EZER™ Candida albicans/Gardnerella vaginalis/Trichomonas vaginalis antigen Combo Rapid Test

INTENDED USE

The EZER™ Candida albicans/ Gardnerella vaginalis/ Trichomonas vaginalis antigen Combo Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of Candida albicans/Trichomonas vaginalis /Gardnerella vaginalis antigens from vaginal swabs. It is intended to be used as a screening test and as an aid in the diagnosis of infection with *Candida albicans, Trichomonas vaginalis and Gardnerella vaginalis.* Any reactive specimen with the Candida albicans/Gardnerella vaginalis/Trichomonas vaginalis antigen Combo Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

BACKGROUND

Vulvovaginal candidiasis (VVC) is a common clinical gynecological disease, and the infection rate is second only to bacterial vaginitis, which greatly affects women's reproductive health. Its pathogens mainly include *Candida albicans, Candida glabrata, Candida tropicalis, Candida paralpara, Monilia guilliermondii*. Among them, *C. albicans* is a common pathogen of the disease, and the infection rate is about 80%. Symptoms of VVC which include: acute itching, vaginal soreness, irritation, rash on the outer lips of the vagina and genital burning that may increase during urination, are nonspecific. Clinicians should keep in mind that a broad variety of infectious and noninfectious diseases can cause a similar array of symptoms. To obtain an accurate diagnosis, a thorough evaluation is necessary. In women who complain of vaginal symptoms, the standard tests should be performed, such as saline and 10% potassium hydroxide microscopy, Microscopy is the mainstay in the diagnosis of VVC, yet studies show that, in academic settings, microscopy has a sensitivity of at best 50% and thus will miss a substantial percentage of women with symptomatic VVC, To increase the accuracy of diagnosis, yeast cultures have been advocated by some experts as an adjunctive diagnostic test, but these cultures are expensive and underutilized, and they have the further disadvantage that it may take up to a week to get a positive result. Inaccurate diagnosis of candidiasis may delay treatment and cause more serious lower genital tract diseases.

Trichomonas infection is responsible for the most common, non-viral sexually transmitted disease (vaginitis or trichomoniasis) worldwide. Trichomoniasis is a significant cause of morbidity among all infected patients. Effective diagnosis and treatment of Trichomonas infections have been shown to eliminate symptoms. Conventional identification procedures for Trichomonas from vaginal swabs or vaginal washes involve the isolation and subsequent identification of viable pathogens by wet mount microscopy or by culture, a process that will cost 24-120 hours. Wet mount microscopy has a reported sensitivity of 58% versus culture.

Gardnerella vaginalis is an anaerobic bacterium that resides in the normal vaginal flora. Normally, vaginal flora is predominated by the *Lactobacilli* species, but when organisms such as *Gardnerella* begin to overgrow and become the dominant species, this leads to bacterial vaginosis (BV). Bacterial vaginosis is characterized by the presence of clue cells, which are epithelial cells of the cervix that are covered with rod-shaped bacteria.

PRINCIPLE

Candida albicans/Gardnerella vaginalis/Trichomonas vaginalis antigen Combo Test is a qualitative, membrane based immunoassay for the detection of *Candida albicans, Trichomonas vaginalis, and Gardnerella vaginalis* antigens through visual interpretation of color development on the internal strip. When the appropriate amount of test samples treated with dilution buffer is added to the sample well of the test cassette, the sample will move forward along the test strip by capillary action. If the sample contains *Candida albicans, Trichomonas vaginalis, and Gardnerella vaginalis* antigen, and the concentration is higher than the limit of detection, the antigen will form immune complexes with corresponding *Candida albicans, Trichomonas vaginalis, or Gardnerella* vaginalis antibody labeled with colored particles impregnated, which are captured by CA line, TV line and GV line. If test sample contains *Candida albicans,* forming a red CA line, indicating a positive result for *Candida albicans*. If test sample contains *Trichomonas vaginalis* , forming a red TV line, indicating a positive result for *Trichomonas vaginalis.* If test sample contains *Gardnerella vaginalis* , forming a red GV line, indicating a positive result for *Gardnerella vaginalis* .

