GENTAUR

DATA SHEET

REF B6-100FRT

VER 15.10.10

IVD For *in Vitro* Diagnostic Use

CE

Trichomonas vaginalis Real-TM

for use with RotorGene™ 3000/6000 (Corbett Research), SmartCycler® (Cepheid), iQ iCycler™ and iQ5™ (Biorad), Applied Biosystems® 7300/7500 Real Time PCR Systems (Applera), MX3000P® and MX3005P® (Stratagene)

Key to symbols used

REF	List Number	2°C-8°C / -15°C-25°C	Store at 2-8°C/-20°C
IVD	For *in Vitro* Diagnostic Use	!	Caution!
LOT	Lot Number	VER	Version
	Expiration Date	i	Consult instructions for use
Σ	Contains reagents		

NAME

Trichomonas vaginalis Real-TM

INTRODUCTION

STDs (sexually transmitted diseases) refer to a variety of bacterial, viral and parasitic infections that are acquired through sexual activity. Some STDs, such as syphilis and gonorrhea, have been known for centuries — while others, such as HIV, have been identified only in the past few decades. STDs are caused by more than 25 infectious organisms. As more organisms are identified, the number of STDs continues to expand. Common STDs include: chlamydia, gonorrhea, herpes, HIV, HPV, syphilis, gardnerella and trichomoniasis.

The development of tests based on nucleic acid amplification technology has been the most important advance in the field of STD diagnosis. Because nucleic acid amplification is exquisitely sensitive and highly specific, it offers the opportunity to use noninvasive sampling techniques to screen for infections in asymptomatic individuals who would not ordinarily seek clinical care.

INTENDED USE

Kit **Trichomonas vaginalis Real-TM** is a test for the qualitative detection of *Trichomonas vaginalis* in the urogenital swabs, urine, prostatic liquid and other biological materials.

PRINCIPLE OF ASSAY

Kit **Trichomonas vaginalis Real-TM** is based on two major processes: isolation of DNA from specimens and Real Time amplification. *Trichomonas vaginalis* DNA is extracted from the specimens, amplified in Real Time PCR and detected using fluorescent reporter dye probes specific for *Trichomonas vaginalis* DNA and Internal Control. Internal Control (IC) serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. IC is detected in a channel other than the *Trichomonas vaginalis* DNA.

MATERIALS PROVIDED

- PCR-mix-1-FRT, 1,2 ml;
- PCR-Buffer-FRT, 2 x 0,35 ml;
- TaqF Polymerase, 2 x 0,03 ml;
- Pos C+, 0,2 ml;
- Negative Control C-, 1,2ml;*
- Internal Control IC, 1,0 ml;**
- DNA-buffer, 0,5 ml;

Contains reagents for 110 tests.

*must be used in the isolation procedure as Negative Control of Extraction.

**add 10 µl of Internal Control during the DNA isolation directly to the sample/lysis mixture (see DNA-Sorb-A REF K-1-1/A protocol).

MATERIALS REQUIRED BUT NOT PROVIDED

- Real Time Thermal cycler
- Reaction tubes
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes
- Vortex mixer
- Freezer, refrigerator

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose all specimens and unused reagents in accordance with local regulations
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
8. Material Safety Data Sheets (MSDS) are available on request.
9. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
10. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

Trichomonas vaginalis Real-TM must be stored at 2-8°C. **TaqF Polymerase** must be stored at -20°C. The **Trichomonas vaginalis Real-TM** kit can be shipped at 2-8°C but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY

Trichomonas vaginalis Real-TM is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

QUALITY CONTROL

The complete kit has been tested on an RotorGene 6000 (Corbett Research).
Certificates of Analyses are available on request at info@sacace.com.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Trichomonas vaginalis Real-TM can analyze DNA extracted with from:

- *cervical, urethral, conjunctival swabs:* insert the swab into the nuclease-free 1,5 ml tube and add 0,2 ml of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.
- *urine sediment* : collect 10-20 ml of first-catch urine in a sterile container. Centrifuge for 30 min at 3000 x g, carefully discard the supernatant and leave about 200 µl of solution. Resuspend the sediment. Use the suspension for the DNA extraction.
- *prostatic liquid* stored in "Eppendorf" tube;
- *seminal liquid:* maintain semen for 40 min in darkness until liquefaction. Use 100 µl for the DNA extraction.

