

Instructions for Use

# RealLine Mycoplasma hominis / M. genitalium Fla-Format

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










*In vitro* Diagnostics



<b>RealLine Mycoplasma hominis / M. genitalium (Fla-format)</b>	<b>VBD0493</b>	<b>100 Tests</b>
<b>valid from :</b>	<b>September 2019</b>	

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### Explanation of symbols used in labeling

	<i>In vitro</i> diagnostic medical device
	Batch code
	Catalogue number
	Contains sufficient for <n> tests
	Use-by-date
	Temperature limit
	Consult instructions for use
	Keep away from sunlight
	Manufacturer



BIORON Diagnostics GmbH

In den Rauhweiden 20  
67354 Römerberg  
Germany

Phone +49 6232 298 44 0

Fax: +49 6232 298 44 29

info@bioron.de

### Trademarks:

Rotor-Gene® is a registered trademark of Qiagen Group, Germany.

# RealLine *Mycoplasma hominis* / *M. genitalium* Fla-Format

## Table of content:

1. INTENDED USE	4
2. KIT CONTENTS	5
3. PRINCIPLE OF THE METHOD	5
4. SPECIFICATIONS	6
5. WARNING AND PRECAUTIONS	7
6. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED	7
7. PREPARATION OF SPECIMEN	8
8. PROCEDURE	9
9. DATA ANALYSIS AND INTERPRETATION	11
10. STORAGE AND TRANSPORTATION	12
ANNEX I: Settings for RealLine Cyclers and DT96:	12
ANNEX II: Programming the device and analysis of results using rotor-type cyclers:	13

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### 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 (Fla-format)** 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 (Fla-format)** kit is designed for the analysis of clinical materials: scrapings of the epithelial cells, semen, prostatic juice, urine.

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

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

The **Fla-format** Kit contains 10 vials with the lyophilized Mastermix, each vial with 10 reactions, for volume of 50 µl per reaction. The kit contains reagents required for 100 tests, including the positive control samples.

The kit is intended for use with block cyclers iQ™ iCycler, iQ5™ iCycler, CFX96™ (*Bio-Rad, USA*), DT96 (*DNA-Technology, Russia*) and RealLine Cyclers; and rotor type cyclers Rotor-Gene® 3000, 6000 and Rotor-Gene® Q (*Qiagen, Germany*).

For the **Eco™48 Realtime PCR System** (PCRmax, UK) the **RealLine Fla-format** kits can be recommended. The practice with this cycler to use 10 µl of the diluted Mastemix and 10 µl of extracted DNA, was validated. The protocol for using and cycling can be provided.

##### 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.**

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### 2. KIT CONTENTS

<b>Universal Positive Control sample (PC)</b>	1 tube, 1 ml
<b>Master Mix (MM)</b> , lyophilized	10 tubes (10 tests each);
<b>Recovery Solution (RS)</b>	2 vials, 2 ml each;

### 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.

## RealLine *Mycoplasma hominis* / *M. genitalium* Fla-Format

### 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.

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### 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.
- ☞ Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent vials.
- ☞ 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 Kits: **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.
- Plates or Tubes suitable for the used device with caps or a sealing foil for PCR
- Internal Control reagent (VBC8881), 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.

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### 7. PREPARATION OF SPECIMEN

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

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 their instruction manuals. If samples of isolated DNA were stored frozen prior the assay, thaw them and keep at least 30 minutes at a temperature of (18 – 25) °C.

*The isolated DNA can be stored at (2 – 8) °C for no more than 24 hours.*

*After initial opening shelf life of Positive Control sample at (2 – 8) ° C is 1 month or in 50 µl aliquots at minus (18 – 24) °C for no more than 3 months.*



## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### 8. PROCEDURE

#### 8.1 Preparation of the Kit Components.

Prior the test take the kit out of the refrigerator, open the package, take the necessary number of tubes with **Master Mix (MM)** closed in the package at (18 – 25) °C for at least 30 minutes. with MM (including prepared samples and controls: 1 NC and 1 PC).

Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close with the clip.

*After initial opening store MM at (2 – 8) °C for the entire shelf life of the kit.*

To prepare diluted Master Mix, add **300 µl** of Recovery Solution (RS) to each tube with MM. Mix gently, hold at room temperature for 15 minutes, and then carefully re-mix.

*Attention! Each tube with MM is intended for 10 tests.*

*Store diluted MM at (2 – 8) °C for no more than 7days*

*After initial opening shelf life of Recovery Solution is 3 months at (2 – 8) °C.*

**8.2** Prepare an appropriate number of 0.2 ml tubes or PCR-plate. Label each tube for each specimen and control.

**Attention!** Labels should be placed on the caps of tubes for rotor-type cyclers. For block-type cyclers labels should be placed on the lateral side of the tubes.

**8.3** Add **25 µl** of diluted Master Mix to each 0.2 ml tube.

**8.4** Add **25 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.

**8.5** Place the tubes into the real-time PCR system.

**8.6** Program real time PCR system.

**For Rotor-Gene® 3000 (6000/Q):**

Stage 1:	50°C	2min	50 cycles
Stage 2:	95°C	2min	
Stage 3:	94°C	10 sec	
	60°C*	40 sec	

\* Measure the fluorescence at 60°C

**For iQ™ iCycler, iQ™5 iCycler, CFX™96, DT-96 and RealLine Cycler:**

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

\* Measure the fluorescence at 60°C

## RealLine *Mycoplasma hominis* / *M. genitalium* Fla-Format

8.7 Select the amplification detection channels:

- Collect data through **FAM** channel (iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000) and **Green** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of **IC DNA**;
- Collect data through **HEX** channel (iQ5 iCycler, CFX96, DT-96), **JOE** (Rotor-Gene 3000), **Yellow** (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of ***Mycoplasma hominis* DNA**.
- Collect data through **ROX** channel (iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000), and **Orange** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of ***Mycoplasma genitalium* DNA**.