To serve as a procedural control, a colored line will always appeared in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

The control area (C) of the test card is coated with sheep anti-mouse IgG. Regardless of the presence of *Candida albicans* Antigen in the sample, mouse antibody-colloidal gold complex combine to it and forms a purplish red band. Otherwise, the test results are invalid and must be repeated with another test card.

CONTENTS

Test device	20	Tests
Sample buffer, 10mL	2	Vials
Sterile Swabs	20	pcs.
Extraction Tubes	20	pcs.
Extraction Tube Tips	20	pcs.
Tube Stand	1	pc.
Package insert	1	pc.

STORAGE CONDITIONS

Store the EZER™ Candida albicans/Gardnerella vaginalis/Trichomonas vaginalis antigen Combo Rapid Test at room temperature or refrigerated (2-30°C). All reagents are stable until the expiration dates printed on the kit box or the label inside.**DO NOT FREEZE**. Do not use beyond the expiration date.

SAMPLE COLLECTION AND PREPARATION

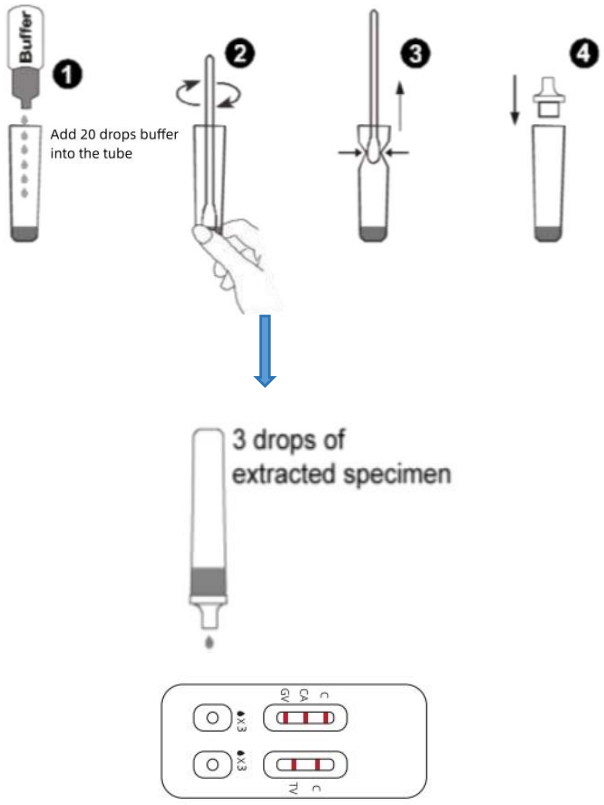
1. The quality of the specimen is essential and the swab shall be ensured to contain a sufficient amount of microorganisms to be tested.
2. Use a sterilized swab take the vaginal secretions in the posterior vaginal vault, preferably a cheese-like, tofu-like white clot. In the peep-free condition, a long swab can also be extended into the vaginal to take material.
3. Put the swab to the extraction tube, if the test is to be performed immediately. If immediate testing is not possible, the patient specimen should be placed in a dry transport tube for storage or transport. The swabs may be stored for 4~6 hours at room temperature (15-30℃) or 24~72 hours at 4℃ or no more than 6 months at -20℃. All specimens should be allowed to reach a room temperature of 15-30℃before testing. The specimen should not freeze and melt more than once (i. e., if the specimen is removed from-20℃ and restored to room temperature, it should be tested immediately, and should not be stored in-20℃ again).

