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artus® T. vaginalis QS-RGQ Kit Handbook

72

Version 1
For use with QIAsymphony® SP/AS and Rotor-Gene® Q instruments

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Sample to Insight

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Contents

Intended Use 5

Summary and Explanation 5

Pathogen information 5

Principle of the Procedure 8

Materials Provided 9

Kit contents 9

Materials Required but Not Provided 10

Reagents, materials, and consumables for sample preparation and collection 10

Adapters for QIAsymphony SP 10

Reagents and consumables for QIAsymphony SP 11

Adapters and reagent holders for QIAsymphony AS 11

Reagents and consumables for QIAsymphony AS 12

Equipment 12

Warnings and Precautions 13

Warnings 13

Precautions 14

Reagent Storage and Handling 15

Kit components 15

Procedure 16

Specimen collection, storage and transport 16

Vaginal and endocervical specimens 16

Urine (male or female specimens) 17

Sample storage 17
Sample transport 17
Sample preparation 17
Detection of *T. vaginalis*-specific DNA 19
Controls 20
Positive control 20
Negative control 20
Specimen process controls 20
Preparation of carrier RNA and internal control (T. vaginalis IC) 21
Calculation of mixture by "IC Calculator" 23
Assay control sets and assay parameter sets 23
Protocol: DNA isolation and assay setup on the QIAsymphony SP/AS 24
Important points before starting 24
Things to do before starting 25
Procedure 26
Protocol: PCR on the Rotor-Gene Q instrument 40
Important points before starting 40
Things to do before starting 40
Procedure 41
Interpretation of Results 44
Limitations 50
Quality Control 50

Performance Characteristics 51
Limit of detection 51
Analytical reactivity (inclusivity) 52
Cross-reactivity and interference 55
Total precision and reproducibility 58
Carryover 61
Interfering substances 61
Diagnostic performance evaluation 65
Discordant analysis 70
References 73
Symbols 75
Troubleshooting Guide 78
Ordering Information 85

Intended Use

The *artus* T. vaginalis QS-RGQ Kit is an in vitro real-time polymerase chain reaction (PCR) assay for the direct qualitative detection of *Trichomonas vaginalis* DNA purified from clinician-collected vaginal swabs, endocervical swabs, and urine samples (male and female) from symptomatic and asymptomatic subjects as an aid in the diagnosis of trichomoniasis. This diagnostic test is configured for use with the QIAsymphony SP/AS and Rotor-Gene Q instruments for target amplification and detection.

Summary and Explanation

The *artus* T. vaginalis QS-RGQ Kit constitutes a ready-to-use system for the detection of *T. vaginalis* DNA using PCR on Rotor-Gene Q instruments with sample preparation and assay setup using QIAsymphony SP and AS instruments.

Pathogen information

Trichomoniasis is a sexually transmitted infection (STI) that is caused by the motile protozoan *T. vaginalis*. It is a common STI (1,2) and the World Health Organization estimates the worldwide incidence of trichomonas infection at over 170 million cases annually (3).

The high worldwide prevalence of *T. vaginalis* infection and its coinfection with other STIs make trichomoniasis a compelling public health concern. Research has shown that infection with *T. vaginalis* increases the risk of human immunodeficiency virus (HIV) transmission in both men and women (1, 4). Trichomoniasis is also associated with adverse pregnancy outcomes, infertility, postoperative infections, and cervical neoplasia (5).

T. vaginalis infection is one of the top 3 causes of vaginitis (6). Women with trichomoniasis may be asymptomatic or may experience various symptoms, including a frothy yellow-green

vaginal discharge and vulvar irritation. Men with trichomoniasis may experience non-gonococcal urethritis, but are frequently asymptomatic (6). Trichomoniasis is thought to be widely under diagnosed due to a variety of factors, including a lack of routine testing (2), the low sensitivity of the commonly used diagnostic technique of wet mount microscopy (6, 7, 8), and nonspecific symptomatology. The two other most common causes of vaginal discharge are the overgrowth of anaerobic bacteria in normal flora and candidiasis caused by *Candida albicans* infection (6).

In women, *T. vaginalis* is isolated from the vagina, cervix, urethra, bladder, and Bartholin and Skene glands. In men, the organism is found in the anterior urethra, external genitalia, prostate, epididymis, and semen.

Humans are the only known host of *T. vaginalis* and transmission occurs predominantly via sexual intercourse. The organism is most commonly isolated from vaginal secretions in women and urethral secretions in men. It has not been isolated from oral sites and the rectal prevalence appears to be low in men who have sex with men (5).

Symptoms of trichomoniasis typically occur after an incubation period of 4–28 days (9, 10). While the infection may persist for months or even years in women, the infection in men is often self-limiting and generally lasts for fewer than 10 days (11,12, 13). As such, a positive test result from a sample collected from a man is seen infrequently.

Risk factors for *T. vaginalis* infection include:

- New or multiple partners
- A history of STIs
- Current STIs
- Sexual contact with an infected partner
- Exchanging sex for money or drugs
- Using injection drugs
- Not using barrier contraception

Testing is recommended for *T. vaginalis* in all women seeking care for vaginal discharge and screening for *T. vaginalis* is advised in women at high risk of STIs (9,10). Sexual partners of infected women should also be treated. Both the woman and their partner should abstain from sex until the pharmacological treatment of the *T. vaginalis* infection has been completed and they have no symptoms. Infected women who are sexually active have a high rate of reinfection; thus, rescreening at 3 months post treatment should be considered (9, 14, 15). Currently, no information on rescreening of men is available.

Oral metronidazole remains the treatment of choice for trichomoniasis. In cases where this first-line agent is ineffective, other nitroimidazoles or higher doses of metronidazole may be used. Topical metronidazole and other antimicrobials are not efficacious and should not be used to treat trichomoniasis. In most cases, treatment of the infection takes 7–10 days. All sexual partners of individuals with a *T. vaginalis* infection should also be treated.

Principle of the Procedure

The *artus* T. vaginalis QS-RGQ Kit contains reagents and enzymes for the specific amplification and direct detection of a multiple repeat region within *T. vaginalis* genomic DNA. The *T. vaginalis* target is detected using the fluorescence channel Cycling Green on the Rotor-Gene Q instrument.

In addition, the *artus* T. vaginalis QS-RGQ Kit contains an exogenous internal control (IC), which identifies possible PCR inhibition and monitors reagent integrity. The IC is detected in fluorescence channel Cycling Orange on the Rotor-Gene Q instrument. This distinguishes the IC from the *T. vaginalis* target detected in fluorescence channel Cycling Green.

Materials Provided

Kit contents

***artus* T. vaginalis QS-RGQ Kit**			**(72)**
Catalog number			**4571366**
Number of reactions			**72**
Blue	T. vaginalis Master	MASTER	3 x 800 µl
Yellow	T. vaginalis Magnesium Solution	MG-SOL	3 x 200 µl
Green	T. vaginalis Internal Control	IC	3 x 500 µl
Red	T. vaginalis Positive Control	CONTROL +	3 x 1500 µl
White	T. vaginalis Negative Control	CONTROL –	3 x 100 µl
	Handbook		1

Note: The contents of the *artus* T. vaginalis QS-RGQ Kit are sufficient for 72 tests in one to three batches of 24 reactions on the QIAsymphony RGQ. The rotor in the Rotor-Gene Q instrument holds up to 72 reaction tubes.

Materials Required but Not Provided

Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. This kit requires the use of QIAsymphony SP and QIAsymphony AS, QIAsymphony software version 4.0 or higher, Rotor-Gene Q MDx 5plex HRM instrument* with Rotor-Gene AssayManager® version 1.0.X where X is ≥4.

Reagents, materials, and consumables for sample preparation and collection

- eNAT® Collection Kit (300) including eNAT tube, 2 ml, regular FLOQSwab™ in peel pouch, and a pipet (cat. no. 4669848)
- Recommended: Biological materials for Positive and Negative Specimen Process Controls see "Specimen process controls", page 20

Adapters for QIAsymphony SP

- Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730) in combination with the QIAsymphony SP/AS Transfer Frame
- 13 mm tube Insert 1A (cat. no. 9242058)
- Optional 2.0 ml tube Insert 3B (cat. no. 9242083)

* Rotor-Gene Q 5plex HRM instruments with a production date of January 2010 or later can be used as an alternative to Rotor-Gene Q MDx 5plex HRM instruments. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format "mmyynnn" where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "nnn" indicates the unique instrument identifier.

Reagents and consumables for QIAsymphony SP

- QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
- Buffer ATL (4 x 50 ml) (cat. no. 939016)
- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Elution Microtubes CL (EMTR) (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)
- Microtubes 2.0 ml Type H, without skirted base (cat. no. 72.693) or Microtubes 2.0 ml Type I, with skirted base (Sarstedt®, cat. no. 72.694) for use with internal control
- Tubes, 14 ml, 17 x 100 mm polystyrene round-bottom (Corning®, cat. no. 352051, for use with samples and internal control

  Note: BD™ was the previous supplier of this tube
- Empty eNAT Tube (e.g., a tube not filled with eNAT transport medium), 12 x 80 mm, for loading T. vaginalis Positive Control (cat. no. 4670002)
- Screw-Cap for eNAT Tube , 12 mm, for reclosure of the eNAT collection tube (cat no. 4670003)

Adapters and reagent holders for QIAsymphony AS

- Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
- RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)

Reagents and consumables for QIAsymphony AS

- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)
- Tubes, conical, 5 ml, Qsym AS (cat. no. 997104)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Filter-Tips, 50 µl (cat. no. 997120)
- Tip disposal bags (cat. no. 9013395)

Equipment

- Dedicated adjustable pipets* and sterile pipet tips with filters
- Vortex mixer
- Benchtop centrifuge with rotor for 2 ml reaction tubes
- Rotor-Gene Q MDx 5plex HRM instrument† and Rotor-Gene AssayManager version 1.0.X where X is ≥4
- QIAsymphony SP (cat. no. 9001297) or QIAsymphony AS (cat. no. 9001301) and QIAsymphony software version 4.0 or higher

* Ensure pipets have been checked and calibrated according to the manufacturer's recommendations.

† Rotor-Gene Q 5plex HRM instruments with a production date of January 2010 or later can be used as an alternative to Rotor-Gene Q MDx 5plex HRM instruments. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format "mmyynnn" where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "nnn" indicates the unique instrument identifier.

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the test.

For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

For safety information for the QIAsymphony DSP Virus/Pathogen Midi Kit, see the *QIAsymphony DSP Virus/Pathogen Kit Instructions for Use (Handbook)* supplied with the QIAsymphony DSP Virus/Pathogen Midi Kit. For safety information regarding instruments, see *QIAsymphony RGQ MDx User Manual* and *Epsilon Plug-in User Manual*.

Warnings

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipet tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.

- Use separated and segregated working areas for specimen preparation, reaction setup and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area, and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separate from all other components of the kit.
- Repeated thawing and freezing of the eluates should be avoided, as this may reduce assay performance.
- Do not open the reaction tubes post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

Precautions

- Make sure that the required adapters are precooled to 2–8°C.
- Work quickly and keep the PCR reagents on ice or in the cooling block before loading.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- Use sterile pipet tips with filters.
- During manual steps, keep tubes closed when possible and avoid contamination.
- When thawed, mix the components by pipetting repeatedly up and down or by pulse vortexing and centrifuge briefly. Make sure that no foam or bubbles are present in the reagent tubes.

- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between the QIAsymphony AS and the Rotor-Gene Q instrument.
- All reagents loaded on the QIAsymphony AS are for use in that run only. Do not use the residual components for a second PCR.
- Do not mix or combine components from kits with different lot numbers.
- Follow universal safety precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Make sure that maintenance has been performed and replaceable parts, such as tip guards, have been reinstalled.
- Make sure that the Application Process files and required Rotor-Gene AssayManager plug-ins are installed. If not installed, refer to the applicable user manual or contact QIAGEN customer support or technical services for advice.

