0
<i>Treponema pallidum</i>	1	0
<i>Trichomonas vaginalis</i>	1	0
TOTAL	50	0

In addition, an in-silico analysis of the primers/probes sequences comparing to other microorganisms that could be found in clinical samples was performed.

The results were as follows:

Microorganism	Homology >80%		
	MH	UP	UU
<i>Atopobium parvulum</i>	No	No	No
<i>Bacteroides caccae</i>	No	No	No
<i>Bacteroides fragilis</i>	No	No	No
<i>Bifidobacterium adolescentis</i>	No	No	No
<i>Candida metapsilosis</i>	No	No	No
<i>Haemophilus ducreyi</i>	No	No	No
Hepatitis A virus	No	No	No
Hepatitis B virus	No	No	No
Hepatitis C virus	No	No	No
<i>Lactobacillus acidophilus</i>	No	No	No
<i>Lactobacillus crispatus</i>	No	No	No
<i>Mobiluncus mulieris</i>	No	No	No
<i>Peptostreptococcus anaerobius</i>	No	No	No
<i>Prevotella bivia</i>	No	No	No

No significant homology was observed between the primers/probes used in this assay and the analyzed microorganisms.

ANALYTICAL SENSITIVITY

A preliminary LoD (limit of detection) was determined by testing serial dilutions of quantified MH, UP and UU samples. Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad). Once an approximated LoD is determined, the final concentration was confirmed by testing 3 serial dilutions. A minimum of 20 replicates is tested for each dilution. The LoD is determined as the lowest concentration where ≥ 95% of the replicates are positive.

Urine samples

	MH	UP	UU
LoD (copies/µl)	4.7	2.4	2.1
LoD (copies/ml)	1253	640	560
LoD (copies/reaction)	23.5	12	10.5

Urogenital samples

	MH	UP	UU
LoD (copies/µl)	3.2	3.5	3.6
LoD (copies/ml)	853	933	960
LoD (copies/reaction)	16	17.5	18

LINEARITY / MEASURING RANGE

The measuring range (MR) was determined by testing 8 levels of concentration of a quantified sample.

The results were as follows:

Mycoplasma hominis

R²= 0.989

MR=0.5 to 500000 copies/µl

Ureaplasma parvum

R²= 0.997

MR=0.5 to 500000 copies/µl

Ureaplasma urealyticum

R²= 0.994

MR=0.5 to 500000 copies/μl

INCLUSIVITY

An in-silico analysis for the primered genes included in the assay was performed to determine the inclusivity for the different MH, UP and UU sequences available.

The criteria selected for including the different sequences in the analysis was geographic and the date when the sequence was deposited. Different lineages, types or subtypes were included in the analysis of each microorganism.

GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) was used for accessing sequences.

The results of the in-silico analysis show that the kit is predicted to detect all genome variants included in the analysis.

SYMBOLS USED IN LABELS



In vitro diagnostic medical device



Use-by (expiry date)



Store at x-y °C



Contains sufficient for <n> test



Batch code



Catalogue number



Consult instructions for use



Reconstitute in <X> μl



Manufacturer

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6
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