PROCEDURE

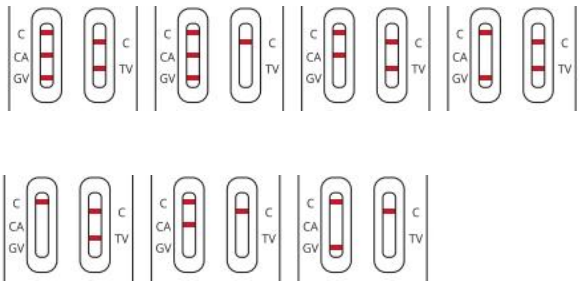
Allow the test, specimen swab, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Place a clean extraction tube in the designated area of the workstation. Add 20 drops buffer into the tube.
2. Put the specimen swab into the tube, vigorously mix the solution by rotating the swab forcefully against the side of the tube for least ten times (while submerged). Best results are obtained when the specimen is vigorously mixed in the solution.
3. Allow the swab to soak in the extraction buffer for 2 minutes prior to the next step. Squeeze out as much liquid as possible from the swab by pinching the slide of the flexible extraction tube as the swab is removed. At least 1/2 of the extraction buffer solution must remain in the tube for adequate capillary migration to occur.
4. Discard the swab in a suitable bio-hazardous waste container, then fit on the extraction tube tip onto the extraction tube.
5. Remove the test cassette from its sealed pouch, and place it on a clean and level surface. To obtain a best result, the assay should be performed within one hour.
6. Add 3 drops (approx. 100µl) of extracted specimen from the extraction tube to the specimen well on the test cassette. Please avoid trapping air bubbles in the specimen well and do not drop any solution in observation window.
7. Wait for the colored line(s) to appear. The result should be read at 15 minutes, do not interpret the results after 20 minutes.

Note: It is suggested not to use the buffer, beyond 6 months after opening the vial.



INTERPRETATION OF RESULTS



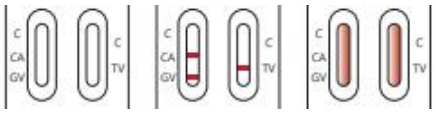
Positive Result:

If the quality control C line appears, and one or more red lines appear in the CA/ GV/ TV detection line area, indicating that the sample contains one or more pathogenic microorganisms.

※ **NOTE: The intensity of the color in the test line region(T)will vary depending on the concentration of *Candida albicans, Trichomonas vaginalis, Gardnerella vaginalis* antigens in the specimen.**

Therefore, any shade of color in the test line region(T)should be considered positive.

Negative Result:
The colored line in the control line region (C) appears. No line appears in the CA/ GV/ TV detection line area.



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

QUALITY CONTROL

Internal control
Each *EZER*™ Candida albicans/ Gardnerella vaginalis/ Trichomonas vaginalis antigen Combo Rapid Test contains internal/procedural controls. The appearance of a control line at the Control "C" position validates the proper reagent function and assures that the correct test procedure was followed.

PRECAUTIONS FOR ASSESSMENT

- For *in vitro* diagnostic use only.
- Do not use after the expiration date.
- Ensure foil pouch containing test device is not damaged before opening for use.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.
- Do not exchange or mix buffer and test cassettes from kits of different lots.
- Be sure to add sufficient extracted specimen to the cassette's specimen well. Invalid result may occur if inadequate extracted specimen is added.

PERFORMANCE CHARACTERISTICS

1.Detection Limitation

The EZER™ Candida albicans/Gardnerella vaginalis/Trichomonas vaginalis antigen Combo Rapid Test can detect Candida albicans as low as 1.4 × 10^5CFU/ml, Gardnerella vaginalis as low as 1.07 × 10^5CFU/ml, and Trichomonas vaginalis as low as 1.17 × 10^4 cells/ml.

2.Clinical Sensitivity, Specificity and Accuracy

The clinical research was evaluated by comparing the EZER™ Candida albicans/ Gardnerella vaginalis/ Trichomonas vaginalis antigen Combo Rapid Test with other rapid test method for BV and CA, Microscopic examination for *Trichomonas vaginalis* respectively, to evaluate the clinical sensitivity and specificity of the Candidate Kit. The Clinical Test results of the test kit and the reference method are summarized in the 2×2 table below：

3.*Candida albicans* antigen test result vs. Other Rapid Test

Method		Other Rapid Test result		Total Results
Candida albicans antigen test result	Results	Positive	Negative	
	Positive	12	1	13
	Negative	1	69	70
Total Results		13	70	83

Relative Sensitivity: 92.3% (95%CI*: 64%~99.8%);

Relative Specificity: 98.6% (95%CI*: 92.3%~＞99.9%);

Overall Accuracy: 97.6% (95%CI*: 91.6%~99.7%).

*Confidence Intervals

4.*Gardnerella vaginalis* antigen test result vs. Microscopic examination

Method		Other Rapid Test result		Total Results
Gardnerella vaginalis antigen test result	Results	Positive	Negative	
	Positive	12	1	13
	Negative	1	69	70
Total Results		13	70	83

Relative Sensitivity: 92.3% (95%CI*: 64%~99.8%);

Relative Specificity: 98.6% (95%CI*: 92.3%~＞99.9%);

Overall Accuracy: 97.6% (95%CI*: 91.6%~99.7%).