Reagent Storage and Handling

Kit components

The components of the *artus* T. vaginalis QS-RGQ Kit should be stored at –15°C to –30°C and are stable until the expiration date stated on the label. Do not thaw and freeze the components more than 3 times as this may reduce assay performance. All reagents that are loaded on QIAsymphony AS are for use in that run only. Do not reuse residual components in a second PCR.

Procedure

Specimen collection, storage and transport

Human vaginal and endocervical swabs and urine specimens can be used with the *artus* T. vaginalis QS-RGQ Kit.

Specimens must be collected using an eNAT Collection Kit including eNAT tube, 2 ml, regular FLOQSwab in peel pouch and pipet.

Vaginal and endocervical specimens

1. Each vaginal/endocervical specimen is clinician collected using the sterile swab included in the eNAT Collection Kit.
2. Aseptically unscrew and remove the cap from the tube filled with 2 ml eNAT medium. After collecting the vaginal/endocervical specimen, insert the FLOQswab into the tube and bend the swab shaft at the breakpoint against the tube to break the shaft. Discard the broken handle part of the swab shaft into an approved medical waste disposal container.

   Note: During specimen collection when handling the swab applicator, the operator must not touch the area below the marked breakpoint indication line, as this will lead to contamination of the applicator shaft thus invalidating the test results. Do not use excessive force, pressure, or bending when collecting swab samples as this may result in accidental breakage of the swab shaft.
3. Replace cap on the tube and secure tightly. The action of screwing the cap onto the tube moves the end of the broken swab shaft into a funnel shaped molded docking receptacle in the cap. This molded funnel shape captures the end of the broken applicator shaft and secures it firmly in the dock by friction grip.
4. Write patient information on the tube label or apply patient identification label.

Urine (male or female specimens)

1. Each urine specimen is patient collected (first 20–30 ml of the urine stream), and then transferred to the clinician.
2. Aseptically unscrew and remove the cap from the tube filled with 2 ml eNAT medium. Using the pipet provided with the kit, transfer 4 ml of the urine specimen into the eNAT tube in two separate 2 ml transfer steps.

   Note: When transferring the urine specimen, the operator must not touch below the squeeze bulb on the transfer pipet, as this will lead to contamination of the pipet thus invalidating the test results.
3. Replace cap on the tube and secure tightly.
4. Write patient information on the tube label or apply patient identification label.

Sample storage

Specimens in eNAT, including time needed for transport, are stable at 4–22°C (± 2°C) for up to 4 weeks for the *artus* T. vaginalis QS-RGQ test.

Sample transport

Within 1 week of specimen collection, ship specimens in shatterproof transport. Ship the labeled swab and urine specimens according to legal instructions for the transport of pathogen material.*

Sample preparation

1. Mix each specimen in the eNAT tube thoroughly with a vortex mixer for 10–15 seconds at high speed.

   Note: If a vortex mixer is not available, invert the eNAT tubes for 20 times to mix the specimen.

* International Air Transport Association. Dangerous Goods Regulations.

2. Place the eNAT (urine or swab) tubes into a QIAsymphony SP tube carrier with a 1A tube insert.
3. Carefully remove and discard each specimen cap (urine) or cap and swab (vaginal or endocervical, using the tube cap as a handle) for each sample loaded into the tube carrier, and then load it into the QIAsymphony SP as described in "Protocol: DNA isolation and assay setup on the QIAsymphony SP/AS", page 24.

   Note: When the eNAT tube cap is unscrewed and removed, the swab applicator stick is securely attached to the cap. This feature allows the operator to conveniently remove the swab using the tube cap as a handle to hold and manipulate the swab.
4. After the Protocol DNA isolation and assay setup on the QIAsymphony SP/AS is finished, reclose the eNAT tube with a fresh cap, 12 mm and keep at 4°C in case a retest is needed (see "Interpretation of Results", page 44).

Detection of *T. vaginalis*-specific DNA

Table 1. General information

Kit	***artus* T. vaginalis QS-RGQ Kit (cat. no. 4751366)**
Sample material	Human urine, human vaginal swab or human endocervical swab collected in eNAT tube, prefilled with 2 ml eNAT medium
Front-end purification	QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	2000 µl for vaginal or endocervical specimens 6000 µl for urine specimens 1500 µl for positive control
Assay parameter set	artus_T.vag_swab/urine_V1
Default assay control set	Complex_T.vaginalis_V1
Elution volume	60 µl
QIAsymphony software version	Version 4.0 or higher
QIAsymphony SP/AS configuration profile	Default profile 1
Master mix volume	25 µl
Template volume	15 µl
Number of reactions	24–72* (including all controls)
Runtime on QIAsymphony SP/AS module	Approximately 105 minutes for 24 reactions Approximately 305 minutes for 72 reactions
Runtime on Rotor-Gene Q instrument	Approximately 100 minutes

* Ensure that the limit of 72 reactions and 1 assay rack adaptor is not exceeded. Avoid extended incubation time (>30 minutes) between completion of the assay run and transfer to the Rotor-Gene Q instrument.

Controls

Positive control

The T. vaginalis Positive Control (supplied with the *artus* T. vaginalis QS-RGQ Kit) monitors the efficiency of sample preparation and the downstream PCR amplification. This positive control is loaded onto QIAsymphony SP before DNA purification (see page 31 for further details on loading the positive control).

Negative control

The T. vaginalis Negative Control (called "NTC", for "no template control", in the QIAsymphony software) is loaded onto QIAsymphony AS before amplification in place of an extracted sample and monitors the PCR for contamination (see page 31 for further details on loading the negative control).

Specimen process controls

Positive and negative control strains should be routinely tested in each laboratory according to the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations for monitoring total process performance. The Positive Specimen Process Control (PSPC) is intended to monitor the entire process. The Negative Specimen Process Control (NSPC) detects reagent or environmental contamination by *T. vaginalis* DNA. It is recommended that a negative urine or vaginal specimen inoculated with approximately 1×10^3 trichomonads/ml *T. vaginalis* (e.g., American Type Culture Collection, ATCC® 30001) or a well characterized clinical isolate of *T. vaginalis* is used as a Positive Specimen Process Control while a negative urine or vaginal specimen inoculated with approximately 1×10^5 trichomonads/ml *Pentatrichomonas hominis* (e.g., ATCC 30000) or any other non-*T. vaginalis* organism is used as a Negative Specimen Process Control. Using an eNAT Collection Kit, each PSPC and NSPC sample should be transferred to a labeled eNAT tube with a FLOQswab for vaginal / endocervical samples, or the pipet for urine samples, before loading them into the tube carrier of QIAsymphony SP. Speciment process controls should

be tested on the QIASymphony in the same wat as test samples. For further details on loading test samples, see page 33.

Preparation of carrier RNA and internal control (T. vaginalis IC)

Using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* T. vaginalis QS-RGQ Kit requires introduction of the internal control (T. vaginalis IC) into the purification procedure to monitor the efficiency of sample preparation and the downstream assay.

The internal control (T. vaginalis IC), supplied with the *artus* T. vaginalis QS-RGQ Kit, must be added to the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture. The total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture is 120 µl per sample.

To prepare the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, add 1350 µl Buffer AVE (AVE), supplied with the QIAsymphony DSP Virus/Pathogen Midi Kit, to resuspend the lyophilized carrier RNA (CARRIER). Invert the tube 20 times to mix.

For internal control (IC) calculation, the "IC Calculator" within the QIAsymphony Management Console (QMC) should be used.

Table 2 represents the preparation of internal control per sample at a ratio of 0.1 µl per 1 µl elution volume. We recommend preparing fresh mixtures for each run just before use.

Table 2. Preparation of carrier RNA and internal control (T. vaginalis IC)

	Reactions	
Component	**Volume (µl) for n≤13 in Sarstedt tubes***	**Volume (µl) for n>13 in BD tubes†**
Stock carrier RNA (CARRIER)	(n + 3) × 3	(n + 5) × 3
Internal Control (T. vaginalis Internal Control)	(n + 3) × 9	(n + 5) × 9
Buffer AVE	(n + 3) × 108	(n + 5) × 108
Final volume per sample (excluding dead volume)	120	120
Total volume for n samples	(n + 3) × 120	(n + 5) × 120

* Microtubes 2.0 ml Type H and Microtubes 2.0 ml Type I (Sarstedt, cat. no. 72.693 and 72.694). If preparing IC as a stock solution in a larger tube, multiply the total volume of each component by the number of IC tubes used. Internal control mixture corresponding to 3 additional samples (i.e., 360 µl) is required. Do not fill more than 1.92 ml total volume (corresponding to a maximum of 13 samples). If using more than 13 reactions in 2.0 ml microtubes, set up the reactions in a larger tube and load in multiple tubes. Make sure that for each tube the required excess volume of 3 additional reactions is added.

† Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Corning, cat. no. 352051. BD was the previous supplier of this tube; Corning, Inc. is the new supplier). Internal control mixture corresponding to 5 additional samples (i.e., 600 µl) is required. Do not fill more than 13.92 ml total volume (corresponding to a maximum of 111 samples).

Calculation of mixture by "IC Calculator"

1. Open the QMC.
2. Select the IC Calculator icon.
3. Select "Complex _T.vaginalis_V1" from the ACS drop-down list.
4. Enter the required number of samples.
5. Select the labware used for the IC.
6. Select an elution volume of 60 µl.
7. Select "Internal Control/Eluate" and "0.1 µl" for internal control mode.
8. Press "Calculate" to start calculation of IC mixture.

The IC calculator displays the different volumes of reagents to be mixed for the internal control mixture and the tube type to be used on the right side of the screen.

Assay control sets and assay parameter sets

Assay control sets are the combination of a protocol plus additional parameters, such as internal control, for sample purification on the QIAsymphony SP. A default assay control set is preinstalled for each protocol.

Assay parameter sets are the combination of an assay definition with additional parameters defined, such as replicate count and number of assay standards, for assay setup on the QIAsymphony AS.

For the integrated run on the QIAsymphony SP/AS, the assay parameter set, artus_T.vag swab/urine_V1, is directly linked to the upfront assay control set, Complex_T.vaginalis_V1, specifying the associated sample purification process.

Protocol: DNA isolation and assay setup on the QIAsymphony SP/AS

Important points before starting

- Make sure that you are familiar with operating the QIAsymphony SP/AS instruments. Refer to the user manual supplied with your instrument and the most current versions available online at www.qiagen.com/products/qiasymphonyrgq.aspx for operating instructions.
- Download the Application Package from "Protocol Files" on the "Resources" tab of the *artus* T. vaginalis QS-RGQ Kit web catalog at www.qiagen.com/products/artustvaginalisqsrgqkitce.
- Before using a reagent cartridge (RC) for the first time, make sure that Buffers QSL2 and QSB1 in the RC do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the RC and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the RC is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete RC for 30 minutes at 37°C with occasional shaking in a water bath.* Allow the reagents to cool down to room temperature (15–25°C).
- Check that Buffer ATL (ATL) does not contain a precipitate. If a precipitate has formed, dissolve by heating the buffer at 70°C with gentle agitation in a water bath.†† Aspirate bubbles from the surface, and let the buffer cool to room temperature (15–25°C).
- Avoid vigorous shaking of the reagent cartridge (RC) and Buffer ATL (ATL) bottle. Otherwise foam may be generated, which can lead to liquid-level detection problems.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- The reagent volumes are optimized for 3 batches of 24 reactions per kit per run.
- Make sure that eluates from the sample preparation and all components of the *artus* T. vaginalis QS-RGQ Kit remain on the QIAsymphony SP/AS for no more than the normal time required for sample purification and assay setup for 72 samples, including

* Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

up to 30 minutes transfer time from the QIAsymphony AS to the Rotor-Gene Q instrument.