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

The following kit is recommended:

⇨ **DNA-Sorb-A** (Sacace, REF K-1-1/A)

Please carry out DNA extraction according to the manufacture's instruction. Add 10 µl of Internal Control during DNA isolation procedure directly to the sample/lysis mixture.
(Note: the Sacace Internal Control is the same for all urogenital infection Real Time kits)

PROTOCOL

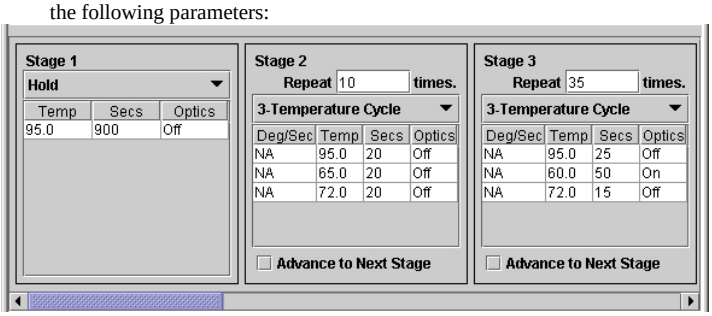
1. Prepare required quantity of reaction tubes for samples (N) and controls (N+2).
2. Prepare in the new sterile tube **10*N µl** of **PCR-mix-1-FRT**, **5*N µl** of **PCR-Buffer-FRT** and **0,5*N µl** of **TaqF DNA Polymerase.** Vortex and centrifuge briefly.
3. Add to each tube **15 µl** of **Reaction Mix** and **10 µl** of **extracted DNA**. Mix by pipetting.
4. Prepare for each panel 2 controls:
   - add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
   - add **10 µl** of **Positive Control C+** to the tube labeled Amplification Positive Control;
5. Insert the tubes in the thermalcycler.

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line.
Trichomonas vaginalis is detected on the FAM (Green) channel, *IC DNA* on the JOE(Yellow)/HEX/Cy3 channel

Real Time Amplification with SmartCycler® (Cepheid)

Program SmartCycler as follows:

1. Transfer the SmartCycler tubes into the rotor of the minicentrifuge and centrifuge briefly (5-7 sec).
2. Select in the main menu **Define Protocols** and in the lower left corner select option **New Protocol**. Assign a name to the protocol and set the following parameters:



3. Choose **Save Protocol**.
4. Select in the main menu option **Create Run** and in the window **Run Name** give a name to the experiment.
5. Click button **Dye Set** and select **FCTC25**.
6. Choose **Add/Remove Sites** and select in the new window the **Protocol** and **Sites** for analysis. Click **OK**.
7. Transfer reaction tubes into the SmartCycler and start the experiment by pressing **Start Run** button.
8. In the menu **View Results** press **Results Table** and insert in the column **Sample ID** the name of the samples.

