INSTRUCTION MANUAL
KleverTest TV PCR kit

INTENDED USE

KleverTest TV PCR kit is designed for the detection of *Trichomonas vaginalis* bacterial DNA by polymerase chain reaction with real-time detection (PCR-RT) results in clinical laboratory practice.

CHARACTERISTICS OF THE KIT

KleverTest TV PCR kit is a highly sensitive kit for DNA detection: the detection limit is 500 copies/ml when extracted from 100 µl sample.

KleverTest TV PCR kit contains the enzyme uracil-DNA glycosylase (UDG) and dUTP to avoid contamination of the PCR mixture with products of previous PCR reactions and false-positive results.

KleverTest TV PCR kit uses a rapid and simple amplification protocol, identical to the protocols of other KleverTest series kits, resulting in reliable assay results in 70-90 minutes with minimal risk of operator error, significantly improving the accuracy of laboratory analyses.

KleverTest TV PCR kit is designed to perform 100 tests and its components are listed in Table 1.

Table 1. The content of the KleverTest TV PCR kit

Component	Volume (no less than)
1. PCR Reagent TV	1.0 ml
2. Primers TV	1.0 ml
3. Positive Control TV	0.5 ml
4. Negative Control	1.0 ml

For the extraction of DNA from the sample, the reagents recommended are: PuriMag S Total DNA/RNA Isolation kit, PuriSpin S Total DNA/RNA Isolation kit.

The endogenous internal control amplification result is registered on the FAM/Green, the pathogen DNA amplification result is registered on the HEX/VIC/Yellow channel channel.

PRECAUTIONS

Follow the current technical regulations for clinical laboratory PCR and the manufacturer's guidelines when handling with the reagent kit.

EQUIPMENT AND MATERIALS REQUIRED

– real-time PCR analyser;
– laminar flow cabinet;
– centrifuge/vortex;

– set of variable volume pipettes;
– disposable tips with filter for variable-volume pipettes;
– 1.5 - 2.0 ml disposable polypropylene sealed tubes;
– optically transparent PCR-RT tubes adapted for the amplifier used;
– racks for tubes and tips;
– refrigerator from +2°C to +8°C with a freezer from -24°C to -16°C;
– lab coat and disposable gloves;
– container for tip discharge;
– workplace treatment kit.

REACTION SETUP

Isolation of DNA from clinical material is carried out according to the instructions of the manufacturer of the DNA purification reagents.

Preparation of PCR mix

0. Thaw all reagents (if necessary), mix (inverting tubes several times) and remove droplets by short centrifugation.

I. Each amplification run should include 3 control samples in addition to the test samples: negative PCR control (NC), positive PCR control (PC), negative extraction control (NEC – SFE buffer from Purimag S or Purispin S extracted in parallel with the samples).

Prepare Master Mix in a 1.5 - 2 ml tube according to the formula:

10*(N+1) µl PCR reagent TV + 10*(N+1) µl Primers TV

where N is the total number of amplification reactions, including control samples. Values may be rounded up.

II. Mix the Master Mix by rotating the tube 5 times, precipitate by brief centrifugation and add 20 µl to each PCR tube.

III. Using a filter tip, add 10 µl of DNA of the tested samples into the prepared tubes.

IV. Perform control amplification reactions:

1) NC – add 10 µl of the Negative Control to the PCR tube;
2) PC – add 10 µl of the Positive Control to the PCR tube;
3) NEC – add 10 µl of NEC extracted in parallel with the samples;

Seal tubes tightly with the caps. If bubbles or droplets appear in the solution on the walls of the tube, remove them by brief centrifugation.

Preparation of the amplification reaction

I. Place the tubes prepared for the PCR reaction in the thermocycler.

II. Program the thermocycler according to the instruction manual and Table 2.

Table 2. Amplification parameters

Step	Temperature [°C]	Time	Number of cycles
Initial denaturation	95	15 min	1
Denaturation	95	5 s	5
Annealing	63	10 s	
Elongation	67	10 s	
Denaturation	95	5 s	40
Annealing / Detection on channels: FAM/Green, HEX/VIC/Yellow	63	10 s	
Elongation	67	10 s	

III. Set the position of the tubes in the thermocycler according to the equipment instructions. If it is allowed by software, set it during or by the end of amplification.

IV. Name the experiment and save it on the disc (the results of the experiment will be automatically saved in this file). Start the instrument run*.