Note: Do not use an Elution Microtubes CL rack that has already been used on a different QIAsymphony SP instrument. Do not enter a rack ID manually.

Things to do before starting

- Before each use, all assay reagents in the *artus* T. vaginalis QS-RGQ Kit need to be thawed completely, mixed by repeated up and down pipetting or by quick vortexing, and centrifuged for at least 3 seconds. Avoid bubbling or foaming of the reagents.
- Prepare all required mixtures. Prepare mixtures containing RNA (CARRIER) and internal controls immediately prior to starting. For more information, see "Preparation of carrier RNA and internal control (T. vaginalis IC)", page 21.
- Before starting an integrated run, make sure that all instruments are clean and that the replaceable parts have been loaded (e.g., tip guards) as described in the maintenance instructions in the *QIAsymphony SP/AS User Manual — General Description*, *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP*, *QIAsymphony SP/AS User Manual — Operating the QIAsymphony AS*, and *QIAsymphony Management Console User Manual* supplied. Make sure to perform maintenance regularly to minimize the risk of cross-contamination.
- Make sure that QIAsymphony process profile "Default Profile 1" is active. The selected profile is shown at the bottom, right corner of the touchscreen. The profile may be changed in the "Configuration" menu of the "Tools" tab by a user logged in as "Supervisor".

Procedure

Preparing the "Waste" drawer

1. Close all drawers and the hoods of the QIAsymphony SP/AS module.
2. Switch on the instrument. Wait until the "Sample Preparation" screen appears and the initialization procedure has finished.

   Note: The power switch is located at the bottom, left corner of the QIAsymphony SP.
3. Log in to the QIAsymphony.
4. Prepare the "Waste" drawer of the QIAsymphony SP module.
5. Open the "Waste" drawer.
6. Empty and install the liquid waste bottle. Make sure to remove the lid before placing the liquid waste bottle into the drawer.
7. Insert the tip chute.

   Note: Different tip chutes must be used for benchtop and QIAsymphony Cabinet SP/AS operation.
8. Insert the tip park station.
9. Insert the empty unit boxes (see Table 3 and Figure 1). Make sure that there is at least one empty unit box in slot 4 (closest to you).
10. Install an empty tip disposal bag.

    Note: The empty tip disposal bag is installed below the waste drawer for benchtop operation or in the waste bin for QIAsymphony Cabinet SP/AS operation.
11. Close the "Waste" drawer and perform an inventory scan.

Table 3. Required plastic ware for 1–3 sample batches

	1 batch, 24 samples	2 batches, 48 samples	3 batches, 72 samples
Empty unit boxes	2	3	4

Figure 1. Position of unit boxes.

Loading the "Eluate" drawer

1. Place the Elution Microtubes Rack QS adapter onto the transfer frame.
2. Open the "Eluate" drawer.
3. Place the adapter and transfer frame onto slot 1 of the "Eluate" drawer.
4. Select "Elution Slot 1" on the touchscreen.
5. Remove the bottom from a new Elution Microtubes CL rack by twisting the rack until the bottom comes out.
6. Scan the bar code on the Elution Microtubes CL rack using the handheld bar code scanner.

7. Insert the Elution Microtubes CL rack in the adapter on “Elution Slot 1”.
8. Remove the lid of the Elution Microtubes CL rack.
9. Close the “Eluate” drawer.
10. Press “OK”.
11. Wait until the scan has finished.

Preparing the reagent cartridge (RC)

1. Place the reagent cartridge (RC) in the gray reagent cartridge holder.
2. Remove the trough containing the magnetic particles from the reagent cartridge (RC).
3. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes to resuspend the magnetic particles.
4. Place the trough containing the magnetic particles back in to the reagent cartridge (RC).
5. Remove the cover from the trough containing the magnetic particles.
6. Remove the caps from the enzyme tubes, and place the caps of the enzyme tubes onto the cap holders on the gray reagent cartridge holder.
7. Make sure the enzyme tubes do not contain air bubbles. If air bubbles are present, aspirate the bubbles from the surface.
8. Mount the enzyme rack (ER) on the reagent cartridge (RC).
9. Mount the piercing lid (PL) onto the reagent cartridge (RC) and gently click into place.

Loading the "Reagents and Consumables" drawer

1. Open the "Reagents and Consumables" drawer.
2. Place prepared reagent cartridge(s) (RC) onto position RC 1 and/or RC 2. One new reagent cartridge (RC) is sufficient for up to 48 samples.
3. Close the "Reagents and Consumables" drawer.
4. Press the "R+C" button on the touchscreen.
5. Press the "Bottle ID" button.
6. Press the text field and scan the bar code of the Buffer ATL (ATL) bottle using the handheld bar code scanner.

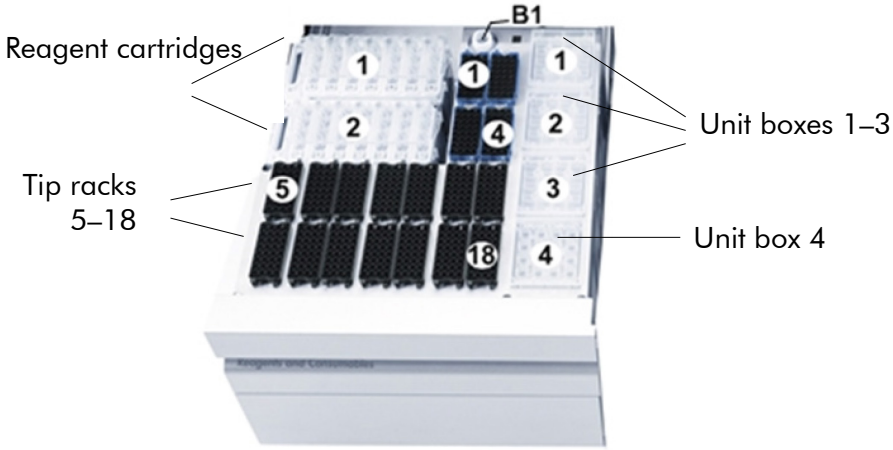

Figure 2. Position of the reagents and consumables on the QIAsymphony SP.

7. Open the bottle of Buffer ATL (ATL) and make sure that it does not contain a precipitate. If Buffer ATL (ATL) contains a precipitate, follow the instructions on page 24.
8. Place the bottle of Buffer ATL (ATL) into position B1.

   Note: Position B1 is next to the reagent cartridge slot 1 (RC 1).

Note: Try to avoid vigorous shaking of the buffer bottle otherwise foam may be generated. This can lead to liquid-level detection problems.

9. Load sufficient racks of disposable 200 µl filter-tips in tip rack holder positions 1–4 (see Table 4, page 31). Make sure to click all racks into place.

   Note: There are 32 filter-tips per tip rack.

10. Load sufficient racks of 1500 µl disposable filter-tips in tip rack holder positions 5–18 (see Table 4, page 31). Make sure to click all racks into place.

    Note: There are 32 filter-tips per tip rack.

    Recommendation: Load more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

11. Remove the lid of the sample prep cartridges and load sufficient sample prep cartridges in unit box holder positions 1–3 (see Table 4, page 31).

    Note: There are 28 sample prep cartridges per unit box.

    IMPORTANT: Plastic consumables may shift during transit or storage. Check that all plastics are aligned properly inside the unit box before loading on the QIAsymphony SP.

12. Remove the lid of the 8-Rod Covers and load sufficient 8-Rod Covers in unit box holder position 4 (see Table 4, page 31).

    Note: There are twelve 8-Rod Covers per unit box.

    IMPORTANT: Plastic consumables may shift during transit or storage. Check that all plastics are aligned properly inside the unit box before loading on the QIAsymphony SP.

13. Press "OK" in the consumables screen.

14. Close the "Reagents and Consumables" drawer and perform an inventory scan.

Table 4. Required plastic ware for sample batches

	1 batch, 24 samples*	2 batches, 48 samples*	3 batches, 72 samples*
Disposable filter-tips, 200 µl*†	34 (2 racks)	60 (2 racks)	86 (3 racks)
Disposable filter-tips, 1500 µl*†	123 (4 racks)	205 (7 racks)	295 (10 racks)
Sample prep cartridges	18 (1 unit box)	36 (2 unit boxes)	54 (2 unit boxes)
8-rod covers	3 (1 unit box)	6 (1 unit box)	9 (1 unit box)

* Performing more than one inventory scan requires additional disposable filter-tips.

† The number of required filter-tips includes filter-tips for one inventory scan per reagent cartridge.

Load the tube carrier with controls

1. Using an empty eNAT tube, 12 x 80 mm containing no transport medium, pipet 1.5 ml of the T. vaginalis Positive Control supplied with the *artus* T. vaginalis QS-RGQ Kit.

   IMPORTANT: Make sure the eNAT tube used in this step is empty and does not contain any eNAT transport medium.

2. Place the tube with the T. vaginalis Positive Control in position 1 of the first sample carrier.

   IMPORTANT: Make sure to load the positive control in the correct position. Rotor-Gene AssayManager will not import the result file if the positive control is placed in any other position. Do not load the positive control into additional carriers for the same AS batch.

   Note: The position of samples and controls on the assay rack can be displayed before the start of the run. After creation of the AS batch (page 34), press the “Assays” drawer button on the touch screen and select the respective “Assay” slot. The sample type of each position will be displayed (“Type”), if the toggle button “Sample” is pressed.

3. Load the tube carrier with specimen process controls if used in the run (see page 33 for more information).
4. Open an eNAT Collection Kit for each specimen process control. Take a known T. vaginalis Positive Specimen Process Control (PSPC) or a known T. vaginalis Negative Specimen Process Control (NSPC) and transfer the PSPC or NSPC into a tube filled with 2 ml eNAT solution.
5. Load the specimen process controls for each swab type acccording step a or b as follows:

   5a. For each swab (vaginal/endocervical) specimen process control (PSPC and NSPC), unscrew and remove the cap from the eNAT tube. Then one at a time transfer ~0.1 ml of the PSPC or NSPC specimen into each tube using the sterile swab to absorb (~0.1 ml) and transfer the sample into the eNAT tube, and then bend the swab shaft at the breakpoint against the tube to break the shaft and replace the cap. Invert the tubes or vortex to homogenize the specimen processing controls in the eNAT solution. Unscrew the eNAT tube cap and remove the swab applicator stick, which is securely attached to the cap, before loading the tubes into the QIAsymphony SP tube carrier.

   5b. For each urine specimen process control (PSPC and NSPC), unscrew and remove the cap from the eNAT tube. Then one at a time transfer 4 ml (2 ml × 2 transfer steps) of the PSPC or NSPC specimen into each eNAT tube using the pipet, and then replace the cap. Invert the tubes or vortex to homogenize the specimen processing controls in the eNAT solution. Unscrew and remove the eNAT tube cap before load the tubes into the QIAsymphony SP tube carrier.
6. Place the tube containing the known Positive Specimen Process Control (PSPC) into the next available position, for example, position 2 of the first sample carrier.
7. Place the tube containing the known Negative Specimen Process Control (NSPC) into the next available position, for example position 3 of the first sample carrier.

   Note: The specimen process controls will be analyzed as regular samples. Althought the results will be reported for each PSPC and NSPC, wrong result will not automatically

invalidate the run by Rotor Gene AssayManager. The results of all full process controls require user interpretation**.**

Note: If the sample run contains vaginal/endocervical specimens and urine specimens, PSPC and NSPC representating all specimen types may be included. The additional full process controls can be placed into the next available positions, for example position 4 and 5 of the first sample carrier.

Load the "Sample" drawer with the samples

1. Load prepared samples (see page 17) in eNAT tubes in the sample tube carrier already containing the controls.
2. If required, prepare further sample tube carriers in the same way, but without controls. Do not add further controls to sample tube carriers to be combined in the same AS batch.