Results Analysis

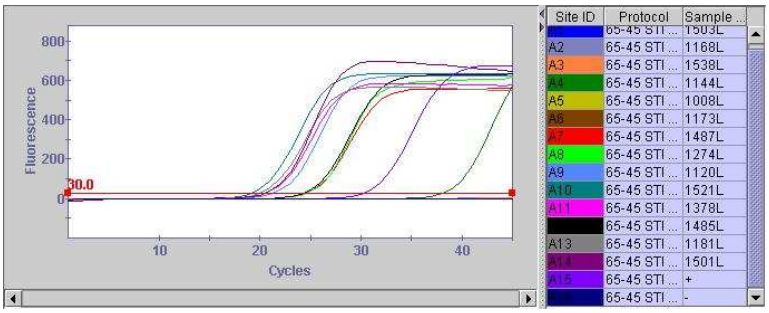
1. Press **Analysis settings** and in the column **Manual Thresh Fluor Units** set value of the threshold line to **30** for the channels Fam and Cy3. Click **Update Analysis.**
2. Click **Save Run** in the menu **Results Table**.
3. The sample is considered to be **Positive** if in the column **FAM Std/Res** the result is indicated as **POS** (value of **FAM Ct** is different from zero). If the Ct value of the IC is higher than 40 a retesting of the sample is required.
4. The sample is considered to be **Negative** if in the column **FAM Std/Res** the result is indicated as **NEG** (value of **FAM Ct** = zero) and in the column **CY3 Std/Res** the result is indicated as **POS.** Negative samples Ct values on the Cy3 channel in a range of 40-42 cycles testify an inefficient DNA extraction (if Ct > 40 retesting of sample is required).
5. The result is invalid if in the column **FAM Std/Res** and in the column **CY3 Std/Res** the result is indicated as **NEG** *(Ct = 0).* Repeat the entire test including sample preparation and amplification.
6. Result is accepted as significant only when Positive and Negative Controls of amplification and DNA isolation are valid.

Table 2. Results for controls

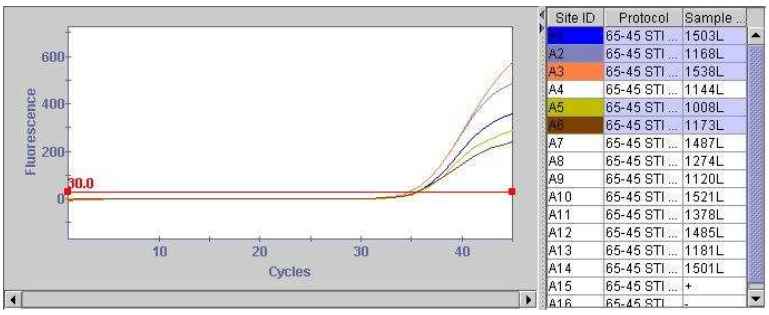
Control	Stage for control	Ct channel Fam	Ct channel Cy3	Interpretation
NCS	DNA isolation	NEG	**POS**	Valid result
DNA-buffer	Amplification	NEG	NEG	Valid result
C+	Amplification	**POS**	**POS**	Valid result

Example:

FAM Channel –Trichomonas vaginalis DNA detection (Positive samples):



Cy3 Channel – Internal Control DNA detection:



Real Time Amplification with Rotor-Gene™ 3000/6000

1. Create a template for "Urogenital Assays" by activating in the window *New Run* the programming regime *Advanced*. Choose *Dual Labeled Probe/Hydrolysis probes* and click the button *New*.
2. Select in the new window the carousel type *36-Well Rotor or 72-Well Rotor* and *Reaction Volume (µL)* 25.
3. Set in the window *Edit Profile* program **"STD 65-60-45 RG-TaqF"** (this program is universal for all Sacace™ Urogenital Assays):

1.	**Hold**	**95 deg – 15 min**
2.	**Cycling**	**95 deg - 20 secs**
		65 deg - 20 secs
		72 deg - 20 secs
		Cycle repeats – 10 times.
3.	**Cycling 2**	**95 deg - 20 secs**
		60 deg - 30 secs – Acquaring on Fam (Green), Joe (Yellow) *
		72 deg -15 secs
		Cycle repeats – 35 times

