RIDA®GENE Trichomonas vaginalis

REF PG4975

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Trichomonas vaginalis is a multiplex real-time PCR for the direct, qualitative detection of *Trichomonas vaginalis* from human genital swabs and urine.

The RIDA®GENE Trichomonas vaginalis real-time PCR is intended for use as an aid in diagnosis of Trichomoniasis caused by *Trichomonas vaginalis*.

2. Summary and explanation of the test

Trichomonas vaginalis is a human pathogenic parasite that infects the genital area. It can lead to trichomoniasis where both sexual organs and also the urinary tract can be affected. Trichomoniasis is transmitted by sexual intercourse and is either transmitted through vaginal secretion or sperm indicating the infection potential for both men and women. Worldwide, 120 million cases are described yearly, whereas a higher prevalence rate is known in women.[1]
According to the Centers of Disease Control and Prevention (CDC), about 3.7 million people are infected with *Trichomonas vaginalis* in the US. However, only 30% of the infected show symptoms.[2] Symptoms reach from discomfort in the vaginal area and with urination up to discharge. During a *Trichomonas vaginalis* infection in women, also a miscolonisation of the vaginal flora with other pathogens is observed. For example *Gardnerella vaginalis* or various stool pathogens often accompany a *Trichomonas vaginalis* infection. In pregnant women, an infection with *Trichomonas vaginalis* may lead to further complications such as premature labour or premature membrane rupture.[3] Complications in men are amongst others infertility or prostatitis. Besides the miscolonisation of the vaginal flora, *Trichomonas vaginalis* also plays an important role as co-factor during transmission of HIV.[2] The gold standard for diagnosis is still culture, however sensitivity is only about 80% and with the time to result of up to 7 days it is not suitable for timely diagnosis.

3. Test principle

The RIDA®GENE Trichomonas vaginalis is a real-time PCR for the direct, qualitative detection of *Trichomonas vaginalis* from genital swabs, as well as from urine.
After DNA isolation, amplification of the gene fragment (ITS1, if present) specific for *Trichomonas vaginalis* occurs.
The amplified target for *Trichomonas vaginalis* is detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-Polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Trichomonas vaginalis assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and/or to determine possible PCR inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µl	yellow
2	Taq-Polymerase	1x	80 µl	red
D	Internal Control DNA	2x	1700 µl	orange
N	No Template Control	1x	450 µl	white
P	Positive Control	1x	200 µl	blue

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw and fully defrost reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 5 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all reagents should be stored cold in an appropriate way (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Trichomonas vaginalis multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2: Necessary equipment

Extraction platforms	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
bioMérieux	NucliSENS easy®MAG™
Real-time PCR instruments	
Roche	LightCycler® 2.0, LightCycler® 480II
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- Sterile, media-free Rayon or Nylon flocked swabs (e.g. Copan Diagnostic Inc., catalogue no. 155C or 552C)
- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II
- RIDA®GENE Color Compensation Kit II (PG0002) for use with the LightCycler® 2.0
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (BioScience grade, nuclease-free, DEPC treated water)

7. Precautions for users

For *in vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

8. Collection and storage

8.1 DNA-Preparation

For DNA isolation from dry swabs, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.
To isolate DNA from dry swabs the following procedure is recommended: Add 400 µl PCR water into a preparation tube. Insert the swab into the water, squeeze it and cut or break the swab stem. Cap the preparation tube tightly, vortex shortly and continue according to manufacturer's instruction of the DNA extraction kit or DNA extraction system (see also Maxwell® RSC Application ER101).

For DNA isolation from urine, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions (see also Maxwell® RSC Application ER100).

The RIDA®GENE Trichomonas vaginalis assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master- Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure and as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure.
The Internal Control DNA should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each assay run.

We recommend calculating an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and briefly centrifuge the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 4: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl No Template Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the negative control.

Sample: Add 5 µl DNA extract to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the positive control.

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument set-up (see Tab. 5, Tab. 6, Tab.7, Tab.8).