   Note: If samples contain bar codes, orient samples in the tube carrier so that the bar codes are completely visible.
3. Check that sample and control tubes are correctly loaded and clicked into place.
4. Insert all sample carriers in "Sample" drawer slots 1–4. (The LED light turns orange if loaded correctly.)

   Note: First load the sample tube carrier containing the controls and samples into slot 1. Do not load more than 71 samples and controls in one run. A negative control (NTC), which must be loaded on the QIAsymphony AS, will result in one additional reaction and therefore requires one output position.
5. Using the "Integrated run" setup on the QIAsymphony touchscreen, enter the required information for each batch of samples to be processed.
6. Press the "Integrated Run" tab on the touchscreen.
7. Press "Define run".
8. Select "SP Batch 1" (or appropriate batch number of sample carrier with "Full Process Controls", if performing continuous loading).

9. Press “Edit samples”.

   Note: Make sure that the correct labware “COP#606C eNAT Tube” is assigned to the samples. If necessary, correct the labware assignment.

10. Press “ID/Type”.
11. Select the first position and press “Sample ID”.
12. Press the text field and enter T. vaginalis Positive Control, then press “OK”.
13. Select the first position and press “EC+”.
14. If necessary, resolve any bar code errors for sample and insert IDs.
15. Press “OK”.

    IMPORTANT: Do not assign the Sample Type “EC+” to tubes other than the positive control supplied with the *artus* T. vaginalis QS-RGQ Kit. Rotor-Gene AssayManager will reject runs with incorrect control patterns. Do not assign the Sample Type “EC+” to the Positive Specimen Process Control (PSPC). Do not assign the Sample Type “EC–” to the Negative Specimen Process Control (NSPC). Make sure to have the Sample Type “Sample” assigned to PSPC and NSPC.

16. Define the assay(s) to run.
17. Press the corresponding “SP Batch” button.
18. Press “Define assays”.
19. Select the samples to be processed with the assay.
20. Select the assay “artus_T.vag swab/urine_V1” under the category “artus QS-RGQ”.
21. Press “OK”.
22. Repeat step 17 for all batches and samples to be processed.
23. Define the QIAsymphony AS batch.
24. Select all batches that should be processed in one integrated QIAsymphony RGQ run.
25. Press “Create AS batch”.

Note: All QIAsymphony SP batches assigned to the same QIAsymphony AS batch (integrated QIAsymphony RGQ run) will be processed in the same assay setup procedure.

26. Press "OK" to queue the run.
27. Load the "Sample" drawer with the IC mixture.
28. Place the previously prepared tube(s) of IC mixture (see page 20) into the sample carrier (use Tube Insert 3B for 2 ml microtubes).
29. Insert sample carrier in slot A of the "Sample" drawer.

    Note: For certain liquid levels in unlabeled 14 ml tubes (see "Reagents and consumables for QIAsymphony SP", page 11) scan errors can occur due to the clear liquid and tube. To avoid this, attach a blank label to the tube or mark the tube area facing the barcode scanner with a permanent marker.
30. Define the IC positions.
31. Press the "Define ICs" button.
32. Select the positions of the IC mixture.
33. Select the corresponding IC "Complex_T.vaginalis_V1" from the folder "Required".
34. Make sure that the correct labware is assigned. If not, correct labware assignment by pressing "IC Tubes".
35. Press "OK".
36. Start the run.
37. To start the run press the "Run" button.
38. Read and confirm the message that appears.
39. We recommend waiting beside the instrument until it has performed liquid level detection of the IC tubes (QIAsymphony SP carrier status changes to "running").

    IMPORTANT: Do not pause or stop the run during processing (unless an emergency occurs), as this will lead to the respective samples and assay reactions being flagged as "unclear". Rotor-Gene AssayManager will invalidate "unclear" assay reactions.

Note: It is possible to continuously load samples and add them to this run (until reagents are loaded) or to a new QIAsymphony RGQ run.

Loading the QIAsymphony AS drawers for assay setup

1. Install an empty tip disposal bag and tip chutes.
2. Install an empty tip disposal bag below the "Waste" drawer for benchtop operation or in the waste bin for QIAsymphony SP/AS Cabinet operation.
3. Open the "Eluate and Reagents" drawer and the "Assays" drawer of QIAsymphony AS.
4. Open the hood and insert the tip chute inside the instrument.

   Note: Different tip chutes must be used for benchtop and QIAsymphony Cabinet SP/AS operation.
5. Close hood, and read and confirm message.
6. Load "Assays" drawer with assay rack.
7. Press slot 5 "Assay" (yellow).
8. Fill the required number of strip tubes (4 tubes = 1 segment) in a pre-cooled Rotor-Gene Strip Tubes 72 QS cooling adapter as indicated on the touchscreen.

   Note: Load complete strip tubes. Do not break strip tubes.
9. Load adapter with strip tubes on slot 5 of the "Assays" drawer.
10. Press "Rack ID" on the touchscreen, enter a user-defined rack ID, and press "OK".

    Note: It is also possible to use the automatic ID function.
11. Press "Load".
12. Load "Assays" and "Eluate and Reagents" drawer with filter-tips.
13. Load at least the number of filter-tips provided in the "Assay Setup | Loading Information" screen.

    Note: We recommend loading more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling. Use tip rack positions near the cooling slots in both QIAsymphony AS drawers only.

14. Load “Eluate and Reagents” drawer with reagents.
15. Before each use, all assay reagents need to be thawed completely, mixed, and centrifuged for at least 3 seconds. Avoid bubbling or foaming of the reagents (see procedure described in “Important points before starting”, page 24).
16. Press slot 3 “Reagent” (yellow) on the touchscreen.
17. Prepare a precooled reagent holder as requested on the touchscreen.
18. Select the tube positions on the touchscreen, load an empty tube for the master mix, and fill at least the required volume of the correct reagents and Negative Control (NTC) in the required tubes in the corresponding positions as indicated on the touchscreen.

    Note: It may be necessary to combine the same reagent types (T. vaginalis Master or Mg-Sol) into one tube if required volume exceeds filling volume of the corresponding reagents. One tube each of the T. vaginalis Master and Mg-Sol is sufficient for 24 QIAsymphony SP eluates (including controls) plus one NTC.

    Note: Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the T. vaginalis Master into the respective tube.

    Note: Alternatively, select “List View” on the touchscreen and prepare the reagent adapter accordingly. A “Loading Information File” can also be downloaded via the QMC or USB port (and printed) after the QIAsymphony AS batch is defined and queued.
19. Press the “Scan Kit Barcode” button on the touchscreen and press the light-blue kit bar code line.
20. Press the text field and scan the kit bar code on the upper side of the *artus* T. vaginalis QS-RGQ Kit using the handheld bar code scanner.

    IMPORTANT: If the kit bar code is not scanned at this step, the Rotor-Gene AssayManager will reject the QIAsymphony AS result file during import.
21. Load the prepared reagent adapter onto slot 3 of the “Eluate and Reagents” drawer.
22. Press the “Load” button.
23. Close both drawers.
24. Press “Scan” to enter the scan dialog.

25. Press “Scan” to perform an inventory scan of all QIAsymphony AS components.

    Note: We recommend waiting beside the instrument until the scan is completed.

26. Assay setup will start automatically when sample preparation on the QIAsymphony SP has finished.

27. Check the time for the end of the QIAsymphony AS batch to remove assay rack.

28. After the QIAsymphony AS scan has finished, the calculated integrated run time is shown on the “Integrated Run Overview” screen. The maximum time permitted from the end of the QIAsymphony AS run until the start of the Rotor-Gene Q run is 30 minutes. Make sure to transfer the assay rack to the Rotor-Gene Q instrument within 30 minutes of the assay run finishing.

Removal of assay rack and transfer of result file

1. Remove the QIAsymphony AS batch and the assay rack.
2. Open the “Assays” and the “Eluate and Reagents” drawers.
3. Remove the adapter with the strip tubes and close the tubes with the appropriate caps.
4. Press slot 5 “Assay”.
5. Press the “Remove” button.
6. Remove the reagents adapter and discard the reagents according to your local safety regulations.
7. Press slot 3 “Reagent”.
8. Press the “Remove” button.
9. Close the “Assays” and the “Eluate and Reagents” drawers.
10. Press “Scan” to enter the scan dialog.
11. Press “Scan” to perform an inventory scan for adapters on the left and right drawers (typically preselected).
12. Press the “Integrated Batch” button (green) to remove the integrated run.
13. Read and confirm the message.

14. The final QIAsymphony AS result file is created and can be transferred to either a USB stick or to a defined folder (\log\Results\AS) via the QMC.

15. Transfer the result file to a defined folder. To transfer the result file using the USB stick, follow step 15a. To transfer the result file using the QMC, follow step 15b.

    15a. Transfer result file using the USB stick.

    I. Insert the USB stick.
    II. Select "Tools".
    III. Select "File Transfer".
    IV. Select "Result Files" in the "Save to USB Stick" column.
    V. Press the "Transfer" button.
    VI. Read and confirm the message.
    VII. After successful transfer, press "OK" and remove the USB stick.
    VIII. Proceed to "Protocol: PCR on the Rotor-Gene Q instrument", page 40

    15b. Transfer result file using the QMC.

    I. Log in to the correct QIAsymphony SP/AS
    II. Select the transfer file icon.
    III. Choose file format "Result File AS".
    IV. Select result file with the correct time stamp and batch ID from the list of "Remote Site" files (right column).
    V. Transfer result file to the "Local Site" (the file is saved under the path defined in "Tools", "Options", "File Transfer", under \log\Results\AS).
    VI. Proceed to "Protocol: PCR on the Rotor-Gene Q instrument", page 40.

Note: If multiple batches on the QIAsymphony AS are configured in an integrated run, check the tip disposal bag for remaining capacity, and reload the QIAsymphony AS drawers, starting at step 1 in the instructions for Loading the QIAsymphony AS drawers for assay setup.

Note: We recommend marking the strip tube caps to ensure correct positioning and to use a cooled transport frame to avoid contamination.

Note: Perform daily, weekly, and annual preventive maintenance as described in the *QIAsymphony SP/AS User Manual — General Description*.

Protocol: PCR on the Rotor-Gene Q instrument

Important points before starting

- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. See the specific user manual for your instrument for details.
- The *artus* T. vaginalis QS-RGQ Kit must be run on the Rotor-Gene Q instrument using automated interpretation of results with Rotor-Gene AssayManager. The cycling parameters are locked for the run.
- After installing the plug-in and importing the assay profile (see "Things to do before starting" below), the Rotor-Gene AssayManager can use the information given in the QIAsymphony AS result file to set up a run for real-time PCR amplification and subsequent automated interpretation of results.

Things to do before starting

- For system-wide process safety, it is necessary to ensure that the following settings for the closed mode are activated on the Rotor-Gene AssayManager: "Material number required", "Valid expiry date required", and "Lot number required" (Under "Configuration", "Settings", "Global Settings", "Work List". User role "Administrator" is required to access "Configuration").
- For automated interpretation of results using the *artus* T. vaginalis QS RGQ Kit with Rotor-Gene AssayManager, the latest Epsilon Plug-in must be installed to your Rotor-Gene AssayManager. Start the installation process for the Plug-in by by double-clicking on the msi installer file and then following the installation instructions. For a detailed description refer to "Installing Plug-ins" (see the *Rotor-Gene AssayManager Core Application User Manual* supplied).

- To use the *artus* T. vaginalis QS-RGQ Kit, the file AP_artus_Tvag_swab_urine800_QS_V1_0_x.iap (with x ≥0) must be imported to Rotor-Gene AssayManager. To import the assay profile into Rotor-Gene AssayManager, navigate to the "Configuration Environment" and change to the "Assay Profile" tab. Click on "Import" and select the AP_artus_Tvag_swab_urine800_QS_V1_0_x.iap file in the open file dialog. Click on "Open", and the assay profile is loaded and added to the list of available assay profiles.