4. Make the adjustment of the fluorescence channel sensitivity: *Channel Setup →Calibrate (Gain Optimisation… for* Rotor-Gene 6000) → *Auto Gain Calibration (Optimisation) Setup →Calibrate Acquiring (Optimise Acquiring)* and select *Perform Calibration (Optimisation) Before 1-st Acquisition*. For *Fam/Sybr (Green)* channel indicate *Min Reading* **5**, *Max Reading* **10**, for *Joe (Yellow)* channel *Min Reading* **4**, *Max Reading* **8.** In the column *Tube position* schedule position of the tubes in the carousel of the Rotor-Gene 2000/3000/6000 (the 1st position must contains reaction tube with reagents). Close the window *Auto Gain Calibration Setup.*
5. Save the protocol in the folder Programm files / RotorGene 6 / Templates (for RG6000 Programm files / RotorGene 6000 Software / Templates) and close the window *New Run Wizard*. The new template appears in the list of templates in the window *New Run* and can be used for all Sacace™ Real-TM Urogenital Assays.

START AMPLIFICATION AND DETECTION

1. Select *Quick Start* (*Advanced Start* for RG6000) in window *New Run* and choose from the templates **"STD 65-60-45 RG-TaqF".**
2. Select carousel type and mark *No Domed 0,2 Tubes* for 36-Well Rotor or *Locking Ring Attached* for 72-Well Rotor. Click *Next*.
3. Program position of the tubes in the window *Sample Setup*.
4. Click *Next* and *Start Run* to begin the experiment.

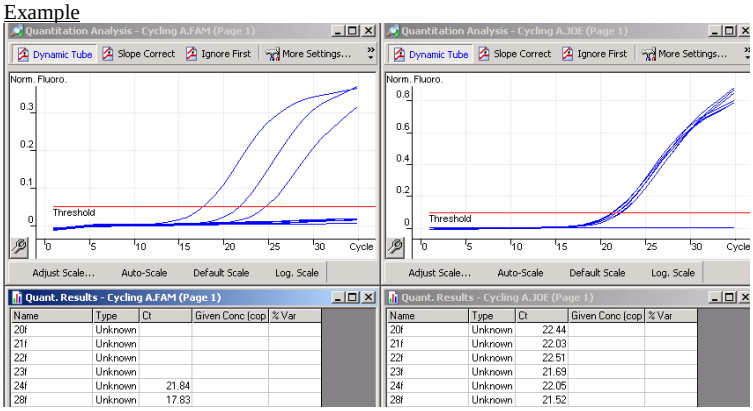
RESULTS ANALYSIS:

1. The results are interpreted with the software of **Rotor-Gene 3000/6000** through the presence of crossing of fluorescence curve with the threshold line. *Trichomonas vaginalis* is detected on the FAM (Green) channel, *Internal Control* on the JOE (Yellow) channel
2. Press *Analysis* then select button *Quantitation*. Perform the operation for the Fam (Green) channel (*Cycling A FAM or Cycling A. Green for RG6000)*, then for the Joe (Yellow)channel (*Cycling A JOE or Cycling A. Yellow)*
3. For the Fam (Green) channel (*Trichomonas vaginalis*) select *Dynamic Tube, More Setting (Outlier Removal for RG6000) 0%,* and *Threshold*: 0,05.
4. For the Joe (Yellow) channel (*IC)* select *Dynamic Tube, More Setting (Outlier Removal for RG6000) 5%, Threshold*: 0,1
5. Specimens with Ct < 33 in the channel Fam (Green) (*Quant. Resultes – Cycling A. FAM/Green)* are interpreted as positive.
6. Specimens with Ct < 30 in the channel Joe (Yellow) (*Quant. Resultes – Cycling A.JOE/Yellow)* and absent fluorescence signal in the channel Fam (Green) are interpreted as negative.
7. Specimens with absent signal in the FAM (Green) and JOE (Yellow) *(or Ct > 30)* are interpreted as invalid.