9.3 PCR instrument set-up

9.3.1 DNA real-time PCR profile

Tab. 5: Real-time PCR profile for LightCycler® series and Rotor-Gene Q

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 6: Real-time PCR profile for Mx3005P, ABI 7500 and CFX96™

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.3.2 Universal real-time PCR profile

Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RIDA®GENE RNA real-time PCR assays in one run.

Tab. 7: Universal real-time PCR profile for LightCycler® series

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 8: Universal real-time PCR profile for Mx3005P, ABI7500, Rotor-Gene Q and CFX96™

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.4 Detection channel set-up

Tab. 9: Selection of appropriate detection channels

Real-time PCR Gerät	Detection	Detection Channel	Note
Roche LightCycler® 2.0	*Trichomonas vaginalis*	530	**RIDA®GENE Color Compensation Kit II (PG0002) is required**
	ICD	560	
Roche LightCycler® 480II	*Trichomonas vaginalis*	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICD	533/580	
ABI 7500	*Trichomonas vaginalis*	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
Agilent Techn. Mx3005P	*Trichomonas vaginalis*	FAM	**Check that passive reference dye is none**
	ICD	HEX	
Qiagen Rotor-Gene Q	*Trichomonas vaginalis*	Green	**The gain settings have to be set to 5**
	ICD	Yellow	
Bio-Rad CFX96™	*Trichomonas vaginalis*	FAM	**-**
	ICD	VIC	

10. Quality Control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instructions. Negative control and positive control have to show correct results (see Tab. 10, Fig. 1) in order to determine a valid run.

The Positive Control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Tab. 10: For a valid run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target Ct
Positive control	Positive	NA *[1]	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	0

*[1] *No Ct value is required for the ICD to make a positive call for the positive control.*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

