Instructions for Use

RealLine Mycoplasma hominis / M. genitalium Str-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION AND DIFFERENTIATION OF *MYCOPLASMA HOMINIS* AND *MYCOPLASMA GENITALIUM* DNA BY REAL TIME PCR METHOD

In vitro Diagnostics

CE

RealLine Mycoplasma hominis / genitalium (Str-Format)	**VBD0494**	**96 Tests**
valid from	**September 2019**	

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Explanation of symbols used in labeling

IVD	For in vitro diagnostic use
LOT	Batch code
REF	Catalogue number
	Content of number of tests
	Expiry date
	Temperature limitation
	Consult instructions for use
	Manufacturer
	Keep out of sunlight

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ASSAY KIT FOR THE QUALITATIVE DETECTION AND DIFFERENTIATION OF *MYCOPLASMA HOMINIS* AND *MYCOPLASMA GENITALIUM* DNA
In vitro Diagnostics

1. INTENDED USE

Clinical information:
Mycoplasma hominis and *Mycoplasma genitalium* are bacterias which cause sexually transferred diseases. *Mycoplasma genitalium* is known to cause the so-called non-gonococcal urethritis and *Mycoplasma hominis* can be associated with post-abortal and post-partum fever.

RealLine Mycoplasma hominis / **genitalium** assay kit is designed to detect *Mycoplasma hominis* DNA and *Mycoplasma genitalium* DNA isolated from specimens using following extraction kits:
RealLine DNA-Express (REF VBC8899)
RealLine DNA-Extraction 2 (REF VBC8897)
RealLine DNA-Extraction 3 (REF VBC8889)
RealLine Extraction 100 (REF VBC8896)

RealLine Mycoplasma hominis / **genitalium (Str-format)** kit is designed for the analysis of clinical materials: urogenital and cervical swabs, pithelial cells, semen, prostatic fluid, urine.

The results of PCR analysis are taken into account in complex diagnostics of disease.

The assay is based on real-time polymerase chain reaction (PCR) method with fluorescent detection of amplified product.

The **Str-Format Kit** contains 96 tubes (0.2ml) in strips with lyophilized Mastermix. 50 µl of extracted DNA have to be pipetted into the tube and the ready mastermix is diluted. The kit contains reagents required for 96 tests, including control samples and the positive control sample.

The kit is intended for use with block-type PCR cyclers: iQ5 iCycler (Bio-Rad, USA), CFX96 (Bio-Rad, USA), DT-96 (DNA-Technology, Russia) and RealLine Cycler (BIORON Diagnostics GmbH).

The use of:

- **Extraction Kits for nucleic acids from clinical specimen from other supplier**
- **other real-time PCR devices**
- **appropriate reaction volumes, other than 50 µl**

has to be validated in the lab by the user. The special notes regarding the internal control IC have to be strongly followed.

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2. KIT CONTENTS

Universal Positive Control sample *(*PC)	1 vial, 1 ml
Ready Master Mix (RMM), lyophilized	96 test-tubes (12 strips x 8 tubes)
The kit also includes optical-quality PCR-film	

3. PRINCIPLE OF THE METHOD

Real time PCR is based on the detection of the fluorescence produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is dual-labeled DNA-probe, which specifically binds to the target region of pathogen DNA. Fluorescent signal increases due to the fluorescent dye and quencher separating by Taq DNA-polymerase exonuclease activity during amplification. PCR process consists of repeated cycles: temperature denaturation of DNA, primer annealing and complementary chain synthesis.

Threshold cycle value – Ct – is the cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold, a fluorescent signal rises significantly above the background fluorescence. Ct depends on initial quantity of pathogen DNA template.
The use of **Internal Control (IC)** prevents generation of false negative results associated with possible loss of DNA template during specimen preparation. IC indicates if PCR inhibitors occur in the reaction mixture. IC template should be added in each single sample (including control samples) prior to DNA extraction procedure. The amplification and detection of IC does not influence the sensitivity or specificity of the target DNA PCR.

Note: IC is a component of the NA extraction kits of RealLine series. Internal Control is added to the sample during NA isolation step and is used throughout the whole process of NA extraction, amplification, detection.