Table 2. Results for controls

Control	Step control	Ct channel Fam (Green)	Ct channel Joe (Yellow)	Interpretation
NCS	DNA isolation	No signal	**< 30**	Valid result
DNA-buffer	Amplification	No signal	No signal	Valid result
C+	Amplification	**< 30**	**< 30**	Valid result

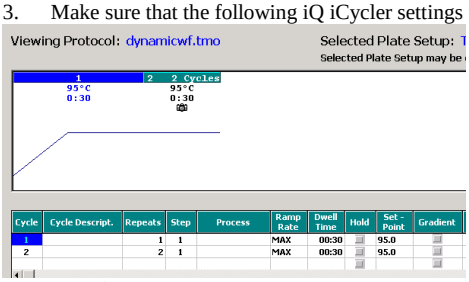
Example



20f, 21f, 22f, 23F – negative samples
24f, 28f, – positive samples

Real Time Amplification with iQ iCycler™ and iQ5 (Bio-Rad)

1. Schedule in the window *Edit Plate Setup* of *Workshop* module the tube positions and the fluorescence signal detection in all tubes on the channels Fam and Hex. Save it and use this scheme by activating the button *Run with selected protocol.*
   - For iQ5 instrument edit the scheme in the regime *Whole Plate loading.* Select *Sample Volume* 25: µl, *Seal Type: Domed Cap*, *Vessel Type: Tubes.* Click the button *Save &Exit Plate Editing.*
2. Start on the iQiCycler or iQ5 the program **"STD 65-60-45 iQ-TaqF"**, choose or create it in the module *View Protocols* and start by activating the button ***Run with selected plate setup***.
   95°C – 13 min 30 sec
   10 cycles: 95°C – 10 sec, 65°C – 20 sec, 72°C – 20 sec
   35 cycles: 95°C – 10 sec, 60°C – 30 sec, 72°C – 20 sec
   fluorescence detection on the channels Fam and HEX on the 2-nd step (60°C)
3. Make sure that the following iQ iCycler settings for **dynamicwf** are selected:



4. Transfer tubes in the thermalcycler in accordance with the previously created model.
5. Select *Experimental Plate* under the line *Select well factor source* and choose the reaction volume 25 µl (for iQ iCycler).
6. Click *Run* button.

DATA ANALYSIS

The results are interpreted with the software of "iQ iCycler" or "iQ5" through the presence of crossing of fluorescence curve with the threshold line.

Trichomonas vaginalis is detected on the FAM channel, *IC DNA* on the HEX channel.

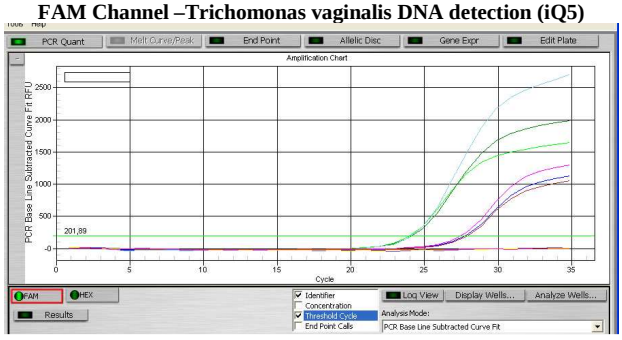
For Fam and Hex channels activate the button "**Log View**". Put the threshold line (with the left button of the mouse) at such level where curves of fluorescence are linear.

- The sample is considered to be positive for *Trichomonas vaginalis* if in the channel Fam (FAM-490 in the window *Select a Reporter*) the value of **Ct** is different from zero (Ct < 33).
- Specimens with Ct ≤ 33 in the channel HEX (HEX-530 in the window *Select a Reporter)* and absent fluorescence signal (*N/A* value) in the channel FAM are interpreted as negative.
- Specimens with Ct absent or > 33 in the FAM and HEX channels are interpreted as invalid.