  Note: The same version of an assay profile cannot be imported twice.

Procedure

1. To prepare the rotor and start the run on the Rotor-Gene Q instrument, first place a 72-Well Rotor on the Rotor Holder.
2. Fill the rotor with strip tubes. Make sure to start at position 1 and to fill the strip tubes in the correct orientation.
3. Check the negative control (NTC) visually by eye to confirm that transfer of the NTC was performed correctly (last strip tube position of QIAsymphony RGQ run).
4. Use empty capped strip tubes to fill all unused positions.
5. Attach the locking ring.
6. Load the Rotor-Gene Q instrument with the rotor and locking ring.
7. If using a USB stick for data transfer directly from the QIAsymphony SP/AS, unzip the result file from the QIAsymphony AS. The result files are stored under log\Results\AS.

   Note: On most computers, files can be unzipped by right-clicking the file and then clicking "Extract" in the menu that opens. Files must be unzipped in order to be imported into Rotor-Gene AssayManager.
8. Start the Rotor-Gene AssayManager.
9. Log in to the closed mode.
10. Select the "Setup" environment, if not already preselected.

11. Import the QIAsymphony AS result file at the bottom of the screen. Select the source "QIAsymphony" as "Import type".
12. In the "Select file" dialog, open the corresponding QIAsymphony AS result file and click "Open".
13. Read and confirm the message.
14. After successful import, select the corresponding work list from the work list manager list and click the "Apply" button.
15. Enter an experiment name.
16. Select the cycler to be used in the "Cycler selection" dialog.
17. Check correct attachment of locking ring and confirm on the screen that the locking ring is attached.
18. Close the Rotor-Gene Q instrument lid.
19. Click the "Start run" button.

    Note: If using multiple cycler runs, change to the corresponding cycler environment to see the progress of the run.
20. When the run is finished, click "Finish run…".
21. For users logged in with the Operator role: Click "Release".
22. For users logged in with the Approver role: Click "Release and go to approval".
23. Release and report results.
24. If you have used "Release" before, select the "Approval" environment.
25. Press "Apply filter" (or choose own filter options beforehand).
26. Select experiment.
27. Click "Start approval".
28. Approve the results of each test sample. Use the "Accepted" button for test samples whose results analyzed by Rotor-Gene AssayManager you agree with. Use the "Rejected" button if the test sample result evaluated by Rotor-Gene AssayManager is not acceptable for any reason.

Note: A result automatically set to "INVALID" by Rotor-Gene AssayManager cannot be converted to a valid result anymore, even if the result is rejected.

29. Optional: Enter assay comments or sample comments.
30. Click "Release / report data…".
31. Click "OK". The report will be generated and stored automatically.

    Note: The user needs approval rights to approve a run.

32. Unload the Rotor-Gene Q instrument and discard the strip tubes according to your local safety regulations.
33. Perform maintenance.

    When all QIAsymphony AS batches of the integrated QIAsymphony SP/AS run have finished, perform the regular maintenance as described in the *QIAsymphony SP/AS User Manual — General Description*. This can be done during the Rotor-Gene Q instrument run.

    Note: This can be performed at any time before the start of the next integrated run as part of a regular maintenance schedule, according to local regulations or priorities. Perform daily, weekly, and annual preventive maintenance as described in the *QIAsymphony SP/AS User Manual — General Description*.

Interpretation of Results

This section describes interpretation of results on the Rotor-Gene Q instrument. Review also the sample status information from the QIAsymphony SP/AS result files for analysis of the complete sample-to-result workflow.

Note: Only samples with a valid status should be used.

The *artus* T. vaginalis QS-RGQ Kit Assay Profile contains rules for interpreting the assay results automatically.

Every sample and control displays an independent result for each target* of *T. vaginalis* (T.vag), and Internal Control (IC/IC_Control). Each result is reported as "Signal detected", "No signal", or "INVALID".

Positive/negative control results:

- All targets for the Positive Control (EC+) and Negative Control (NTC) must be valid to confirm that the assay status is successful and the test results may be reported. If any target of the Positive Control or Negative Control is invalid, results for every sample in the run will display "INVALID". The entire assay run must be retested.
- The Positive Control (EC+) must report a "Signal detected" result for *T. vaginalis* and the Internal Control.
- The Negative Control (NTC) must report "No signal" for both *T. vaginalis* and the Internal Control.

* All targets relating to samples and controls are displayed in separate rows in the column "Output" in the Rotor-Gene AssayManager "Approval" and "Archive" environment and in the report.

Positive Specimen Process Control (PSPC)/Negative Specimen Process Control (NSPC) results:

The PSPC and NSPC are not included, but required, controls (see "Specimen process controls", page 20). Therefore the *artus* T. vaginalis QS-RGQ Kit Assay Profile does not contain rules for automatic analysis of the PSPC and NSPC. The results of the PSPC and NSPC must be checked manually by the user.

- The PSPC must report "Signal detected" for the *T. vaginalis* target.
- The NSPC must report "No signal" for the *T. vaginalis* target and "Signal detected" for the Internal Control target.

Note: If the status for either process control is different from the status stated above, the entire assay run should be treated as invalid and must be retested.

Sample results:

- See Table 5 for a summary of results interpretation.
- A sample is considered positive for *T. vaginalis* if the result for target *T. vaginalis* is reported as "Signal detected" (scenario A).
- The Internal Control target result may be reported as "No signal" in samples where *T. vaginalis* signal is detected. In these cases all targets for the sample will be reported. No retesting is necessary (scenario A).

  Note: It is expected that in some positive *T. vaginalis* samples the Internal Control PCR may be inhibited due to competition from amplifying *T. vaginalis*, which will cause a "No signal" target result for the Internal Control (scenario A).
- A sample is considered negative for *T. vaginalis* if the result for target *T. vaginalis* is reported as "No signal" and the result for the target Internal Control is reported as "Signal detected" (scenario B).

- The Internal Control signal must be detected in samples where no *T. vaginalis* signal is detected (scenario B). If the Internal Control signal is not detected or is "INVALID" in samples where no *T. vaginalis* signal is detected, all target results for the sample will be reported as "INVALID". The sample must be retested (scenario C).
- If the target result for *T. vaginalis* is reported as "INVALID", the sample must be retested (scenario C).

Table 5. Result interpretation

Scenario	Target result		*T. vaginalis* detection in the sample
	T. vaginalis	Internal Control	
A	Signal detected	Signal detected/no signal	Yes
B	No signal	Signal detected	No
C	INVALID	INVALID	Error/retest sample*

* Repeat the QIAsymphony RGQ run with new specimens or from specimens already collected and handled according to the instructions described on page 16. If specimens have already been processed once on the QIAsymphony RGQ, make sure that the eNAT collection tube still contains at least 1050 µl of liquid

Targets reported as "INVALID" will be provided with one or more flags which explain why this target is invalid. The automated analysis may provide the following corresponding flags, see Table 6.

Table 6. Flags assigned during the automated analysis

Flag	Behavior	Description
ASSAY_INVALID	Invalid	Assay is set to invalid because at least one external control is invalid.
AUDAS_CONFLICT	Invalid	Results from the automatic data scan (AUDAS) are in conflict with results from the core analysis. An unambiguous automatic assessment of data validity is not possible.
CT_ABOVE_ ACCEPTED_RANGE	Invalid	The detected C_T value is higher than the defined cut-off C_T.
CT_BELOW_ ACCEPTED_RANGE	Invalid	The detected C_T value is lower than the defined cut-off C_T.
CURVE_SHAPE_ ANOMALY	Invalid	The raw data amplification curve shows a shape that deviates from the established behavior for this assay. There is a high likelihood for incorrect results or a result misinterpretation.
FLAT_BUMP	Invalid	The amplification curve shows a shape like a flat bump, deviating from the established behavior for this assay. There is a high likelihood for incorrect results or result misinterpretation (e.g., wrong C_T value determination).
IC_INVALID	Invalid	The internal control is invalid. Target and internal control share the same tube.
IC_NO_SIGNAL	Invalid	No internal control signal detected. Target and internal control share the same tube.
MULTI_THRESHOLD_ CROSSING	Invalid	The amplification curve crosses the threshold more than once. An unambiguous C_T cannot be determined.
NO_BASELINE	Invalid	No initial baseline has been found. The subsequent analysis cannot be performed.

Flag	Behavior	Description
NO_CT_DETECTED	Invalid	No C_T is detected for this target.
NORM_FACTOR_ ALTERATION	Warning	Curve not normalized properly due to low signal. **Note:** If a valid sample is tagged with this flag, the approver is asked to pay special attention to the information provided by this flag before deciding to accept or reject the result.
OTHER_TARGET_INVALID	Invalid	Another target for the same sample is invalid.
SATURATION	Invalid	The raw data fluorescence is saturating strongly before the inflection point of the amplification curve.
SATURATION_IN_PLATEAU	Warning	The raw data fluorescence is saturating in the plateau phase of the amplification curve. **Note:** If a valid sample is tagged with this flag, the approver is asked to pay special attention to the information provided by this flag before deciding to accept or reject the result.
SPIKE	Warning	A spike in the raw data fluorescence is detected in the amplification curve but outside the region where the C_T is determined. **Note:** If a valid sample is tagged with this flag, the approver is asked to pay special attention to the information provided by this flag before deciding to accept or reject the result.
SPIKE_CLOSE_TO_CT	Invalid	A spike is detected in the amplification curve close to the C_T.
STEEP_BASELINE	Invalid	A steeply rising baseline for the raw data fluorescence is detected in the amplification curve.

Flag	Behavior	Description
STRONG_BASELINE_ DIP	Invalid	A strong drop in the baseline for raw data fluorescence is detected in the amplification curve.
STRONG_NOISE	Invalid	Strong noise is detected outside the growth (exponential) phase of the amplification curve.
STRONG_NOISE_ IN_GROWTH_PHASE	Invalid	Strong noise is detected in the growth (exponential) phase of the amplification curve.
UPSTREAM	Variable	Sample status was set to invalid or unclear by an upstream process (e.g., QIAsymphony Assay Setup). **Note:** For samples that are flagged as unclear, the behavior of Rotor-Gene AssayManager is defined in the "Configuration" environment of the AssayManager software. "Invalid" flags from upstream processes always result in an invalid corresponding sample in Rotor-Gene AssayManager.
WAVY_BASE_ FLUORESCENCE	Invalid	A wavy baseline for the raw data fluorescence is detected in the amplification curve.

Limitations

- All reagents may exclusively be used in in vitro diagnostics.
- The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.
- It is important that the operator read the instructions for use thoroughly before using the system.
- The *artus* T. vaginalis QS-RGQ Kit is to be used by laboratory professionals trained in the use of the QIAGEN QIAsymphony RGQ system, Rotor-Gene AssayManager, and the *artus* T. vaginalis system.
- Strict compliance with the instructions for use is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Although rare, mutations within the highly conserved regions of the target genome covered by the kit's primers and/or probe may result in failure to detect the presence of the target in these cases. Validity and performance of the assay design are evaluated at regular intervals.
- Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* T. vaginalis QS-RGQ Kit is tested against predetermined specifications to ensure consistent product quality.

Performance Characteristics

Limit of detection

The limit of detection of the *artus* T. vaginalis QS-RGQ Kit (in combination with the QIAsymphony DSP Virus/Pathogen Midi Kit) was assessed by using two *T. vaginalis* strains, with one metronidazole-susceptible strain (ATCC 30001) and one metronidazole-resistant strain (ATCC 50143). Both strains were propagated in an anaerobic workstation and quantitated for the presence of viable and non-viable cells. Known quantities of each *T. vaginalis* strain were then spiked into the two (2) sample matrices: *T. vaginalis* negative human urine specimen matrix and *T. vaginalis* negative natural vaginal fluid matrix.