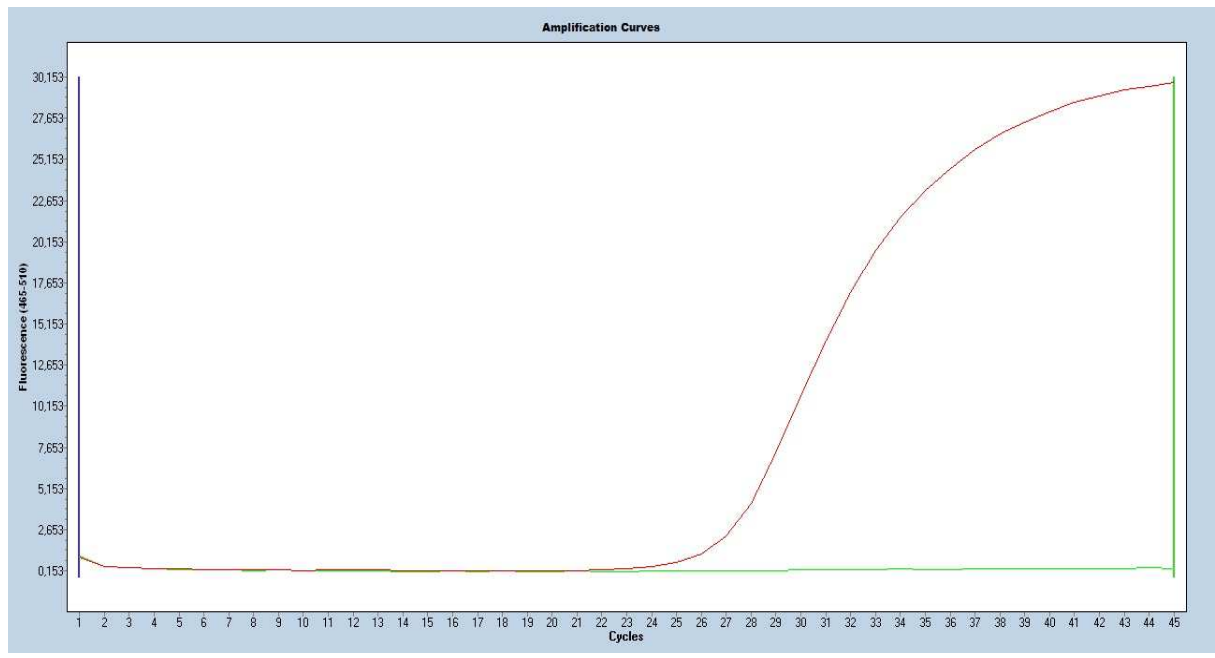

Fig.1: Correct run of the positive control and negative control (*Trichomonas vaginalis*) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Tab. 11.

Tab.11: Sample interpretation

Target genes		
T. vaginalis	**ICD**	**Result**
positive	**positive/negative**	***Trichomonas vaginalis* detected**
negative	**positive**	**Target gene not detected**
negative	negative	**Invalid**

Trichomonas vaginalis is detected, if the sample DNA and the Internal Control DNA show an amplification signal in the detection system.

Trichomonas vaginalis is also detected, if the sample DNA shows an amplification signal but none for the Internal Control DNA in the detection system. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA.

Trichomonas vaginalis is not detected, if the sample DNA shows no amplification signal, but an amplification signal for the Internal Control DNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA.

A sample is invalid, if the sample DNA and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and amplified again, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for human genital swabs and urine samples.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Trichomonas vaginalis assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target gene (ITS1).

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE Trichomonas vaginalis multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction. The following figure 2 shows a dilution series of *Trichomonas vaginalis* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II.

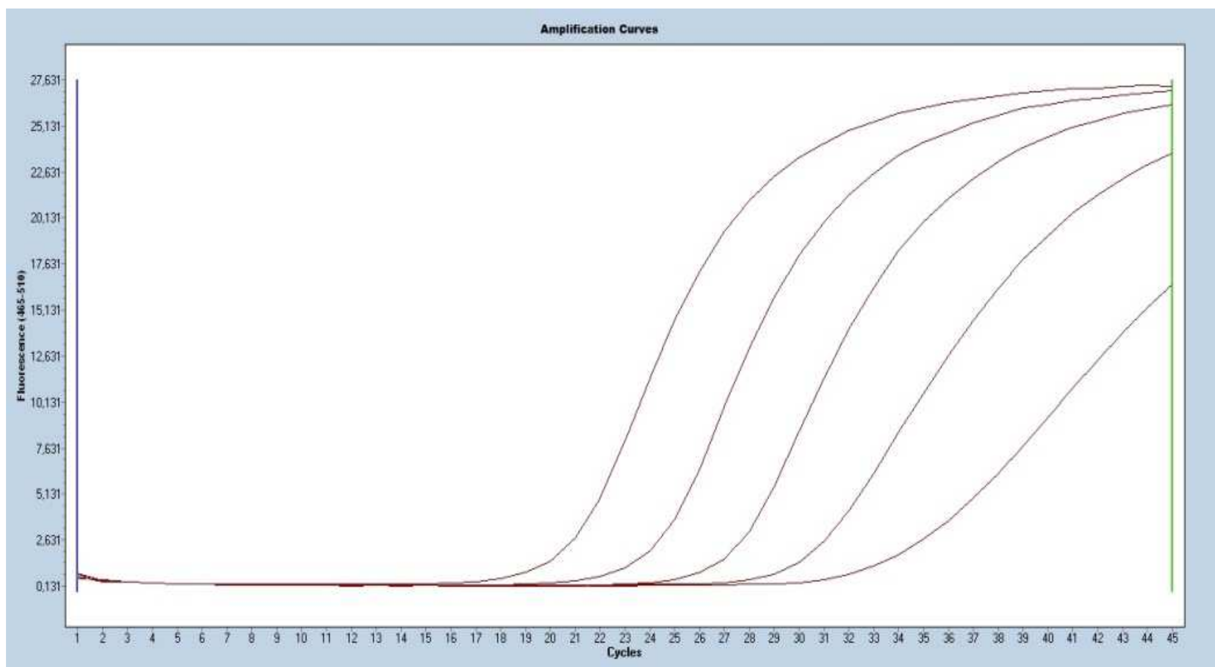

Fig. 2: Dilution series *Trichomonas vaginalis* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