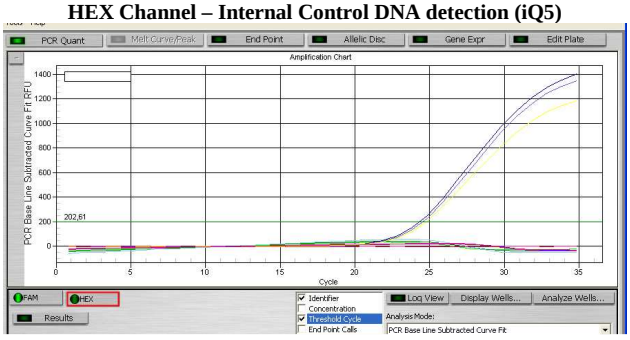
Table 2. Results for controls

Control	Step control	Ct channel Fam	Ct channel HEX	Interpretation
NCS	DNA isolation	No signal	**≤ 33**	Valid result (Negative)
DNA-buffer	Amplification	No signal	No signal	Valid result (Negative)
C+	Amplification	**≤ 33**	**≤ 33**	Valid result (Positive)

FAM Channel –Trichomonas vaginalis DNA detection (iQ5)



HEX Channel – Internal Control DNA detection (iQ5)



Program Applied Biosystems® 7300/7500 Real Time PCR Systems as follows:

1. Select in the main menu option "**New**" and set the data of new document: select in the window **Assay** the option **Absolute Quantitation**, in the window **Template** the option **Blank Document**. Press **OK**.
2. In the new window in the **Tools** menu click button **Detector Manager**.
3. In the lower left corner of the window click **File** and select **New**. Set in the window **New detector** probes features:
    a) Detection of "POS": in the lines **Name** and **Description** indicate **POS**; in the line **Reporter Dye – Fam** and in **Quencher Dye – None**. Select the **Color** (for example, red). Click button **Create Another**.
    b) The window **New detector** is opened against. Set the following parameters for Internal Control: in the lines **Name** and **Description** indicate **IC**; in the line **Reporter Dye – Joe** and in **Quencher Dye – None**. Select the **Color** (for example, blue). Click **OK**.
4. Close the window **Detector manager** with probes information.
5. Select window **Instrument**.
6. Activate **Thermal profile** and set the following amplification program:

Stage	Profile	Reps
1	95°C – 15:00	1
2	95°C – 0:20 65°C – 0:20 72°C – 0:20	10
3	95°C – 0:25	35
	60°C – 0:50*	
	72°C – 0:15	

*****Fluorescence detection on the Fam, Joe channels

7. Indicate reaction volume, **25 µl**. Choose **9600 Emulation**.
8. Save created document: in the menu **File** select **Save as...**, in the line **File type** select **SDS Templates (*.sdt)** and click **Save**.
9. In the upper right corner of the window choose **Setup**. In the opened window **Plate,** with the mouse select the cells in which the amplification has been planned. In the menu **View** click button **Well inspector**.
10. Click button **Add Detector** and select probes **POS** and **IC** from the window **Detector manager**. To do this, select lines with mouse and click button **Add to Plate Document** and **Done**.
11. In the column **Use** of the window **Well inspector** select probes **POS** and **IC**.
12. In the lower right corner of the window in the line **Passive Reference** set **none**.
13. In the field **Sample Name** insert name of the samples.

Results Analysis

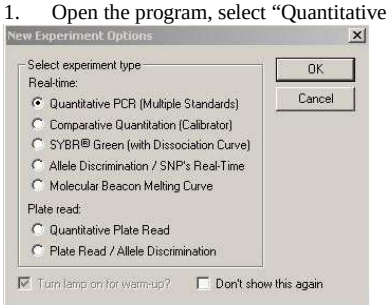
1. The results are interpreted with the software of Applied Biosystems® 7300/7500 Real Time PCR Systems through the presence of crossing of fluorescence curve with the threshold line. *Pos* result is detected on the FAM channel, *Internal Control* on the JOE channel
    a. Specimens with Ct < 40 in the *Fam* channel are interpreted as **Positive** regardless of the *Joe* channel (IC) results
    b. Specimens with absent Ct ("Undet.") in the *Fam* channel and Ct < 40 in the Joe channel are interpreted as **Negative**
    c. The result is invalid if in the column *Fam* and in the column *Joe* the result is indicated as "**Undet."** or with Ct >40. Repeat the entire test including sample preparation and amplification.
2. Result is accepted as significant only when Positive and Negative Controls of amplification and DNA isolation are valid.