Six different concentration levels were evaluated with 24 replicates performed for each dilution level. All replicates at each dilution level were prepared using the QIAsymphony SP/AS instrument with the QIAsymphony DSP Virus/Pathogen Midi Kit and then analyzed on Rotor-Gene Q MDx. Combined data (hemocytometer quantitation and PCR results) were analyzed by probit analysis using R software. The limit of detection (LoD) for each stain is shown in Table 7. This means that there is a 95% probability that the titer listed for each strain will be detected. The standard error was calculated and is also listed in Table 7. The LoD determined for each strain was verified successfully with 20 additional replicates for each matrix.

Table 7. Limit of detection

T. vaginalis ATCC strain	Test matrix	LoD (C_{95}) (cell/sample)	Standard error	LoD (C_{95}) (cell/ml)	Verification (positive/20)
30001	Urine	0.149	0.034	0.025	20/20
	NVF	0.088	0.021	0.044	20/20
50143	Urine	0.123	0.032	0.021	20/20
	NVF	0.530	0.138	0.265	20/20

ATCC: American Type Culture Collection; LoD: limit of detection; NVF: natural vaginal fluid.

Analytical reactivity (inclusivity)

The analytical reactivity of the *artus* T. vaginalis QS-RGQ Kit was assessed by testing a panel of 43 different *T. vaginalis* strains (see Table 8) at approximately 2-3 × LoD in replicates of three (3). All 43 strains were detected by the *artus* T. vaginalis QS-RGQ Kit and the inclusivity was 100%.

Note: Following the study plan, if any replicates give a negative result, a further 3 replicates were re-tested. The organism was deemed "detectable" if the re-test results were all positive (100%).

Table 8. Relevant genotypes tested in analytical reactivity (inclusivity) studies

Sample no.*	Panel no.	Strain	#Detected/3 (urine)	#Detected/3 (SVF)	Comments
1	30001	C-1:NIH	3/3	3/3	
2	30092	11769	3/3	3/3	
3	30093	45422	3/3	3/3	
4	30184	123414	3/3	3/3	
5	30185	129155-8	3/3	5/6†	Retested†
6	30186	123413	3/3	5/6†	Retested†
7	30187	165307-1	3/3	3/3	
8	30188	RP	3/3	3/6†	Retested†
9	30235	JH 30A #4	3/3	3/3	
10	30236	JH 31A #4	3/3	3/3	
11	30237	JH 32A #2	3/3	3/3	
12	30238	JH 32A #4	3/3	3/3	
13	30239	JH 34A #4	3/3	3/3	
14	30240	JH 37A #2	3/3	3/3	
15	30241	JH 37A #4	3/3	3/3	
16	30242	JH 161A #4	3/3	3/3	
17	30243	JH 162A #4	3/3	3/3	
18	30244	JH 191A #4	3/3	3/3	
19	30245	TVC	3/3	3/3	
20	30246	TVC1	3/3	5/6†	Retested†
21	30248	TV 3	3/3	3/3	
22	30488	RFC-1	3/3	3/3	
23	50138	IR 78	3/3	3/9†	Retested†
24	50139	RU 357	3/3	3/3	
25	50140	RU 384	3/3	5/6†	Retested†

Sample no.*	Panel no.	Strain	#Detected/3 (urine)	#Detected/3 (SVF)	Comments
26	50141	RU 382	3/3	3/3	
27	50142	RU 393	3/3	3/3	
28	50143	CDC 085	3/3	3/3	
29	50144	CDC 337	3/3	3/3	
30	50145	CDC 409	3/3	3/3	
31	50146	NYH 209	3/3	3/3	
32	50147	NYH 272	3/3	5/6†	Retested†
33	50148	NYH 286	3/3	5/6†	Retested†
34	50167	B7RC2	3/3	3/3	
35	50183	HsD:NIH	3/3	3/3	
36	50747		3/3	3/3	
37	PRA-91	JRS-TV-120	3/3	3/3	
38	PRA-92	JRS-TV-141	3/3	3/3	
39	PRA-95	JRS-TV-VB102	3/3	3/3	
40	PRA-96	MT87	3/3	3/3	
41	PRA-97	BL++	3/3	3/3	
42	PRA-98	G3	3/3	3/3	
43	801805	Z070	3/3	3/3	

ATCC: American Type Culture Collection; no.: number; SVF: simulated vaginal fluid.
* Samples number 1 to 42 were obtained from ATCC and the panel no. in the table corresponds to the ATCC number. Sample number 43 was provided by Zeptometrix and the panel no. is their reference no.
† Following the study plan, if any replicates give a negative result, a further 3 replicates were re-tested. The organism was deemed "detectable" if the re-test results were all positive (100%).

Cross-reactivity and microbial interference

Potential cross-reactivity and microbial interference with the *artus* T. vaginalis QS-RGQ Kit were tested using a panel of bacteria, fungi, protozoa or viruses (Table 9). In the cross-reactivity study, organisms were spiked at 1×10^6 CFU/ml for bacteria and yeast, 1×10^5 PFU/ml for viruses, and 1×10^5 cells/ml for protozoa in either negative human urine or natural vaginal fluid matrix and tested. In the microbial interference study, the same organisms were spiked into a sample containing *T. vaginalis* (ATCC 30001) at a level close to a limit of detection (e.g., 3 × LoD). None of the pathogens tested demonstrated cross-reactivity. None of the pathogens tested caused interference.

Table 9. Panel of organisms tested for cross-reactivity and microbial interference

Species of microorganism	Cross-reacts? Yes/No	Interferes? Yes/No
Acinetobacter lwoffi	No	No
Acinetobacter baumannii	No	No
Actinomyces israelii	No	No
Atopobium vaginae	No	No
Bacteroides (Parabacteroides) merdae	No	No
Bacteroides fragilis	No	No
Bifidobacterium adolescentis	No	No
Bifidobacterium bifidum	No	No
Moraxella (Branhamella) catarrhalis	No	No
Campylobacter jejuni	No	No
Candida albicans	No	No
Candida glabrata	No	No
Candida parapsilosis	No	No

Species of microorganism	Cross-reacts? Yes/No	Interferes? Yes/No
Candida tropicalis	No	No
Chlamydia trachomatis	No	No
Clostridium difficile	No	No
Clostridium perfringens	No	No
Corynebacterium genitalium	No	No
Cryptococcus neoformans	No	No
Entamoeba histolytica	No	No
Enterobacter aerogenes	No	No
Enterococcus faecalis	No	No
Escherichia coli	No	No
Fusobacterium nucleatum	No	No
Gardnerella vaginalis	No	No
Haemophilus ducreyi	No	No
Herpes Simplex Virus Type1 (HSV-1)	No	No
Herpes Simplex Virus Type1 (HSV-2)	No	No
Human papillomavirus 16 (HPV-16, SiHa)	No	No
Human papillomavirus 18 (HPV-18)	No	No
HIV Type 1 (HIV-1)	No	No
Klebsiella oxytoca	No	No
Lactobacillus acidophilus	No	No
Lactobacillus jensenii	No	No
Lactobacillus vaginalis	No	No
Listeria monocytogenes	No	No
Mobiluncus curtisii	No	No
Mycobacterium smegmatis	No	No

Species of microorganism	Cross-reacts? Yes/No	Interferes? Yes/No
Mycoplasma hominis	No	No
Neisseria gonorrhoeae	No	No
Pentatrichomonas hominis	No	No
Peptococcus niger	No	No
Peptostreptococcus anaerobius	No	No
Porphyromonas asaccharolytica	No	No
Prevotella bivia	No	No
Prevotella melaninogenica	No	No
Propionibacterium acnes	No	No
Proteus mirabilis	No	No
Pseudomonas aeruginosa	No	No
Salmonella enterica (typhimurium)	No	No
Shigella flexneri	No	No
Staphylococcus aureus MRSA	No	No
Staphylococcus epidermidis	No	No
Staphylococcus saprophyticus	No	No
Streptococcus agalactiae	No	No
Streptococcus pyogenes	No	No
Trichomonas tenax	No	No
Ureaplasma urealyticum	No	No
Veillonella parvula	No	No

MRSA: methicillin-resistant *Staphylococcus aureus*; n/a: not applicable

Table 10. Panel of organisms tested *in silico* for cross-reactivity

Species of microorganism	Cross-reacts? Yes/No
Mycoplasma genitalium	No

Note: This strain was not available for testing and cross-reactivity analysis was therefore performed *in silico*. It was not possible to assess microbial interference.

Total precision and reproducibility

The intermediate precision and reproducibility of the *artus* T. vaginalis QS-RGQ Kit was assessed using an 8-member panel consisting of *T. vaginalis* strain ATCC 30001. Panel members were formulated either in human urine matrix or in simulated vaginal fluid (SVF) (17) with *T. vaginalis* at a concentration of 3 × LoD, 1 × LoD, and below the LoD. The negative panel members, R1 and R5, were prepared using the matrix only. For reproducibility, the 8-member panel was tested in triplicates on 3 instrument systems at 3 sites with 2 runs per day over 5 days using 3 lots of the *artus* T. vaginalis QS-RGQ Kit combined with 3 lots of the QIAsymphony DSP Virus/Pathogen Midi Kit. The results are summarized in Table 11 on page 59.

The total precision and reproducibility were also evaluated in terms of Ct values for each target detected. The standard deviation (SD), coefficient of variance (CV) and variance between run, between day, between lot, between site (reproducibility) and within run (repeatability) are presented in Table 12 on page 60.

Table 11. Summary of the site-to-site reproducibility for the *artus* T. vaginalis QS-RGQ Kit

Panel description	Matrix	ID	Total rep.	Site 1 no. +	Site 2 no. +	Site 3 no. +	Total + (%)	Acceptance criteria
T. vaginalis ATCC 30001 PSPC	Urine	PSPC-1	30	10	10	10	100	100% positive
	SVF	PSPC-2	30	10	10	10	100	100% positive
P. hominis Hs-3:NIH ATCC 30000 NSPC	Urine	NSPC-1	30	0	0	0	0	100% negative
	SVF	NSPC-2	30	0	0	0	0	100% negative
Negative	Urine	R1	90	0	0	0	0	100% negative
Below LoD	Urine	R2	90	9	16	16	45.60	20–80% positive
1 × LoD	Urine	R3	90	30	30	30	100	≥95% positive
3 × LoD	Urine	R4	90	30	30	30	100	100% positive
Negative	SVF	R5	90	0	0	0	0	100% negative
Below LoD	SVF	R6	90	9	19	13	45.60	20–80% positive
1 × LoD	SVF	R7	90	30	30	30	100	≥95% positive
3 × LoD	SVF	R8	90	30	30	30	100	100% positive

LoD: limit of detection; rep.: replicate; no.: number; NSPC: Negative Specimen Process Control; PSPC: Positive Specimen Process Control; SVF: simulated vaginal fluid; Total+%: total percentate of positive samples.

