Original Article

Evaluation of the Sensitivity and Specificity of Rapid Immunochromatographic Strip Using PCR Assay for Detection of Candidiasis, Trichomoniasis, and Gonorrhea in Iraqi Patients

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Abstract

Background: Sexually transmitted diseases (STDs) are infections transmitted from an infected person to an uninfected person through sexual contact. STDs can be caused by bacteria, parasites, or viruses. Examples include gonorrhea, trichomoniasis, chlamydia, and syphilis. **Objective:** The objective of this study was to compare the immunochromatographic (IC) strip with the molecular assay in order to assess the sensitivity and specificity of each method for detecting *Candida albicans*, *Trichomonas vaginalis*, and *Neisseria gonorrhea* in the Karbala and Babylon cities. **Materials and Methods:** From November 2022 to the end of June 2023, 300 patients (150 male and 150 female) seeking treatment for abnormal vaginal discharge, itching, dysuria, dyspareunia, urethritis, and prostatitis at private clinics in Karbala and Babylon cities participated in a cross-sectional study. The entire patient database was contained in a single questionnaire. Using an immunochromatographic kit for detection of *C. albicans*, *T. vaginalis*, and *N. gonorrhea*. **Results:** The current study compared the sensitivity and specificity of polymerase chain reaction (PCR) and IC strips, which finding that the sensitivity was 91.76%, specificity 94.91%, and accuracy 93.06% for *Candida albicanis*, and the sensitivity and specificity of both PCR and IC strip for detection of *T. vaginalis*, showed that the value of sensitivity was 87.23%, specificity 97.93% and accuracy 94.44%, as well as the sensitivity and specificity of both PCR and IC strip for detection of *N. gonorrhea*, presented that the value of sensitivity was 41.67%, specificity 95.37% and accuracy 81.94%. **Conclusion:** The new IC strip has more sensitivity and specificity than routine diagnostic tests for *C. albicans*, *T. vaginalis*, and *N. gonorrheae*.

Keywords: *Candida albicans*, immunochromatographic strip, *Neisseria gonorrhea*, *Trichomonas vaginalis*

Introduction

More than one million sexually transmitted diseases (STDs) are picked up every single day around the globe, with the vast majority of those cases going undiagnosed. An estimated 374 million new cases of STDs that may be treated arise every year. These STDs include trichomoniasis, gonorrhea, chlamydia, and syphilis.[1]

Due to the great frequency of STDs and the variety of testing for each disease, choosing diagnostic tests is difficult. Many current STD tests have disadvantages that may limit their effectiveness in STD control. In an era with limited resources, applying the numerous tests available for a given purpose can be difficult. Prioritization should include the likelihood of an illness, its impact on people and populations, its complications, test performance aspects, test costs, and the purpose of the testing.[2]

Immunochromatographic capillary flow strip that detects *Candida albicans, Trichomonas vaginalis*, and *Neisseria gonorrhea* membrane proteins in about 10–15 min. Rapid

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diagnostic strips that identify *C. albicans*, *T. vaginalis*, and *N. gonorrhea* antigens or nucleic acids have an advantage over microscopy and culture in that they are not limited by timely transportation and specimen processing. The Rapid test has a sensitivity of 77–98% and a specificity of 99–100% when performed on vaginal secretions or swabs; however, it should not be utilized in asymptomatic females or males. False positives are possible, particularly in low-prevalence groups.[3,4]

A study was conducted at the Institute of Microbiology and Immunology (IMI), Faculty of Medicine, University of Ljubljana, to compare the efficacy of three techniques for detecting *C. albicans*, *T. vaginalis*, and *N. gonorrhea* in urogenital swabs: wet mount microscopy, culture, and real-time polymerase chain reaction (PCR). Specimens were obtained from 75 male and 80 female patients who had STD symptoms, sexual risk behavior, or a sex partner with definite STDs, where the results of the study showed that wet mount microscopy revealed that all 155 specimens (100%) were negative. After culture, 154 (99.4%) vaginal swabs were negative, and one (0.6%) was positive, however, six specimens tested positive using real-time PCR (five vaginal swabs from five female patients with an average age of 31.4 years and one urethral swab from a 31-year-old male patient).[5]