8.8 Program the position of the tubes with the specimens, **PC** and **NC** according to the Instruction Manual for the cycler in use.

8.9 Run the program.

## RealLine *Mycoplasma hominis* / *M. genitalium* Fla-Format

### 9. DATA ANALYSIS AND INTERPRETATION

- 9.1** The program should detect in **Positive Control** sample:
- increase of the IC DNA amplification signal along channel **FAM/Green** and determine the threshold cycle, IC **Ct**;
  - increase of the ***Mycoplasma genitalium* DNA** amplification signal along channel **ROX/Orange** and determine the **Ct** value;
  - increase of the ***Mycoplasma hominis* DNA** amplification signal along channel **HEX/JOE//Yellow** and determine the **Ct** value.
- 9.2** For **NC** the program should detect the increase of the amplification signal of IC DNA along channel **FAM/Green** and determine the threshold cycle, IC **Ct**. No significant **HEX/JOE/Yellow** and **ROX/Orange** fluorescent increase should appear.  
If **Ct** value for NC through **HEX/JOE/Yellow** or **ROX/Orange** 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 along channel **FAM/Green** and determine IC **Ct**.
- 9.4** Calculate (IC **Ct**)<sub>av</sub> 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**)<sub>av</sub> should be ignored. Recalculate the (IC **Ct**)<sub>av</sub> for the remaining values after the screening.
- 9.5** The sample is considered **negative**, if **Ct** value via **HEX/JOE/Yellow** and **ROX/Orange**) channels for this sample is **above 40** or is not determined.  
If IC **Ct** value for such sample differs from the (IC **Ct**)<sub>av</sub> 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/Orange** 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/JOE/Yellow** channel for this sample is **less than or equals to 40**.
- 9.7** When Ct value for NC through **ROX/Orange** or **HEX/JOE/Yellow** channel is **less than or equal 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

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### 10. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 – 8) °C in the manufacturer's packing.
- Transport at (2 – 8) °C, transportation at up to 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 (MM): unused MM at (2 – 8) °C for the entire shelf life of the kit.
- Diluted MM: at (2 – 8) °C for no more than 7days
- Recovery Solution: at (2 – 8) °C for 3 months.

#### **ANNEX I: Settings for RealLine Cyclers 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.

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### ANNEX II: Programming the device and analysis of results using rotor-type cyclers:

Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (Qiagen, Germany)

Hereinafter, detection channels and terms corresponding to different versions of cyclers and software are listed in the following order: Rotor-Gene 3000 (Rotor-Gene 6000, Rotor-Gene Q).

#### Program real-time PCR cycler:

- 1) Click **New** button.
- 2) Select an **Advanced** template from the tab of the New Run wizard. Click **New** button.
- 3) Select **36-Well Rotor** type, check that No Domed 0.2 ml Tubes are used. Click **Next** button.
- 4) In the new window, determine Reaction volume as **50 µl**. Click **Next** button.
- 5) The temperature profile of real-time PCR should be set. Click **Edit Profile** button.
 

Stage 1:	50°C	2min	50 cycles
Stage 2:	95°C	2min	
Stage 3:	94°C	10 sec	
	60°C*	40 sec	
- \* Measure the fluorescence at 60°C
- 6) Then temperature profile is set, click **OK** button.
- 7) In the **New Run Wizard** window click **Calibrate (Gain optimization)** button. The window **Auto Gain Calibration Setup** opens. In the line **Channel Settings** choose **ROX (Orange)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **Ok**. In the line **Channel Settings** choose **JOE (Yellow)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **OK**. In the line **Channel Settings** choose **FAM (Green)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **Ok**.
- 8) Tick off **Perform Calibration Before 1st Acquisition**. Click **Close** button.
- 9) Click **Next** button, start the amplification process by clicking **Start Run** button.
- 10) Save a file in the Rotor-Gene/templates folder, named RealLine with \*.ret extension. In subsequent work RealLine template would be presented in New run wizard.
- 11) Save reaction result file with Rotor-Gene Run File \*.rex extension.
- 12) Record the positions of the control samples and specimens according to the instruction manual of the PCR cycler. Click **Start run** button.

#### Results for Internal Control DNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. FAM (Cycling A. Green)**, click **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button. Settings should change to **Log. scale**.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings (Outlier Removal)** button, determine **NTC threshold** value as **5 %**.
- In the column **CT Calculation (right part of the window)** determine **Threshold** value as **0,04**.
- In the result table (**Quant. Results window**) **Ct** will be displayed.

## RealLine Mycoplasma hominis / M. genitalium Fla-Format

### Results for *Mycoplasma hominis* DNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. JOE** (*Cycling A. Yellow*) click, **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings (Outlier Removal) button**, determine **NTC threshold** value as **10 %**.
- In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0,04**.
- In the result table (**Quant. Results window**) **Ct** will be displayed.

### Results for *Mycoplasma genitalium* DNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. ROX** (*Cycling A. Orange*) click, **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings (Outlier Removal) button**, determine **NTC threshold** value as **5 %**.
- In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0,04**.
- In the result table (**Quant. Results window**) **Ct** will be displayed.

Technical support:techsupport@bioron.de

**RealLine Mycoplasma hominis / M. genitalium  
Fla-Format**

SPACE FOR YOUR NOTES:

**RealLine Mycoplasma hominis / M. genitalium  
Fla-Format**