Table 12. *artus* T. vaginalis QS-RGQ Kit precision components and total precision

Test	Parameter	PSPC-1	PSPC-2	R2	R3	R4	R6	R7	R8
	C_{tmean}	30.12	30.49	35.09	31.09	29.91	35.74	31.64	30.40
	No.	30	30	81	90	90	81	90	90
Within run	SD	0.202	0.176	0.991	0.410	0.441	1.121	0.232	0.207
	%CV	0.67	0.58	2.82	1.32	1.47	3.14	0.73	0.68
	Var_{tot}	0.041 (89.69)	0.031 (52.10)	0.981 (78.42)	0.168 (78.37)	0.194 (74.32)	1.256 (95.65)	0.054 (67.28)	0.043 (65.81)
Between-run/ op.	SD	0.000	0.000	0.485	0.000	0.000	0.185	0.097	0.000
	%CV	0.00	0.00	1.38	0.00	0.00	0.52	0.31	0.00
	Var_{tot}	0.000 (0.00)	0.000 (0.00)	0.236 (18.82)	0.000 (0.00)	0.000 (0.00)	0.034 (2.62)	0.009 (11.74)	0.000 (0.00)
Between-day	SD	0.000	0.090	0.000	0.151	0.000	0.000	0.000	0.011
	%CV	0.00	0.30	0.00	0.49	0.00	0.00	0.00	0.04
	Var_{tot}	0.000 (0.00)	0.008 (13.70)	0.000 (0.00)	0.023 (10.68)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.000 (0.18)
Between-lot	SD	0.000	0.137	0.000	0.099	0.019	0.000	0.000	0.030
	%CV	0.00	0.45	0.00	0.32	0.06	0.00	0.00	0.10
	Var_{tot}	0.000 (0.00)	0.019 (31.78)	0.000 (0.00)	0.010 (4.57)	0.000 (0.14)	0.000 (0.00)	0.000 (0.00)	0.001 (1.35)
Between-site	SD	0.069	0.038	0.186	0.117	0.258	0.151	0.130	0.146
	%CV	0.23	0.12	0.53	0.38	0.86	0.42	0.41	0.48
	Var_{tot}	0.005 (10.32)	0.001 (2.41)	0.035 (2.76)	0.014 (6.38)	0.067 (25.54)	0.023 (1.73)	0.017 (20.98)	0.021 (32.66)
Total precision	SD	0.213	0.243	1.119	0.463	0.511	1.146	0.283	0.255
	%CV	0.71	0.80	3.19	1.49	1.71	3.21	0.90	0.84
	Var_{tot}	0.045 (100.0)	0.059 (100.0)	1.252 (100.0)	0.215 (100.0)	0.262 (100.0)	1.313 (100.0)	0.080 (100.0)	0.065 (100.0)

CV: coefficient of variation; No.: Total replicate numbers with non-zero C_t values; op.: operator; PSPC: Positive Specimen Process Control; SD: standard deviation; $Var_{tot.}$: Variance (% total variance).

Carryover

This study included a series of five PCR runs each containing 34 high positive and 34 negative samples in alternating positions (checkerboard pattern). The 5 checkerboard PCR runs (used to assess within-run cross contamination) were interrupted by negative PCR runs containing completely negative samples to assess on potential between run carry-over. The high positive sample used in this study was *T. vaginalis* (ATCC 30001) diluted into urine and simulated vaginal matrices to reach a concentration of 1×10^5 cells/ml. This concentration was designed to represent at least 95% or more of the results obtained from specimens of infected patients in the intended use population.

All positive samples were reported as "Signal Detected" and all negative samples were reported as "Signal not detected". No carryover and cross contamination for the entire workflow were observed.

Interfering substances

A panel of exogenous and endogenous substances (listed in Table 13) that may be present in patient specimens was tested to determine whether these substances cross-reacted or interfered with the performance of the *artus* T. vaginalis QS-RGQ Kit. The substances were tested at clinically relevant concentrations in the presence (interference) and absence (cross-reactivity) of *T. vaginalis* target (ATCC 30001) at 3 × LoD in human urine and natural vaginal fluid matrices, respectively, in triplicate per each substance. None of the substances showed interference/cross-reactivity with the detection of *T. vaginalis* by the *artus* T. vaginalis QS-RGQ Kit.

Table 13. Substances tested for potential interference/cross-reactivity

Interfering substance category	No.	Potential interfering active ingredient	Conc. tested	Cross reacts? Yes / No	Interfers? Yes / No
Vaginal lubricants e.g., K-Y personal lubricant jelly	1	Glycerin with propylene glycol	1% v/v	No	No
Douches, e.g, Summer's Eve Douche Extra Cleansing Vinegar & Water	2	Vinegar, sodium benzoate	1% v/v	No	No
Human whole blood	3	Whole blood	10% v/v	No	No
Human leukocytes	4	Human leukocytes	1×10^6 cells/ml (urine) 2.5×10^6 cells/ml (NVF)	No	No
HeLa cells	5	HeLa cells	1×10^5 cells/ml	No	No
Human genomic DNA	6	Human gDNA	500 ng/ml	No	No
Spermicides, e.g., Options Gynol II vaginal contraceptive gel	7	Nonoxynol 9, 4%	1% w/v	No	No

Interfering substance category	No.	Potential interfering active ingredient	Conc.. tested	Cross reacts? Yes / No	Interfers? Yes / No
Vaginal yeast treatments anti-fungal medications, anti-itch medications	8	Clotrimazole,1%	1% w/v	No	No
	9	Miconazole nitrate, 2%	1% w/v	No	No
	10	Nystatin cream (100,000 USP)	1% w/v	No	No
	11	Phenazopyridine HCl 200 mg	1% w/v	No	No
	12	Itraconazole 100 mg	1% w/v	No	No
	13	Tinidazole 250 mg	1% w/v	No	No
	14	Terconazole 80 mg	1% w/v	No	No
	15	Fluconazole 200 mg	1% w/v	No	No
	16	Metronidazole vaginal gel 0.75%	1% w/v	No	No
	17	Clindamycin vaginal cream 2%	1% w/v	No	No
	18	Isobutane, corn starch, hydrated silica, mineral oil	1% v/v	No	No
Intravaginal hormones, e.g., Crinone 8% gel, Estrace vaginal cream	19	Progesterone	1% w/v	No	No
	20	Estrogen (estradiol)	1% w/v	No	No

Interfering substance category	No.	Potential interfering active ingredient	Conc.. tested	Cross reacts? Yes / No	Interfers? Yes / No
Human seminal fluid	21	Human seminal fluid	5% v/v	No	No
Mucus, e.g., porcine gastric mucus	22	Mucin	1% w/v	No	No
Hemorrhoid cream (vaginal testing only), e.g., Preparation H maximum strength pain relief cream	23	Glycerin 14.4%, phenylephrine HCl 0.25%, pramoxine HCl 1%	1% w/v	No	No
Abnoram urine (urine testing only)	24	High abnormal with urobilinogen (KOVA-Trol I*)	Sub-stituted for urine	No	No
	25	Acidic human urine (pH4.0)	Sub-stituted for urine	No	No
	26	Alkaline human urine (pH9.0)	Sub-stituted for urine	No	No

* Substance purchased from KOVA International. For futher information on the values for pH, protein, glucose, ketones, hemoglobin, bilirubin, nitrites, leukocyte esterase, specific gravity, osmolality, and creatinine can be found on the KOVA International website.

LoD: limit of detection; NVF: natural vaginal fluid.

Diagnostic performance evaluation

A diagnostic performance evaluation of the *artus* T. vaginalis QS-RGQ Kit was performed in a prospective investigational study by comparing results from the *artus* T. vaginalis QS-RGQ Kit with a composite reference method comprising wet mount microscopy and InPouch TV culture/microscopy (Biomed Diagnostics, Inc, White City, OR, USA) for samples from female subjects. For urine samples prospectively collected from male subjects, results from the *artus* T. vaginalis QS-RGQ Kit were compared to a composite reference method comprising InPouch TV culture/microscopy and PCR using primers different from the *artus* T. vaginalis QS-RGQ Kit followed by bi-directional sequencing. The male urine prospective study was supplemented with a contrived male urine panel study, due to the low prevalence of *T. vaginalis* in male subjects enrolled in the prospective study. For the contrived male urine samples, results from the *artus* T. vaginalis QS-RGQ Kit were compared with the reference method of InPouch TV culture/microscopy only. Specimens were collected from five (5) distinct geographical areas (5 collection sites) following the procedures below:

- Three (3) vaginal swabs and one (1) endocervical swab were collected from each female subject by the clinician, and one (1) self-collected urine specimen was collected from each female and male subject enrolled in the study.
- The first (1) vaginal swab, the endocervical swab (Regular FLOQSwab was used for vaginal, endocervical samples), the female urine sample, and the male urine sample were placed into an individual eNAT tube (containing 2 ml eNAT solution for testing with *artus* T. vaginalis QS-RGQ test.
- Wet mount microscopy was performed immediately with the second (2) vaginal swab at the site of collection following the institution's standard of care process for Wet Mount microscopy.
- The third (3) vaginal specimen (for the female study reference method) from the same female subject was collected onto the appropriate device (disposable cotton swab) defined within the labeling for InPouch culture. The InPouch was directly inoculated with the swab in less than one hour from the collection following the InPouch TV IFU.]

- For the male study culture reference method the male urine sample was collected to directly inoculate the InPouch in less than one hour from the collection following the InPouch TV IFU.
- For male urine PCR/sequencing reference testing, a pellet from 10 ml of the first-catch male urine was resuspended in 1ml of eNAT medium and sent to the reference lab for further processing for *T. vaginalis* by PCR and bi-directional sequencing.

A "true *T. vaginalis*-positive" specimen was defined as a specimen where *T. vaginalis* is identified by both tests (*artus* T. vaginalis QS-RGQ PCR and any one of the composite reference methods, for example, wet mount and/or InPouch TV for female specimens; InPouch TV and/or PCR plus sequencing for male specimens).

A "false *T. vaginalis*-positive" specimen was defined as a specimen where *T. vaginalis* is identified only by the *artus* T. vaginalis QS-RGQ Kit and not by the reference tests (both reference methods must be negative).

A "false *T. vaginalis*-negative" specimen was defined as a specimen where *T. vaginalis* is identified only by the reference tests (any one or both reference methods), and not by the *artus* T. vaginalis QS-RGQ test.

A "true negative" specimen was defined as a specimen where *T. vaginalis* was not identified by all tests (*artus* T. vaginalis QS-RGQ PCR and both reference methods must be negative).

Out of a total of 4222 (1408 vaginal, 1408 endocervical, and 1406 female urine) prospective female samples, 84 (20 vaginal, 42 endocervical, and 22 urine) were not available for testing for the various reasons including patient hysterectomy or problems with transportation, and collection.Out of the 4138 samples available for testing (1388 vaginal, 1366 endocervical, and 1384 female urine), 228 gave invalid results. This was due to various reasons, but only 25 samples (0.6% of total samples tested) were to be classified as

unresolved invalid* after root cause analysis, leaving total of 3910 samples evaluable for the statistical analysis results.

A total of 335 male specimens were collected prospectively. Out of 335 specimens, 0 (zero) gave indeterminate or invalid results when tested with the *artus* T. vaginalis QS-RGQ Kit. Of these, 12 out of 335 specimens gave invalid reference sequence results because of insufficient sample volume for DNA extraction for bi-directional sequencing. Valid results were therefore available for 323 specimens in total.

A total of 100 male specimens were manufactured for the contrived portion of the clinical study due to the low prevalence of *T. vaginalis* in male urine population. Of the 100 member of contrived male urine panel tested with *artus* T. vaginalis QS-RGQ kit at EGI MDx and by InPouch cultures, 30 samples were excluded due to technical errors with the InPouch culture testing, leaving 70 evaluable results that were included in statistical analysis.

Sensitivity and specificity by gender, specimen type and symptom status are presented in Table 14 for specimens from vaginal and endocervical swabs and Table 15 for specimens from urine samples.

* when judged by internal, negative and positive controls of the assay.

