**RealLine Ureaplasma urealyticum /
Ureaplasma parvum
Str-Format**

Instructions for Use

RealLine Ureaplasma urealyticum / U. parvum Str-Format

A QUALITATIVE ASSAY KIT FOR THE DIFFERENTIAL DETECTION OF *UREAPLASMA UREALYTICUM* DNA AND *UREAPLASMA PARVUM* DNA BY REAL TIME PCR METHOD

In vitro Diagnostics

RealLine Ureaplasma urealyticum / U. parvum (Str-Format)	VBD2294	96 Tests
valid from	September 2023	

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Explanation of symbols used in labelling

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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In vitro diagnosticum

1. INTENDED USE

RealLine Ureaplasma urealyticum / Ureaplasma parvum assay kit is intended for the differential detection of Ureaplasma urealyticum and Ureaplasma parvum DNA in clinical specimens (urogenital and cervical swabs, semen, prostate fluid, urine) using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

The extraction of DNA from clinical materials can be performed using the extraction kits:

- **RealLine DNA-Express (REF VBC8899-R)**
- **RealLine Extraction 100 (REF VBC8896-R)**
- **INSTANT Virus RNA/DNA Kit – IPC16 (Roboscreen GmbH, Germany)**
- **INSTANT Virus RNA/DNA Kit – FX (Roboscreen GmbH, Germany)**
- **VeriLab UMag (Laboveritas SIA)**

When using NA extraction kits of other manufacturers, it is highly recommended to use Internal Control sample (IC, VBC8881), which can be ordered additionally. Please note that full and valid result interpretation is not possible without IC.

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

The kit is intended to use with block cyclers: iQ™5 iCycler, CFX™96 (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.

2. KIT CONTENTS

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-quality 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.

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4. SPECIFICATIONS

4.1 Sensitivity:

Sensitivity of the detection of *Ureaplasma urealyticum* DNA is determined on five samples prepared from *Ureaplasma urealyticum* DNA SRS (Standard Reference Sample), containing 100 copies of *Ureaplasma urealyticum* DNA per sample. Sensitivity equals 100%.

Sensitivity of *Ureaplasma parvum* DNA detection is determined on five samples prepared from *Ureaplasma parvum* DNA SRS, containing 100 copies of *Ureaplasma parvum* DNA per sample. Sensitivity equals 100%.

4.2 Specificity:

Specificity of *Ureaplasma urealyticum* DNA detection equals 100 %. It is determined on five samples prepared from *Ureaplasma parvum* DNA SRS, containing 100 copies of *Ureaplasma parvum* DNA per sample.

Specificity of *Ureaplasma parvum* DNA detection equals 100 %. It is determined on five samples prepared from *Ureaplasma urealyticum* DNA SRS, containing 100 copies of *Ureaplasma urealyticum* DNA per sample.

4.3 Diagnostic sensitivity:

Diagnostic sensitivity of *Ureaplasma urealyticum* DNA detection: clinical trials conducted on 47 positive samples showed 100 % sensitivity (interval 93.8 % -100 %, with a confidence level of 90 %);

Diagnostic sensitivity of *Ureaplasma parvum* DNA detection: clinical trials conducted on 75 positive samples showed 100% sensitivity (interval 96.1 % -100 %, with a confidence level of 90 %);

4.4 Diagnostic specificity

Diagnostic specificity of *Ureaplasma urealyticum* DNA detection: clinical trials conducted on 130 negative samples showed 100 % specificity (97.7 % -100 % interval, with a 90 % confidence level).

Diagnostic specificity of *Ureaplasma parvum* DNA detection: clinical trials conducted on 113 negative samples showed 100% specificity (interval 97.4 % -100 %, with a confidence level of 90 %).

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

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 HPV Low carcinogenic Risk Types 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 HPV Low carcinogenic Risk Types 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 (e.g., cervical exam) 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 steps 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.

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7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- real time PCR system, like described in p.1
- DNA-Extraction Kits listed in p.1:
- 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
- razor or scalpel.

8. 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 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 to the Instruction Manual for the kit. If an extraction kit with magnetic particles is used, keep the tubes with extracted NA in a magnetic rack.