Table 2. Results for controls

Control	Stage for control	Ct channel Fam	Ct channel Joe	Interpretation
NCS	DNA isolation	NEG	**POS**	Valid result
DNA-buffer	Amplification	NEG	NEG	Valid result
C+	Amplification	**POS**	**POS**	Valid result

Program MX3000P® and MX3005P® (Stratagene) as follows:

1. Open the program, select “Quantitative PCR (Multiple Standarts)” and click “OK”



2. At the top left of the window choose “Plate Setup”
3. In the window “Well type” set “Unknown” for the samples.
4. In the window “Collect fluorescence data” select for all samples the channels Fam and Joe.
5. At the top left of the window select button “Thermal Profile Setup”
6. Set the following parameters of amplification:

Hold **95°C – 15 min**
Cycling 1 **95°C – 20 sec**
65°C – 30 sec
72°C – 20 sec
***Cycle Repeats* – 10 times**
Cycling 2 **95°C – 25 sec**
65°C – 50 sec*
72°C – 20 sec
***Cycle Repeats* – 35 times**

*Fluorescence is measured at 65°C on the 2nd Cycling.

7. Click “Run” button, enter a name for the experiment and save it.

Results Analysis

1. Soon after amplification is over, choose button “Analysis” at the top left of the window .
2. Choose button “Results”
3. At the right angle of the window “Area to analyze” select “Amplification plots”.
4. The results are interpreted with the software of the instrument through the presence of crossing of fluorescence curve with the threshold line. *Trichomonas vaginalis* is detected on the FAM channel, *Internal Control* on the JOE channel
5. Inhibition of IC may occur in specimens with high initial concentration of *Trichomonas vaginalis*.
6. Specimens with Ct < 40 in the *Fam* channel are interpreted as positive for *Trichomonas vaginalis* regardless of the *Joe* channel (IC) results.
7. Specimens with Ct > 40 or absent in the *Fam* channel are interpreted as negative for *Trichomonas vaginalis.*
8. Specimens with absent signal in the FAM and JOE are interpreted as invalid.

Table 2. Results for controls

Control	Stage for control	Ct channel Fam	Ct channel Joe	Interpretation
NCS	DNA isolation	NEG	**POS**	Valid result
DNA-buffer	Amplification	NEG	NEG	Valid result
C+	Amplification	**POS**	**POS**	Valid result

TROUBLESHOOTING

1. Weak or no signal of the IC (Joe/Hex/Cy3 channel) for the Negative Control of extraction.
    - The PCR was inhibited.
        - ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
        - ⇒ Re-centrifuge all the tubes before pipetting of the extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent inhibit reaction.
    - The reagents storage conditions didn't comply with the instructions.
        - ⇒ Check the storage conditions
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.
    - The IC was not added to the sample during the pipetting of reagents.
        - ⇒ Make attention during the DNA extraction procedure.
2. Weak or no signal of the Positive Control.
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
3. Fam (Green) signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        - ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
        - ⇒ Repeat the DNA extraction with the new set of reagents.
4. Any signal with Negative Control of PCR (DNA-buffer).
    - Contamination during PCR preparation procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        - ⇒ Pipette the Positive control at last.
        - ⇒ Repeat the PCR preparation with the new set of reagents.

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *Trichomonas vaginalis* primers and probes. The specificity of the kit **Trichomonas vaginalis Real-TM** was 100%. The potential cross-reactivity of the kit **Trichomonas vaginalis Real-TM** was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit **Trichomonas vaginalis Real-TM** allows to detect *Trichomonas vaginalis* DNA in 100% of the tests with a sensitivity of not less than 500 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

Target region: G3 hypothetical protein