Table 14. *T. vaginalis* clinical agreement study results (*artus* T. vaginalis QS-RGQ Kit vs. composite reference methods): vaginal and endocervical specimens

Status	No.	TP	FP	TN	FN	Prev. %	Sensitivity (95% CI)	Specificity (95% CI)	PPV %	NPV %
Vaginal swabs										
Sym	895	82	20	793	0	9.2	100.0 (95.5–100)	97.5 (96.2–98.4)	80.4 (71.6–86.9)	100.0 (99.5–100)
Asym	403	37	4	362	0	9.2	100.0 (90.6–100)	98.9 (97.2–99.6)	90.2 (77.5–96.1)	100.0 (99.0–100)
All	1298	119	24	1155	0	9.2	100.0 (96.9–100)	98.0 (97.0–98.6)	83.2 (76.2–88.5)	100.0 (99.7–100)
Endocervical swabs										
Sym	872	81	9	782	0	9.3	100.0 (95.5–100)	98.9 (97.9–99.4)	90.0 (82.1–94.7)	100.0 (99.5–100)
Asym	383	31	2	350	0	8.1	100.0 (89.0–100)	99.4 (98.0–99.8)	93.9 (80.4–98.3	100.0 (98.9–100)
All	1255	112	11	1132	0	8.9	100.0 (96.7–100)	99.0 (98.3–99.5)	91.1 (84.7–94.9)	100.0 (99.7–100)

Asym: asymptomatic; CI: confidence interval; FN: false negative; FP: false positive; No.: number; n/a: not applicable; NPV: negative predictive value; Prev.: prevalence; Pop: population; PPV: positive predictive value; Sym: symptomatic; TN: true negative; TP: true positive

Table 15. *T. vaginalis* clinical agreement study results (*artus* T. vaginalis QS-RGQ Kit vs. composite reference methods): female and male urine samples

Status	No.	TP	FP	TN	FN	Prev. %	Sensitivity (95% CI)	Specificity (95% CI)	PPV% (95%CI)	NPV% (95%CI)
Female urine samples										
Sym	939	88	12	837	2	9.6	97.8 (92.3–99.4)	98.6 (97.5–99.2)	88.0 (80.2–93.0)	99.8 (99.1–99.9)
Asym	418	37	3	377	1	9.1	97.4 (86.5–99.5)	99.2 (97.7–99.7)	92.5 (80.1–97.4)	99.7 (98.5–100)
All	1357	125	15	1214	3	9.4	97.7 (93.3–99.2)	98.8 (98.0–99.3)	89.3 (83.1–93.4)	99.8 (99.3–99.9)
Male urine samples										
Sym	91	1	1	89	0	1.1	100.0 (20.7–100)	98.9 (94.0–99.8)	50.0 (9.4–90.6)	100.0 (95.9–100)
Asym	232	7	0	224	1	3.4	87.5 (52.9–97.8)	100.0 (98.3–100)	100.0 (64.6–100)	99.6 (97.5–99.9)
CS	70	25	1	43	1	n/a	96.2 (81.1–99.3)	97.7 (88.2–99.6)	n/a	n/a
All	393	33	2	356	2	n/a	94.3 (81.4–98.4)	99.4 (98.0–99.8)	94.3 (81.4–98.4)	99.4 (98.0–99.8)

Asym: asymptomatic; CI: confidence interval; CS: contrived specimen; FN: false negative; FP: false positive; No.: number; n/a: not applicable; NPV: negative predictive value; Prev.: prevalence; Pop: population; PPV: positive predictive value; Sym: symptomatic; TN: true negative; TP: true positive.

Discordant analysis

For each discordant sample, DNA extraction was performed on the leftover clinical sample in eNAT followed by PCR using different primers to the ones used in the *artus* T. vaginalis QS-RGQ Kit. This was followed with bi-directional sequencing. A BLAST homology search in the NCBI database was then performed on the sequences to confirm the identity and homology to *T. vaginalis*. Samples were considered as *T. vaginalis* positive if the PCR product had a >95% homology with any *T. vaginalis* strain identified in the NCBI database.

A total of 53 discordant female samples and 2 discordant male samples underwent the discordant analysis procedure, with the final performance parameters changed as outlined in Table 16 for vaginal and endocervical specimens and Table 17 for female and male urine samples.

Table 16. *artus* T. vaginalis QS-RGQ Kit vs. composite reference methods agreement - after discordancy resolution: vaginal and endocervical specimens

Status	No.	TP	FP	TN	FN	Prev., %	PPA,% (95% CI)	NPA,% (95% CI)
Vaginal swabs								
Sym	887	89	5	793	0	10.0	100.0 (95.9–100)	99.4 (98.5–99.7)
Asym	401	38	1	362	0	9.5	100.0 (90.8–100)	99.7 (98.5–100)
All	1288	127	6	1155	0	9.9	100.0 (97.1–100)	99.5 (98.9–99.8)
Endocervical swabs								
Sym	871	85	4	782	0	9.8	100.0 (95.7–100)	99.5 (98.7–99.8)
Asym	383	32	1	350	0	8.4	100.0 (89.3–100)	99.7 (98.4–99.9)
All	1254	117	5	1132	0	9.3	100.0 (96.8–100)	99.6 (99.0–99.8)

Asym: asymptomatic; CI: confidence interval; FN: false negative; FP: false positive; No.: number; NPA: negative percent agreement; PPA: positive percent agreement; Prev.: prevalence; Sym: symptomatic; TN: true negative; TP: true positive.

Table 17. *artus* T. vaginalis QS-RGQ Kit vs. composite reference methods agreement - after discordancy resolution: female and male urine samples

Status	No.	TP	FP	TN	FN	Prev., %	PPA,% (95% CI)	NPA,% (95% CI)
Female urine samples								
Sym	939	96	4	839	0	10.2	100.0 (96.2–100)	99.5 (98.8–99.8)
Asym	418	39	1	378	0	9.3	100.0 (91.0–100)	99.7 (98.5–100)
All	1357	135	5	1217	0	9.9	100.0 (97.2–100)	99.6 (99.0–99.8)
Male urine samples								
Sym	91	2	0	89	0	2.2	100.0 (34.2–100)	100.0 (95.9–100)
Asym	232	7	0	225	0	3.0	100.0 (64.6–100)	100.0 (98.3–100)
CS	70	25	1	43	1	n/a	96.2 (81.1–99.3)	97.7 (88.2–99.6)
All	393	34	1	357	1	n/a	97.1 (85.5–99.5)	99.7 (98.4–100)

Asym: asymptomatic; CI: confidence interval; CS: contrived specimen; FN: false negative; FP: false positive; No.: number; n/a: not applicable; NPA: negative percent agreement; PPA: positive percent agreement; Prev.: prevalence; Sym: symptomatic; TN: true negative; TP: true positive.

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Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
	Contains sufficient for 72 tests
	In vitro diagnostic medical device
	CE mark
	Catalog number
	Lot number
	Material number
	Components

Symbol	Symbol definition
CONT	Contains
MASTER	Master
MG-SOL	Magnesium solution
IC	Internal Control
CONTROL +	*T. vaginalis* positive control
CONTROL –	*T. vaginalis* negative control
GTIN	Global Trade Item Number
Rn	R is for the revision of the Instructions for Use (Handbook) and n is the revision number

Symbol	Symbol definition
	Temperature limitation
	Manufacturer
	Use by
	Consult instructions for use

Troubleshooting Guide

Refer to this section for error handling and troubleshooting. If the recommended steps do not resolve the problem, contact QIAGEN Technical Services for assistance, either via our Technical Support Center at **www.qiagen.com/Support**, by calling 00800-22-44-6000, or by contacting one of the QIAGEN Technical Service Departments or your local distributors.

Possible problem or cause	Corrective action
General handling	
Error message displayed in the touchscreen	If an error message is displayed during an integrated run, refer to the user manuals supplied with your instruments.
Precipitate in reagent trough of opened cartridge of the QIAsymphony DSP Virus/Pathogen Kit	
a) Buffer evaporation	Excessive evaporation may lead to increased salt concentration or decreased alcohol concentrations in buffers. Discard reagent cartridge (RC). Make sure to seal buffer troughs of a partially used reagent cartridge (RC) with Reuse Seal Strips when not being used for purification.

Possible problem or cause	Corrective action
b) Storage of reagent cartridge (RC)	Storage of reagent cartridge (RC) at less than 15°C may lead to formation of precipitates. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate in a water bath* at 37°C for 30 minutes with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) in a water bath* at 37°C for 30 minutes with occasional shaking.
Low yield of nucleic acids	
a) Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 minutes before use.
b) Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Possible problem or cause	Corrective action
c) Carrier RNA (CARRIER) not added	Reconstitute carrier RNA (CARRIER) in Buffer AVE (AVE) and mix with appropriate volume of Buffer AVE (AVE) as described in "Preparation of carrier RNA and internal control (T. vaginalis IC)", page 21. Repeat the purification procedure with new samples.
d) Degraded nucleic acids	Samples were stored incorrectly or subjected to too many freeze–thaw cycles. Repeat the purification procedure with new samples.
e) Incomplete sample lysis	Before use, check that Buffers QSL2 and QSB1 do not contain precipitates. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips, and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.*

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Possible problem or cause	Corrective action
f) Clogging of pipet tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIAsymphony purification procedure. To remove insoluble material for applications, centrifuge the sample at 3000 x g for 1 minute, and transfer the supernatant to a new sample tube.
QIAsymphony AS detectsInsufficient Master transferred to tube	Combine the contents of an appropriate number of Master tubes into one tube before use. Combine the contents of an appropriate number of Mg-Sol tubes into one tube before use. Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the Master in the tube. For viscous reagents, we recommend aspirating an extra volume of 5% when using manual pipets (e.g., adjust the pipet to 840 µl for an 800 µl volume). Alternatively, after slowly dispensing the liquid and performing a blowout at the target tube's wall, remove the tip from the liquid, release the pipet plunger, and wait for an additional 10 seconds. Residual liquid will flow down the tip and can be blown out by pressing the pipet plunger a second time. The use of PCR grade filter-tips labeled as "low retention" can improve the recovery of liquid.

Possible problem or cause	Corrective action
No signal with positive controls	
a) Incorrect configuration of the PCR	Make sure that assay setup was performed correctly and that the correct assay parameter set was used. Repeat the PCR, if necessary. See "Assay control sets and assay parameter sets", page 23.
b) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 15)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
c) The *artus* T. vaginalis QS-RGQ Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Weak or no signal of the internal control of a negative sample subjected to purification using the QIAsymphony DSP Virus/Pathogen Kit and simultaneous absence of a sample signal	
a) The PCR was inhibited	Make sure that you use the validated isolation method (see "Protocol: DNA isolation and assay setup on the QIAsymphony SP/AS", page 24) and closely follow the instructions.

Possible problem or cause	Corrective action
b) DNA was lost during extraction	An absent signal of the internal control can indicate the loss of DNA during the extraction. Make sure that you use the validated isolation method (see "Protocol: DNA isolation and assay setup on the QIAsymphony SP/AS", page 24) and closely follow the instructions. See also "Low yield of nucleic acids", above.
c) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 15)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
d) The *artus* T. vaginalis QS-RGQ Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Signals with the negative controls of the analytical PCR	
a) Contamination occurred during preparation of the PCR	Repeat the integrated QS-RGQ run with new reagents. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure that work space and instruments are decontaminated at regular intervals.

Possible problem or cause	Corrective action
b) Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

Ordering Information

Product	Contents	Cat. no.
artus T. vaginalis QS-RGQ Kit (72)	For 72 reactions: Master, magnesium solution, internal control, *T. vaginalis* positive control, *T. vaginalis* negative control	4571366
Related Products		
QIAsymphony DSP Virus/Pathogen Midi Kit (96)	Includes 2 reagent cartridges and enzyme racks and accessories	937055
QIAsymphony SP	QIAsymphony sample prep module, 1-year warranty on parts and labor	9001297
QIAsymphony AS	QIAsymphony assay setup module, 1-year warranty on parts and labor	9001301
Rotor Gene Q AssayManager Software vesions 1.0.X where X ≥4	Software for routine testing in combination with the Rotor-Gene Q and QIAsymphony RGQ instruments; single license software for installation on one computer	9022737
Rotor Gene Q MDx Cycler	Real-time PCR cycler and high resolution melt (HRM) analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002032

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