INSTRUCTIONS FOR USE

TRICHOMONAS VAGINALIS

RAPID IMMUNOCHROMATOGRAPHIC TEST FOR QUALITATIVE DETECTION OF *TRICHOMONAS VAGINALIS* FROM UROGENITAL SWABS AND URINE SPECIMENS

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

Trichomoniasis is most commonly transmitted through unprotected sexual intercourse. The vagina is the most common site of infection in women while the urethra is the most common site of infection in men. Trichomoniasis accounts for 15-20% of the cases of vaginitis. It occurs in both men and women and is caused by an infection with the single-celled parasite *Trichomonas vaginalis*. Infection with *Trichomonas vaginalis* is frequently associated with other sexually transmitted diseases and helps spreading the **HIV** virus.

TRICHOMONAS VAGINALIS is a manual lateral flow chromatographic immunoassay for the qualitative detection of *Trichomonas vaginalis* antigen in vaginal swab, male urethral swab and urine specimens. This device is intended to be used as an aid in the diagnosis of *Trichomonas vaginalis* infection.

2 - PRINCIPLE OF THE METHOD

The TRICHOMONAS VAGINALIS is a qualitative, lateral flow chromatographic immunoassay for the detection of *Trichomonas vaginalis* antigen from vaginal swab, male urethral swab and urine specimens. The test strip components consist of: 1) a colored conjugate pad containing polyclonal antibodies specific to the *Trichomonas vaginalis (TV)* conjugated with colloidal gold, and a control antibody conjugated with colloidal gold; 2) a nitrocellulose membrane strip containing the test line (T) and a control line (C line). The T line is coated with TV antibody for the detection of antigen of *Trichomonas vaginalis,* and the C line is coated with anti-rabbit antibodies.

When *Trichomonas vaginalis* antigens are present in the specimen, the T-line will become a pink-colored band. If antigen to *Trichomonas vaginalis* are not present or are present below the detectable level, no T-line will develop. The C-line should always appear as a pink-colored band regardless of the presence of antigen to *Trichomonas vaginalis*. The C-line serves as an internal qualitative control of the test system to indicate that an adequate volume of specimen has been applied and the flow occurred.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
TRICHOMONAS VAGINALIS	Immunochromatographic test	**VQ81604** (20 tests)	25 sealed in foil pouch containing the strip, with dessicant. Diluent-Extraction buffer: plastic dropper bottle containing 0,5% Tween-80 – 0,01 M PBS, NaN_3 (<0,1%). (1x20 mL). Glass dropper vial containing positive control: non-infectious components and NaN_3 (<0,1%) as preservative. (1x0,5 mL) Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile swabs for collecting sample, Tubes for reaction, Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- TRICHOMONAS VAGINALIS is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by qualified laboratory personnel also minimally skilled.
- This product is not classified as dangerous according to current European legislation.
- Read the package insert instruction before use the kit.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- Each test strip is for single use only.
- Never use reagents from another lot.
- The test strip should remain in the sealed pouch until use.
- Discard opened-unused tests after 1 hour.
- Do not use the test if pouch is damaged.
- Wear gloves when handling the sample.
- Disposable gloves, buffer solutions, plastic tubes and used strips in a propre biohazard container.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

6 - STORAGE CONDITIONS AND STABILITY

Store as packaged in the sealed pouch at room temperature or refrigerated (2°C-30°C). Do not expose the kit over 30°C. Do not freeze the kit. If stored at 2°C-8°C, ensure that the test strip is brought to room temperature before opening. The test strip is stable through the expiration date printed on the sealed pouch.

7 – SPECIMEN COLLECTION AND PREPARATION

URINE SAMPLE

Collect a urine specimen in a clean glass, plastic, or wax coated container. Do not use preservatives. If the test is not run immediately following collection of the sample specimen, but is to be run within 24 hours following collection. Please take the equal volume of sample buffer of kit into test tube, and mix

well, the diluent sample should be refrigerated (2-8°C), and brought back to room temperature (15-28°C) before testing. If testing is delayed more than forty-eight hours, the specimen should be frozen at -20°C or lower. Prior to testing, the frozen specimen must be completely thawed, thoroughly mixed, and brought to room temperature.

SWAB SAMPLE

Collect specimen with a sterile swab from vaginal cavity, or Glans. Process the swab as soon as possible after collection specimen. This test does not require live organisms for processing. If you cannot dry the swabs or cannot perform test immediately, extract the swabs in Sample Buffer as per protocol and store the aqueous extracted specimen at 2–8°C for up to 24 hours. Alternatively, dried swabs can be stored at 2-8°C for up to 24 hours prior to extraction and testing.

8 - TEST PROCEDURE

Allow the tests, samples and reagent to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

Urine sample

It is recommended to collect the first urine of the day for higher accuracy

1. Dispense 3 to 4 drops (~80 µL) of diluent-extraction buffer into test tube.
2. Pipette equal volume 3-4 drops (~80 µL) of urine into test tube.
3. Mix well in 5-10 seconds.
4. Remove a Trichomonas V Test Strip from its foil pouch.
5. Holding the strip vertically, place it in the specimen test tube. Do not immerse the strip past the maximum line.
6. The test strip can be left in the tube or removed from the tube when red dye begins to migrate through the test line.
7. Read the test results at 15 minutes. If the reading time be after in 30 minutes the test will be considered as a failure.
8. Interpretation of Result by eyes.