* *For Rotor-Gene devices, select the function: "Perform Calibration Before 1st Acquisition/Perform Optimization Before 1st Acquisition". For both channels, set the parameters "Min Reading" - 5Fl and "Max Reading" - 10Fl.*

Analysis of results

I. Before starting the analysis, set up the settings according to the instrument's user manual.

a) For Rotor-Gene devices*:

– set the value of the emission parameter (*NTC threshold*) – 5%. If necessary, the background threshold can be changed in the range of 0-30%**;

– set the threshold line parameter value (*Threshold*) – 0,02. If necessary, the threshold can be changed within the range of 0,02 - 0,2**;

– if necessary, the "Dynamic tube" and "Slope Correct" functions can also be switched on.

b) For Bio-Rad CFX96 devices*:

– set the value of the threshold line parameter (Single Threshold) to 50. If necessary, the threshold can be changed in the range of 25 - 500**;

– it is allowed to switch on the functions "Apply Fluorescence Drift Correction" and "Baseline Subtracted Curve fit".

c) For DT-96 devices*:

– set "Method" to "Geometrical";

– set the value of the threshold line parameter to 5% obtained for the C+ sample in the last amplification cycle. If necessary, the threshold can be changed in the range 0-30%.**

d) For QuantStudio devices*:

– set the threshold line parameter value to 10% obtained for the K+ sample in the last amplification cycle. If necessary, the threshold value can be changed in the range 5-30%**.

** Depending on the software version installed, the command names may vary slightly.*

*** The need for emission correction arises with strong fluorescence fluctuations in individual samples.*

II. Select a logarithmic scale to visualise the results and visually check the intersection of the threshold line in the linear part of the amplification curve growth. If the threshold line crosses the amplification curve not in the linear part of the amplification curve - move it manually to the necessary level.

The results of the analysis are interpreted on the basis of presence/absence of the intersection of the fluorescence curve with the threshold line (which corresponds to the presence/absence of the threshold cycle value "Ct" in the corresponding column in the results table).

III. Verify that the PCR assay is valid: the control points of the assay should correspond to the values given in Table 3.

If the control points do not correspond to the required values, the test should be repeated starting from the DNA extraction step.

Table 3. Evaluation of control point analysis results

Checkpoint	Controlled analysis stage	Ct value on channel FAM/Green	Ct value on channel HEX/VIC/Yellow
NEC	DNA extraction	+/–	–
NC	PCR	+/–	–
PC	PCR	+	+

IV. The results of PCR analysis of the tested samples should be interpreted in accordance with Table 4.

Table 4. Sample results interpretation

Sample result	Ct value on channel FAM/Green	Ct value on channel HEX/VIC/Yellow
Positive	+/–	**+**
Negative	< 29	**–**
Not valid	≥ 29 / –	–

„–" means absence of „Ct" value, amplification measurement graph does not cross the threshold line

„+" means presence of „Ct" value, amplification graph exceeds threshold line

„+/–" value of „Ct" for this channel is not analysed

In case of an incorrect result, it is necessary to repeat the PCR test of the appropriate sample starting from the DNA extraction stage or from the stage of collecting material from the patient.

TRANSPORTATION AND STORAGE OF THE REAGENTS KIT

The reagent kit should be transported at a temperature of +2 - +8°C (up to 14 days). It is allowed to transport by any type of transport in conditions ensuring safety under the rules of cargo carriage, operating on this type of transport.

The reagent kit should be stored in the manufacturer's package at the temperature from -24 °C to -16 °C. Freezing/thawing of the reagent kit components up to 10 times is allowed.

The validity period of the KleverTest TV PCR kit is 12 months from the date of manufacture.

TROUBLESHOOTING GUIDE

Problem	Possible cause	Solutions
No signal on FAM/Green channel	Use of inappropriate supplies	Use PCR tubes recommended by the manufacturer or adapted to the equipment used.
	PCR inhibition	Dilute sample 5 times in water, repeat the analysis
		Use a new material sample
		Apply the DNA extraction method using a sorbent
No signal in PC	Incorrect preparation of PCR-mix	Carefully prepare a new PCR mix
	Incorrect amplification parameters	Set amplification settings according to Table 2
	Inadequate storage conditions for reagents	Use new kit that was stored under the right conditions
HEX/VIC/Yellow signal in NC and/or NEC	Contamination	Perform decontamination procedures. Use a new kit