*Confidence Intervals

5. *Trichomonas vaginalis* antigen test result vs. Other Rapid Test

Method		Microscopic examination		Total Results
Trichomonas vaginalis antigen test result	Results	Positive	Negative	
	Positive	247	0	247
	Negative	1	1035	1036
Total Results		248	1035	1283

Relative Sensitivity: 99.6% (95%CI*: 97.77%~99.9%);

Relative Specificity: 100% (95%CI*: 99.64%~100%);

Overall Accuracy: 99.92% (95%CI*: 99.57%~100%).

*Confidence Intervals

6.Cross-reactivity

Cross reactivity with other organisms has been studied using suspensions of 10^6 Colony Forming Units (CFU)/test. The following organisms were found negative when tested with the Candida albicans/Gardnerella vaginalis/Trichomonas vaginalis antigen Combo Rapid Test.

Acinetobacter calcoaceticus *Proteus vulgaris*

Salmonella typhi *Mycoplasma hominis*
Staphylococcus aureus *Acinetobacter spp.*
Neisseria catarrhalis *Neisseria gonorrhea*
Neisseria meningitides *Escherichia coli*
Gardnerella vaginalis *Streptococcus faecalis*
Streptococcus faecium *Pseudomonas aeruginosa*
Chlamydia trachomatis *Ureaplasma urealyticum*
Mycoplasma hominis

7.Interfering Substances

The following compounds have been tested using the Candida albicans/Gardnerella vaginalis/Trichomonas vaginalis antigen Combo Rapid Test and no interference was observed.

Substance	Concentration with no interference
Whole Blood	4%
Mucin	0.3%
urine	4%
nystatin (plug)	5 mg/ml
miconazole (plug)	5 mg/ml
tinidazole (gel)	5 mg/ml
metronidazole (gel)	5 mg/ml
clean shade (lotion)	2% v/v
clean lotion (lotion)	2% v/v

LIMITATIONS OF THE PROCEDURE

1.The Candida albicans/ Gardnerella vaginalis/ Trichomonas vaginalis antigen Combo Rapid Test is for in vitro diagnostic use only. The test should be used for the detection of *Candida albicans*， *Trichomonas vaginalis and Gardnerella vaginalis* antigens in vaginal swab specimens only. Neither the quantitative value nor the rate of increase in *Candida albicans*， *Trichomonas vaginalis* and *Gardnerella vaginalis* antigens concentration can be determined by this qualitative test.
2. A negative result should be confirmed by culture. A negative result may be obtained if the concentration of the *Candida albicans* antigen present is not adequate or is below the detectable limit of the test.
3.The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

AVAILABILITY

Product	Cat. No.	Contents
EZER™ Candida albicans/ Gardnerella vaginalis/ Trichomonas vaginalis antigen Combo Rapid Test	P271102	20 Tests

REFERENCES

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2. François L Mayer, Duncan Wilson, Bernhard Hube. Candida albicans pathogenicity mechanisms. Virulence. 2013 Feb 15;4(2):119-28.
3.Van Der Pol B. Clinical and Laboratory Testing for Trichomonas vaginalis Infection. J Clin Microbiol. 2016 Jan;54(1):7-12.
4.António Machado and Nuno Cerca. Influence of Biofilm Formation by Gardnerella vaginalis and Other Anaerobes on Bacterial Vaginosis. J Infect Dis. 2015 Dec 15;212(12):1856-61.
5. GE Garber. The laboratory diagnosis of Trichomonas vaginalis. Can J Infect Dis Med Microbiol2005， 16(1): 35-38.
6. Sheng TANG ， Heng-xian LIN,et al. Method Establishment and Clinical Performance Evaluationof Immunofluorescence Assay for Gardnerella Vaginalis,Energy Procedia 2012,70: 1805-1810.

Index of Symbols

	Attention, see instructions for use		Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
	Store between 2~30℃	LOT	Lot Number	REF	Catalog #

EC REP **CMC Medical Devices & Drugs S.L.**
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EC REP **Luxus Lebenswelt GmbH**
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