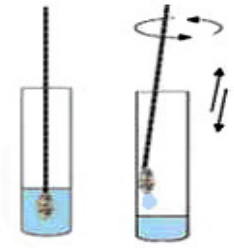
Swab sample

1. Dispense 0,8-1,0 mL (16 drops) of diluent-extraction buffer into a test tube.
2. Place the sample collected by swab into the tube and mix the sample buffer by rotating the swab vigorously in the tube.
3. Allow the swab to soak in sample buffer for 30-60 seconds.
4. Mix again and remove the swab, pressing against the side of the tube to extract as much liquid as possible. Discard the swab.
5. Remove a TV strip test from the foil pouch.
6 Holding the strip vertically, place it in the specimen test tube. Do not immerse the strip past the maximum line.
7. The test strip can be left in the tube or removed from the tube when red dye begins to migrate through the test line.
8. Read the test results in 15 minutes, (positive results may be seen earlier). If the reading time be after in 30 minutes the test will be considered as a failure.
9. Interpretation of Result by eyes.

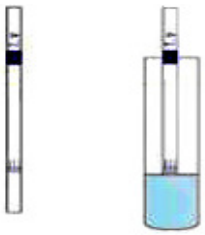
For Positive and Negative controls add 6 drops in a new test tube and use the same procedure (start from point 5 of swab sample).

1. Add diluent-extraction buffer

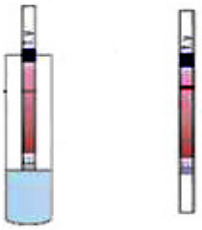
2-4. collect the sample and put the swab into the tube. Mix and remove the swab



5-6. Insert the strip



7. Waiting 15 minutes

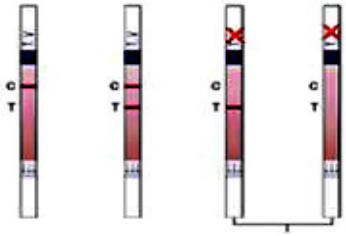


9 – READING AND INTERPRETATION OF RESULTS

POSITIVE RESULT: Two lines appear. One colored line should be in the control line region (in the illustration with "C") and another apparent colored line should be in the test line region (in the illustration with "T").

NEGATIVE RESULT: One colored line appears in the control line region (in the illustration with "C"). No line appears in the test line region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



Negative Positive Invalid

10 - INTERNAL QUALITY CONTROL

The Internal Quality Control procedure is included in each test strip. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

11 – EXPECTED VALUES

The latest data published by the World Health Organisation (WHO), in June 2019, 'Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016', indicate that in 2016, there were an estimated 156 million new cases of Trichomonas vaginalis (Tv) infection worldwide, in women and men aged 15-49 years, and that Tv infection represents the most prevalent 1st of the four reported (127 million new cases of chlamydia infection, 86 million new cases of gonorrhoea and 6.3 million new cases of syphilis). The Western Pacific Region and the African Region reported the highest incidence of Tv infections in both men and women (45 and 43 million new cases per year, respectively).

12 - PERFORMANCES CHARACTERISTICS

A. Correlation, Sensitivity-Specificity

The gold standard was the patient positive to speculum and vaginal examinations to be diagnosis Trichomonas Vaginalis infection. The total 204 cases was compared to wet mount microscopy pouch culture and Mascia Brunelli Trichomonas vaginalis Test Kit. The results are summarized as below table: the Mascia Brunelli Trichomonas vaginalis Test kit will detect soluble antigen present in vaginal samples with at least 20 organisms.

Method (N=204)	Sensitivity	Specificity
Microscopy	72.5%	78.5%
Culture	91.2%	92.8%
Trichomonas Vaginalis kit (Mascia Brunelli)	100%	99%

B. Precision

Due to the nature of the test system, precision was evaluated by testing various samples on consecutive days, by multiple clinicians at different geographic locations. Trichomonas Vaginalis Mascia Brunelli precision studies were run on artificially generated laboratory samples reflecting the known range of T. vaginalis parasite burden levels in female patients. Given the design of the product, the Trichomonas Vaginalis Mascia Brunelli performed according to expectations: the Trichomonas Vaginalis kit performed with >99% precision over the intra-assay (between day, between clinician, and between clinical site) parameter.

13 - LIMITATIONS OF THE TEST

1. TRICHOMONAS VAGINALIS is for in vitro diagnostic use only. This test should be used for the detection of *Trichomonas vaginalis* antigen from vaginal swab, urethral swab and urine specimens. Neither the quantitative value nor the rate of increase in *Trichomonas vaginalis* antigen concentration can be determined by this qualitative test.
2. A positive result does not preclude the possibility of infections by other pathogens.
3. The results obtained with this kit must be interpreted together with other clinical information and laboratory findings available to the physician.
4. This test provides a presumptive diagnosis of Trichomonas V. infections during the acute phase of infections. Samples collected after acute phases of infection may contain titles of antigens below the threshold of sensitivity of the reagent. If the test result is negative and clinical symptoms persist, additional testing using other diagnostic methods is recommended.

14 - REFERENCES

1. Zhang, Z.F., S. Graham, S.Z. Yu, J. Marshall, et al. 1995. Trichomonas vaginalis and cervical cancer. A prospective study in China. Ann. Epidemiol. 5:325-332.
2. World Health Organization. 1995. An overview of selected curable sexually transmitted diseases. Pp. 2-27. In Global Program on AIDS. World Health Organization. Geneva, Switzerland.
3. Cotch, M.F., J.G. Patorek III, R.P. Nugent, et al. 1997. Trichomonas vaginalis associated with low birth weight and preterm delivery. Sex. Transm. Dis. 24:353-360.
4. OMS study, "Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016".

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 5	Updated layout and content.	2023/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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