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4. SPECIFICATIONS

4.1. Sensitivity:

Sensitivity control was performed on 5 samples containing 100 *Mycoplasma hominis* DNA copies per sample, prepared from SRS (Standard Reference Sample containing *Mycoplasma hominis* DNA), and 100 *Mycoplasma genitalium* DNA copies per sample, prepared from SRS (Standard Reference Sample containing *Mycoplasma genitalium* DNA).
The sensitivity equals 100%.

4.2. Specificity:

Specificity of *Mycoplasma hominis and Mycoplasma genitalium DNA* detection was determined using negative DNA-extracts of the Standard Reference Panel , consisting of samples containing IC DNA and not containing DNA of STD agents. Specificity of *Mycoplasma hominis and Mycoplasma genitalium DNA* detection equals 100%.

4.3. Diagnostic sensitivity of *Mycoplasma hominis* detection: clinical trials conducted on 75 positive samples showed 100% sensitivity (interval 96.1% -100%, with a confidence level of 90%).
Diagnostic sensitivity of *Mycoplasma genitalium* detection: clinical trials conducted on 48 positive samples showed 100% sensitivity (interval 94% -100%, with a confidence level of 90%).

4.4. Diagnostic specificity of *Mycoplasma hominis* detection: clinical trials conducted on 112 negative samples showed 100% specificity (interval 97.4% -100%, with a confidence level of 90%).
Diagnostic specificity of *Mycoplasma genitalium* detection: clinical trials conducted on 139 negative samples showed 100% specificity (interval 97.9% -100%, with a confidence level of 90%).

Analysis by the CE-marked reference kit showed full match of results.

5. WARNING AND PRECAUTIONS

- For in vitro use only.
- The kits must be used by skilled personnel only.
- When handling the kit, follow the national safety requirements for working with pathogens.
- To prevent contamination, the stages of DNA isolation and PCR test run must be spatially separated.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.
- The use of sterile disposable pipette tips is recommended.
- Never use the same tips for different samples.
- Do not pool reagents from different lots or from different vials of the same lot.
- Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- Do not use the kit after the expiration date at the side label of the kit..

6. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Real time PCR system, like described in p.1
- DNA-Extraction Kit: RealLine DNA-Express, RealLine DNA-Extraction 2, RealLine DNA-Extraction 3, RealLine Extraction 100
- Internal Control reagent (VBC8881) and Negative Control Sample if the kit is used with the extraction kits of other supplier.
- laminar safety box;
- refrigerator;
- half-automatic variable-volume single-channel pipettes;
- disposable medical non-sterile powder-free gloves;
- disposable pipette tips with aerosol barrier;
- biohazard waste container.

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7. PREPARATION OF SPECIMENS

Each group of samples undergoing the procedure of DNA isolation must include a Positive Control sample (PC) from this kit and a Negative Control sample (NC) which is a component of the DNA extraction kit.

We strongly recommend the implementation of the Internal Control IC, the Negative Control NC and Positive Control PC samples to the extraction procedure.

When using a kit of another supplier for the extraction of nucleic acids as recommended in p1., add **20 µl** of **IC (VBC8881)** to each tube.

- For the NC use **100 µl** of the Negative Control Sample.
- For the PC use **70 µl** of Negative Control Sample and **30 µl** of Positive Control to the tube marked PC.

Sample preparation
The assay is performed on extracted DNA specimens obtained from the clinical material using one of the DNA extraction kits listed in p.1, according to their Instruction Manuals.
Each group of specimens undergoing the DNA extraction procedure should include a Positive Control sample (PC) and a Negative Control sample (NC), which is a component of the DNA extraction kit.

If specimens of extracted DNA were stored frozen prior to the assay, thaw them and keep at (18 – 25) °C for at least 30 min.

Store the extracted DNA at (2 – 8) °C for no more than 24 hours.
After initial opening of the tube, store PC at (2 – 8) °C for no more than 1 month or in 50 µl aliquots at minus (18 - 24) °C for no more than 3 months.

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8. PROCEDURE

8.1 Prior to the test, take the kit out of the refrigerator and keep the **Ready Master Mix for PCR (RMM)** closed in the package at (18 – 25) °C for at least 30 min. Then open the package and cut off the necessary number of tubes with RMM (including the specimens and control samples) with the razor or scalpel. Cut the tubes together with the covering film.
Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close it with a clip.
After initial opening, store RMM at (2 – 8) °C for no more than 3 months.

