Instructions for Use

RealLine Ureaplasma urealyticum Str-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF *UREAPLASMA UREALYTICUM* DNA BY REAL-TIME PCR METHOD

In vitro Diagnostics

CE

RealLine Ureaplasma urealyticum (Str-Format)	**VBD2298**	**96 Tests**
valid from	**February 2020**	

BIORON Diagnostics GmbH
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Explanation of symbols used in labeling

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

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1. INTENDED USE

Ureaplasma species are bacteria belonging to *Mycoplasmatacea* family that lack a cell wall and therefore cannot be seen on Gram stain. Species are subdivided into *U. parvum* (serotypes 1, 3, 6, and 14) and *U. urealyticum* (serotypes 2, 4, 5, and 7 through 13). These subtypes cannot be distinguished from each other with routine microbiological method. Differentiation might be important because nongonococcal urethritis and adverse pregnancy outcome with respect to birth weight, gestational age, and preterm delivery are suggested to be implicated with the presence of *U. urealyticum* and not with *U. parvum*.

Ureaplasma spp. are detected in the vaginal flora of 40-80 % of sexually active women and may cause urethritis and cystitis. Horizontal transmission is by sexual contact and genital infection is usually asymptomatic. The vertical transmission rate varies from 18 to 88 % in different studies. Babies can be infected by intrauterine infection or intrapartum transmission. Newborns infected with *U. urealyticum* were subject to more frequent and longer therapeutic procedures supporting respiration, needed more frequent surfactant and antibiotic administration. *U. urealyticum* and *U. parvum* may also cause neonatal infections, including meningoencephalitis and pneumonia.

In men, although genital tract infection is usually asymptomatic, *U. urealyticum* is one of the most common pathogens associated with male infertility and is isolated in 76 % of infertile men compared with 19 % of fertile men. In addition, *Ureaplasma spp.* have been reported to cause unusual infections, such as prosthetic joint infection and infections in transplant recipients. Although rare, invasive *Ureaplasma* infection (meningitis, renal abscess, mediastinitis and arthritis) has also been reported in both adults and children [1-10]. **RealLine Ureaplasma urealyticum** assay kit detects the fragment of the urease enzyme gene, which is highly conserved in all *Ureaplasma* species. Primers and probe in the kit are designed for better discrimination between *species U. urealyticum* and *U. parvum,* none of cross-specific reactions has been observed.

Ureaplasma urealyticum is part of the normal genital flora of men and women and is found in about 70% of sexually active humans. *U. urealyticum* can be associated with a number of diseases in humans, including non-specific urethritis (NSU), infertility, chorioamnionitis, stillbirth, premature birth, and, in the perinatal period, pneumonia, bronchopulmonary dysplasia and meningitis.

RealLine Ureaplasma urealyticum (Str-format) assay kit is designed to detect *Ureaplasma urealyticum* DNA isolated from clinical specimens using extraction kits:
RealLine DNA-Express (REF VBC8899)
RealLine DNA-Extraction 2 (REF VBC8897)
RealLine DNA-Extraction 3 (REF VBC8889)
RealLine Extraction 100 (REF VBC8896)

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the RealLine Ureaplasma urealyticum (Str-format) kit is designed for the analysis of clinical materials: scrapings of the epithelial cells, semen, prostatic juice, urine. The assay is based on real-time polymerase chain reaction (PCR) method with fluorescent detection of amplified products.

The **Str-Format Kit** contains 96 tubes (0.2ml) in strips with lyophilized Mastermix. 50µl 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

The kit is validated for use with iQ5 iCycler (Bio-Rad, USA). The kit is compatible with other real-time PCR systems such as 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 is additionally supplied with optical-transparent PCR-film	

3. PRINCIPLE OF THE METHOD

The 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.

4. SPECIFICATIONS

4.1 Specificity of *Ureaplasma urealyticum* DNA detection is determined using the Standard Reference Panel of negative DNA-extracts, as a percentage of the samples determined by the kit as negative. Specificity equals 100 %.

4.2 Sensitivity is determined on five samples containing 100 copies of *Ureaplasma urealyticum* DNA per a sample, prepared from the Standard Reference Sample, as a percentage of the samples determined by the kit as positive. Sensitivity equals 100 %.