The PCR analysis is a laboratory method used to determine whether or not a sample contains trace levels of the genetic material DNA. The PCR has a broad range of uses, one of which is the diagnostic of numerous STDs. For instance, if a lab examines a urine sample, they may uncover DNA that indicates the presence of *C. albicans*, *T. vaginalis*, or gonorrhea.[6]

The aim of the study was to determine the specificity and sensitivity of immunochromatographic (IC) strips with a molecular assay to detect *C. albicans*, *T. vaginalis*, and *N. gonorrhea* in Karbala and Babylon cities.

Materials and Methods

The investigation begin from November 2022 to the end of June 2023, a cross-sectional study was conducted on 300 patients (150 male and 150 female) who sought care at private clinics in Karbala and Babylon cities due to symptoms such as abnormal vaginal discharge, itching, dysuria, dyspareunia, urethritis, and prostatitis. Each patient, whose ages varied from 20 and above, had their complete data entered into a unique questionnaire. High vaginal swabs, cervical swabs in females, and urethral swabs for males were collected from each patient using sterile swabs after a thorough clinical examination. Swabs were taken without the use of any antiseptic creams. Phosphate buffer saline was used immediately to soak each swab. All samples collected are transported in an ice pack box until processing for rapid immunochromatographic assay and conventional PCR assay.

Immunochromatographic assay procedure

Five drops of collection solution were added to the extraction tube, the sample swab was placed inside, and the swap was forcefully rotated on the tube's side at least 10 times to mix. Mixing the sample in the solution yields the best results and after saturating in the extraction buffer for at least one minute, the swab was squeezed as much liquid as possible by pinching the side of the flexible extraction tube. Capillary migration requires half the specimen buffer solution in the tube. The extracted tube was tipped. The device identified the patient or control. Three drops of extracted buffer on the test cassette. After 15 min, the result was read.

DNA was produced using one QIAamp® DNA Mini Kit from Qiagen in Valencia, CA, USA, as directed. The QIAamp® DNA Mini Kit came with the following manufacturer's instructions: 20 µL of Proteinase K and 200 µL of Buffer A1 were added to 200 µL of fluid from swabs, which was then incubated at 56°C for 10 min. In a 2 mL collection tube, the mixture was put into a QIAamp® Mini spin column, centrifuged at 16,000*g* for one minute, and the tube containing the filtrate was discarded. 200 µL of ethanol was then added to the mixture. The mini spin column received 500 µL of Buffer AW1 and was centrifuged for 1 min at 16,000*g* then added 500 µL of AW2 and the column was centrifuged at full speed 16,000*g* for one minute. Then100–200 µL of elution Buffer was added to the Mini spin column and centrifuged at 16,000 g for 1 min to obtain DNA and stored at −10°C for PCR assay.

PCR assay

Three primers utilized in the current study were: 5′CATTGATAACGAAGCTCTTTACGAT3′ and 5′GCATGTTGTGCCGGACATAACCAT3′ for *T. vaginalis*,[7] 5′GTTTCAGACGGCCAAAAGCC3′ and 5′GGCATAAAATCCACCGTCCCC3′ for *N. gonorrhea*,[8] and 5′TTTATCAACTTGTCACACCAGA3′ and 5′ATCCCGCCTTACCACTACCG3′ for *C. albicans*.[9] In each of the PCR reaction mixtures, there was 5 µL of DNA, 2 µL of 10× PCR buffer, 5.2 µL of 5 M betaine, and 3.7 µL of distilled water. The primers were each present in 1 µL at a concentration of 10 pmol/µL. Taq polymerase was present in 0.1 µL at a concentration of 5 U/µL.