Store the extracted DNA at (2–8) °C for no more than 24 hours.

9. PROCEDURE

9.1. Preparation of the 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 minutes. Then open the package and cut the necessary number of tubes in strips with RMM (*for prepared samples and controls: 1 PC and 1 NC*) with the razor or scalpel. Cut the tubes together with the covering film.
Put the remaining strips immediately back into the foil pouch, squeeze the air out and tightly close with the clip.
After initial opening store RMM at (2 – 8) °C for no more than 3 months.
After initial opening, store PC at (2 - 8) °C for no more than 1 month or in 50 µl aliquots at (-18 …-24) °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. Carefully remove the covering film so that RMM remains in the tube.

9.4. Add **50** µl of corresponding extracted DNA solution to each tube using a separate pipette tip with filter. Tightly close the tubes with caps or seal with the PCR optical quality film. Start the PCR no later than 30 min after the DNA addition.
Attention! *The optical film should remain clean (free of any labels or marks).*

9.5. Place the tubes into the Real Time PCR system.

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9.6. Program Real Time PCR system as follows:

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

* Measure the fluorescence at 60°C

9.7. 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 ***Ureaplasma urealyticum* DNA**.
- Collect Real Time PCR data through the **ROX** channel for detection of amplification of ***Ureaplasma parvum* DNA**.

9.8. 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.

9.9. Run the program.

10. DATA ANALYSIS AND INTERPRETATION

10.1. For **PC** the program should detect an increase of the IC DNA amplification signal (channel **FAM**) and determine the IC Ct; an increase of the *Ureaplasma urealyticum* DNA amplification signal (channel **HEX**) and determine the PC Ct value and an increase of the *Ureaplasma parvum* DNA amplification signal (channel **ROX**) and determine the PC 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** or **HEX** fluorescent increase should appear.
When **Ct** value for NC through **HEX and ROX** channel **is ≤ Ct 40,** this indicates the presence of contamination (see paragraph 9.8).

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 from the (IC **Ct**)$_{av}$ should be ignored. Recalculate the (IC **Ct**)$_{av}$ for the remaining values after the screening.

10.5. The specimen is considered **negative** (not containing *Ureaplasma urealyticum / Ureaplasma parvum* DNA), if **Ct** value via **HEX** and **ROX** channel for this sample is **above Ct 40** or not determined.
If IC **Ct** value for such sample differs from the (IC **Ct**)$_{av}$ value by more than 2, the result is regarded as equivocal. A repeated analysis of the sample, starting with the DNA isolation step is necessary.

10.6. If **Ct** value via **HEX** channel for this sample is **less than or equals to Ct 40**, the sample is considered **positive** and contains ***Ureaplasma urealyticum*** **DNA**,

If **Ct** value via **ROX** channel for this sample is **less than or equals to Ct 32**, the sample is containing ***Ureaplasma parvum*** **DNA** and considered **positive**.

If **Ct** value via **ROX** channel for this sample is **> Ct 32 and ≤ Ct 40**, the sample is containing *Ureaplasma parvum* DNA and considered as **positive,** but possible clinically insignificant.

Note: *Ureaplasma parvum* is a commensal bacterium in the uterus as part of the microbiome in healthy women. In low concentrations these bacteria do not necessarily need a treatment.

The **RealLine Ureaplasma urealyticum / Ureaplasma parvum** kit detects even lowest amounts of *Ureaplasma parvum*. For results with **Ct 32 to 40** it is advisable to re-check the samples regarding the subsequent treatment decisions, by taking the clinical data, course and appearance of the patient, into account.

10.7. If **Ct** value for NC through **HEX and ROX** channel **is ≤ Ct 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 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

- Transport and store the assay kit at (2 – 8) °C in the manufacturer's packing for the entire shelf life.The shelf life of the kit is 12 months from the manufacture date.
- 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, in 50 µl aliquots 3 month at (-18 …-24)°C .
  Ready Master Mix (RMM): 3 months at (2 – 8) °C.

Technical support: techsupport@bioron.de

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.