4.3 Diagnostic sensitivity of the *Ureaplasma urealyticum* DNA detection: clinical tests performed on 94 positive samples showed 100 % sensitivity (interval 97 % -100 % with a confidence level of 90 %).

4.4 Diagnostic specificity of the *Ureaplasma urealyticum* DNA detection: clinical trials performed on 94 negative samples showed 100% specificity (interval 97% -100%, with a confidence level of 90 %).

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

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5. PRODUCT USE LIMITATIONS

- This assay must not be used on the clinical specimen directly. Appropriate nucleic acids extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- When monitoring a patient the same extraction method must be used in all determinations. Otherwise, results may not be comparable.
- The kit is designed for use in patients with a clinical history and/or symptoms consistent with *Ureaplasma urealyticum* infections. The kit may be used for screening purposes.
- Diagnostic sensitivity of the kit may vary depending on the pathogen prevalence and characteristics of the enrolled cohort.
- Reliable results depend on adequate specimen sampling.
- Positive results indicate active or asymptomatic infection; clinical history and symptoms should be taken into account.
- Negative results indicate lack of detectable DNA but do not exclude the infection or disease.
- Potential mutations within the target regions of the *Ureaplasma urealyticum* genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- The kit is not intended to replace culture and other methods for diagnosis of infections.

6. 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 nuclease 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.

7. 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 or RealLine Extraction 100
- Internal Control reagent (VBC8881) and Negative Control Sample, if the kit is used with the extraction kits of other supplier;
- safety laminar 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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8. PREPARATION OF SPECIMENS

*Each group of samples undergoing the procedure of NA isolation must include a **Positive Control sample (PC)** from this kit and a **Negative Control sample (NC)** that is a component of the RealLine NA extraction kits.*
*The **RealLine Internal Control** (VBC8881 or component of the RealLine NA Extraction Kits) Sample must be added to the extractions procedure to all samples and controls.*

The assay is performed on extracted DNA samples obtained from the clinical material using one of the DNA extraction kits listed in p.1, according to the Instruction Manual to the kit.

If samples of extracted DNA were stored frozen prior the assay, thaw them and keep at least 30 min at (18 – 25) °C.
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 - 60) °C for up to 3 months.

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

9. PROCEDURE

9.1. Preparation of Kit Components

Prior to the test, take the kit out of the refrigerator and keep the **Ready Master Mix (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: 1 NC and 1 PC) 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.

9.2. Label the tubes with RMM for each specimen and control.
Attention! *Labels should be placed on the lateral side of the tubes.*

9.3. 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.

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

9.5. Program real time PCR system as follows:

Step 1:	50°C	2 min	
Step 2:	95°C	2 min	
Step 3:	94°C	10 sec	50 Cycles
	60°C*	20 sec	

* measurement of fluorescent at 60 °C in FAM and ROX

9.6. 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 **ROX** channel for detection of amplification of *Ureaplasma urealyticum* DNA.

9.7. 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.

9.8. Run the program.

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10. DATA ANALYSIS AND INTERPRETATION

10.1 For **PC** the program should detect:

- increase of the IC DNA amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**;
- increase of the *Ureaplasma urealyticum* DNA amplification signal (channel **ROX**) and determine the **Ct** value;

10.2 For **NC** the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine the threshold cycle, IC **Ct**. No **ROX** fluorescent increase should appear (*no Ureaplasma urealyticum DNA amplification*).

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

10.4 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.

10.5 The sample is considered **positive** (containing *Ureaplasma urealyticum* DNA) when **Ct** value via **ROX** channel for this sample is **less than or equals to 40.**

10.6 The sample is considered **negative** (not containing *Ureaplasma urealyticum* DNA), if **Ct** value via **ROX** channel 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.

10.7 If the Ct value for NC through the **ROX** channel is **less than or equal to 40**, it 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 samples of this run that were identified as positive. Samples that showed negative results in this run should be considered as negative.

11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 – 8) °C in the manufacturer's packing.
- Transport at (2 - 8) °C .Transportation up to 25 °C for no more than 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: After initial opening at (2–8) °C for no more than 1 month or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months
- Ready Master Mix (RMM): for not more than 3 month at (2 -8) °C

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12. REFERENCES

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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.

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