8.2 Label the tubes with RMM for each specimen and control sample.
Attention! Labels should be placed on the lateral side of the tubes, leave optical film clean.

8.3 Add **50 µl** of corresponding isolated DNA solution to each tube using a separate pipette tip with filter. Do not touch the pellet! Tightly close the tubes with caps or seal with the PCR transparent film.

8.4 Place the tubes into the real-time PCR system.

8.5 Program real time PCR system as follows:

Stage 1:	50°C	2min	
Stage 2:	95°C	2min	
Stage 3:	94°C	10 sec	50 cycles
	60°C*	20 sec	

* Measure the fluorescence at 60°C

8.6 Select the amplification detection channels:

- Collect real-time PCR data through the **FAM** channel for detection of amplification of **IC DNA**.
- Collect real-time PCR data through the **HEX** channel for detection of amplification of ***Mycoplasma hominis*** **DNA**.
- Collect real-time PCR data through the **ROX** channel for detection of amplification of ***Mycoplasma genitalium*** **DNA**.

8.7 Program the positions of test tubes with samples, positive and negative controls according to the instruction manual for the real time PCR system in use

8.8 Run the program.

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9. DATA ANALYSIS AND INTERPRETATION

9.1. For the Positive Control **PC** the program should detect:

- increase of the **IC DNA** amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**;
- increase of the ***Mycoplasma hominis*** **DNA** amplification signal (channel **HEX**) and determine the PC **Ct** value;
- increase of the ***Mycoplasma genitalium*** **DNA** amplification signal (channel **ROX**) and determine the **Ct** value.

9.2. For the Negative Control **NC** the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine the threshold cycle, IC **Ct**. No significant **ROX** or **HEX** fluorescent increase should appear (*no Mycoplasma genitalium or Mycoplasma hominis DNA amplification*).

If **Ct** value for NC through **ROX** or **HEX** channel **is less than or equal to 40,** this indicates the presence of contamination (see paragraph 9.7).

9.3. For each sample the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine IC **Ct**.

9.4. Calculate (IC **Ct**)$_{av}$ as an average IC **Ct** of all analyzed samples (including PC and NC). IC **Ct** values that differ by more than 2 cycles from the (IC **Ct**)$_{av}$ should be ignored. Recalculate the (IC **Ct**)$_{av}$ for the remaining values after the screening.

9.5. The sample is considered **negative** (not containing *Mycoplasma hominis and Mycoplasma genitalium DNA*), if **Ct** value via **ROX** and **HEX** channels for this sample is **above 40** or is not determined.

If IC **Ct** value for such sample differs from the (IC **Ct**)$_{av}$ value by more than 2 cycles, the result is regarded as equivocal. A repeated analysis of the sample, starting with the DNA isolation step is necessary.

9.6. The sample is considered **positive**, i.e. contains *Mycoplasma genitalium* DNA, when **Ct** value via **ROX** channel for this sample is **less than or equals to 40.**

The sample is considered **positive**, i.e. contains *Mycoplasma hominis* DNA, when **Ct** value via **HEX** channel for this sample is **less than or equals to 40.**

9.7. If Ct value for NC through **ROX** or **HEX** channels **is less than or equals to 40,** this indicates the presence of contamination. In case of contamination all positive results of this individual PCR run are considered equivocal. Actions are required to identify and eliminate the source of contamination, and repeat the analysis of all specimens of this run that were identified as positive. Specimens that showed negative results in this run should be considered negative.

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10. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing.
- Transportation at 25 °C for up to 10 days is allowed.
- Do not freeze the kit!
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction manual for reliable results.
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.

- **Storage and shelf life of solutions and components of the kit after initial opening:**

Positive Control sample: 1 month at (2 – 8) °C or in 50 µl aliquots at minus (18 - 24) °C for no more than 3 months.
Ready Master Mix (RMM): 3 months at (2 – 8) °C.

ANNEX I: Settings for RealLine Cycler and DT96:
for these cyclers the measurement exposure must be adjusted. Choose the **Operation with the device** mode in the **Settings** menu, select the item **Measurement exposition:**

- **FAM** to **250**
- **HEX** and **ROX** to **1000**

Confirm that the current exposure value is saved by pressing **YES**
Attention! The specified exposure values are applicable only for RealLine kits and, if necessary, must be changed for other purposes.

Technical Support: techsupport@bioron.de

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