Instructions for Use

RealLine Treponema pallidum Str-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF *TREPONEMA PALLIDUM* DNA BY REAL-TIME PCR METHOD

In vitro Diagnostics

CE

RealLine Treponema pallidum (Str-Format)	**VBD1898**	**48 tests**
valid from:	**September 2019**	

BIORON Diagnostics GmbH
info@bioron.de - www.bioron.de

RealLine Treponema pallidum Str-Format

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

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

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1. INTENDED USE

Clinical information

Treponema pallidum is a motile spirochaete that is generally acquired by close sexual contact, entering the host via breaches in squamous or columnar epithelium. *T. pallidum* is causing **Syphilis** is a sexually transmitted infection. The primary route of transmission is through sexual contact; it may also be transmitted from mother to fetus during pregnancy or at birth, resulting in congenital syphilis

Typical for this disease is a beginning with painless mucosal ulcers and swollen lymph nodes. In some of the infected there is a chronic course, which is characterized by a variety of skin and organ involvement. In the final stage it comes to the destruction of the central nervous system.

RealLine Treponema pallidum (Str-format) assay kit is designed to detect *Treponema pallidum* 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 Treponema pallidum (Str-format) kit is designed for the analysis of clinical materials such as swabs swabs of the epithelial cells, tissue fluid of erosive–ulcerative skin lesion and mucosa. The assay is based on real-time polymerase chain reaction (PCR) method with fluorescent detection of amplified product.

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

The **Str-Format Kit** contains 48 tubes (6 x 8, 0.2 ml) 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 48 tests, including control samples.

The kit is validated for use with block-type 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 followed strongly.

2. KIT CONTENT

Positive Control Sample (PC) based on plasmid DNA with integrated *Treponema pallidum* DNA fragments in stabilizing solution	1 tube, 1 ml
Ready Master Mix (RMM) for PCR, lyophilized – 48 test-tubes	(6 strips x 8 tubes)
The kit is additionally supplied with PCR transparent 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. Specificity of *Treponema pallidum* DNA detection is determined using the Standard Reference Panel of negative DNA extracts as percentage of samples detected by the kit as negative. Specificity equals 100 %.

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

4.3. Diagnostic sensitivity of *Treponema pallidum* DNA: clinical tests performed in two independent clinical centers on 44 positive samples (epithelial cells swabs) obtained from the patients with syphilitic erosive-ulcerative skin lesions showed 100 % sensitivity (interval 93 % - 100 %, with a confidence level of 90 %).

4.4. Diagnostic specificity of *Treponema pallidum* DNA detection: clinical tests performed in two independent clinical centers on 44 negative samples (epithelial cells swabs) obtained from nominally healthy patients, patients infected with other STD than syphilis (*Chlamydia trachomatis, Ureaplasma species, Mycoplasma hominis, Trichomonas vaginalis, Neisseria gonorrhoeae,* HSV-1, HCV-2, HIV*),* from the patients infected with HPV types 16, 18, 31, 33, 45, 51. Specificity equals 100 % (interval 93% - 100%, 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 *Treponema pallidum* 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 *Treponema pallidum* 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 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 pipettes and 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 box.

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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: **RealLine DNA-Express, RealLine DNA-Extraction 3, RealLine DNA-Extraction 2 or RealLine Extraction 100,**
- Internal Control reagent (VBC8881) and Negative Control Sample if the kit is used with extraction kits of other suppliers;
- 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 THE 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 kits of another supplier for the extraction of nucleic acids as recommended in chapter 1. 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.

Prepare the samples for the assay using one of the DNA extraction kits listed in chapter 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.

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

9. PROCEDURE

9.1. Preparation of the reagents.

Prior 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. Open the package and cut the necessary number of tubes in strips with RMM (*including prepared samples and controls: 1 NC and 1 PC*) 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 the shelf life of RMM is 3 months at (2 – 8) °C.

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. 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:

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

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 ***Treponema pallidum*** **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 an increase of the **IC DNA** amplification signal (channel **FAM**) and determine the threshold cycle, **IC Ct;**
- increase of the ***Treponema pallidum*** **DNA** amplification signal (channel **ROX**) and determine the threshold cycle, **PC Ct.**

10.2 For NC the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine IC **Ct**. No **ROX** fluorescence increase should appear.

10.3 For each specimen the program should detect an 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.

10.5 The sample is considered **negative** (not containing *Treponema pallidum* DNA), if **Ct** value via **ROX** channel for this sample is **above 40** or is not determined.

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

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

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 this case, all positive results of this individual PCR test run are considered **equivocal**. Actions are required to identify and eliminate the source of contamination. Repeat the analysis of all specimens of this run that were determined positive. Specimens that showed negative results in this run should be considered **negative**

11. 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 - 60) °C for up to 3 months*.
  Ready Master Mix (RMM): 3 months at (2 – 8) °C

For help or questions: 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.

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