13.2 Analytical specificity

The analytical specificity of the RIDA®GENE Trichomonas vaginalis multiplex real-time PCR is specific for *Trichomonas vaginalis*. No cross-reaction could be detected for the following species (see Tab. 12):

Tab. 12: Cross-reactivity testing

Adenovirus 1, human, strain Adenoid 71	-	*Citrobacter freundii*	-	*Giardia intestinalis* Portland 1	-	*Proteus mirabilis*	-
Adenovirus 7, human, strain Gomen	-	*Clostridium bifermentans*	-	*Giardia intestinalis* WB Clone 6	-	*Proteus vulgaris*	-
Adenovirus 40, human, strain Dugan	-	*Clostridium difficile*	-	*Giardia lamblia*	-	*Pseudomonas aeruginosa*	-
Adenovirus 41, human, strain Tak	-	*Clostridium novyi*	-	HSV 1	-	Rotavirus	-
Arcobacter butzleri	-	*Clostridium perfringens*	-	HSV 2	-	*Salmonella enteritidis*	-
Aeromonas hydrophila	-	*Clostridium septicum*	-	HPV 6b	-	*Salmonella typhimurium*	-
Astrovirus	-	*Clostridium sporogenes*	-	HPV 16	-	*Serratia liquefaciens*	-
Atopobium vaginae	-	*Clostridium sordellii*	-	HPV 18	-	*Serratia marcescens*	-
Bacillus cereus	-	*Cryptosporidium muris*	-	*Klebsiella oxytoca*	-	*Shigella flexneri*	-
Bacteroides fragilis	-	*Cryptosporidium parvum*	-	*Klebsiella pneumoniae*	-	*Staphylococcus aureus*	-
Campylobacter coli	-	*E. coli* (O157:H7)	-	*Mobiluncus curtisii* subsp. *curtisii*	-	*Staphylococcus epidermidis*	-
Campylobacter jejuni	-	*E. coli* (O26:H-)	-	*Mycoplasma pneumoniae*	-	*Staphylococcus saprophyticus*	-
Campylobacter fetus subsp. *fetus*	-	*E. coli* (O6)	-	*Mycoplasma fermentans*	-	*Streptococcus agalactiae*	-
Campylobacter lari subsp. *lari*	-	*Entamoeba histolytica*	-	*Mycoplasma genitalium*	-	*Treponema pallidum*	-
Campylobacter upsaliensis	-	*Enterobacter cloacae*	-	*Neisseria gonorrhoeae*	-	*Ureaplasma urealyticum*	-
Candida albicans	-	*Enterococcus faecalis*	-	Norovirus GGI	-	*Vibrio parahaemolyticus*	-
Candida glabrata	-	*Gardnerella vaginalis*	-	Norovirus GGII	-	*Yersinia enterocolitica*	-
Chlamydia trachomatis	-						

14. Version history

Version number	Chapter and designation
2014-11-09	Release version
2018-04-18	General revision
2018-04-18	4. Reagents provided 6. Additional necessary reagents and necessary equipment 8. Collection and storage 9. Test procedure 13. Performance characteristics 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

Symbol	Meaning
IVD	For *in vitro* diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Not applicable

16. Literature

1. Sutton M. et al. The prevalence of Trichomonas vaginalis infection among Reproductive-age women in the United States, 2001-2004. Clin Infect Disease, 2007; 45:1319-1326.

2. https://www.cdc.gov/std/trichomonas/stdfact-trichomoniasis.htm accessed 18.04.2018

3. Soper D. Trichomoniasis. Under Control or Undercontrolled? Am J Obstet Gynecol. 2004; 190:281-290.