The PCR protocol included initial denaturing of the DNA for 5 min at 94°C, it was subjected to 40 cycles consisting of 10 s of denaturation at 98°C, 30 s of annealing at 57°C for *C. albicans*, 55°C for *T. vaginalis*, and 52°C for *N. gonorrhea*, and 30 s of extension at 72°C. A simple gel electrophoresis method has been described for the detection of DNA in *C. albicans, T. vaginalis*, and *N. gonorrhea* at 100 V for 1 h.

Table 1: Number of sexually transmitted microorganisms among sex

Types of microorganisms	Female	%	Male	%	Total	%
Positive *Candida*	59	19.67	22	7.33	81	27
Positive *Trichomonas*	34	11.33	9	3	43	14.33
Positive *Neisseria*	6	2	14	4.67	20	6.67
Negative sample	51	17	105	35	156	52
Total	150		150		300	100
Statistical analysis	$\chi^2 = 16.74$, $df = 2$, $P = 0.0002$					

Inclusion criteria

Individuals who do not have regular menstruation or irregular uterine bleeding. Participants who had not used any antibiotics in the two weeks prior to the study beginning and adults (aged 20–59) who have not been treated for a vaginal discharge or urethritis in the week leading up to the sample collection were confusing.

Ethical approval

The study followed the ethical principles of clinical research: respect, benefit, and justice. Patients were informed about the project, its importance, how the sample would be taken, and that it would not harm them. The study was confidential and free. Patients' confidentiality was maintained due to their trust in the research services. Each patient was informed of the study and their permission to participate was sought after it was confirmed that they met the inclusion criteria. In order to collect data on the patients' socioeconomic status, clinical history, and sexual behavior, a questionnaire was distributed to them. Each individual patient provided a sample while working with medical staff at the institution's designated time.

Exclusion criteria

The study excluded the following: not active sexual partner, less than 18 years and above 59 years, urine samples for both sexes, patients taking antibiotics, and sample drying or delayed.

Statistical analysis

Sensitivity, specificity, disease prevalence, as well as accuracy, are expressed as percentages were calculated by using the Statistical Package for the Social Sciences (SPSS) version 25.0 (SPSS, IBM Company, Chicago, IL 60606, USA).

Results

In a study comprising 300 patients, distributed evenly between 150 males and 150 females, the results found 144 positive strips revealed that 81 samples (27%) tested positive for *C. albicans*, 43 samples (14.33%) tested positive for *T. vaginalis*, and 20 samples (6.67%) tested positive for *N. gonorrhea*. The remaining 156 samples (52%) were negative and may be attributed to other causative agents. The distribution of the infection in the clinical samples

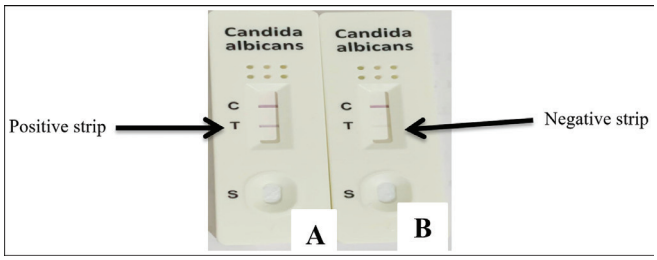

Figure 1: The positive (A) and negative (B) IC strip for *Candida albicanis*. A: Positive results two lines appear at C and T lines. B: Negative results one line appears C-line

was analyzed based on gender. The results indicated that *C. albicans* were present in 19.67% of females, while *T. vaginalis* and *N. gonorrhea* were found in 11.33% and 2% of females, respectively. Additionally, the aforementioned samples exhibited prevalence rates of 7.33%, 3%, and 4.67% in males for *C. albicans*, *T. vaginalis*, and *N. gonorrhea*, respectively. The current investigation observed a distinct and statistically significant disparity (χ^2= 16.74, P = 0.0002) in the prevalence of certain STDs among individuals of different genders. Specifically, *T. vaginalis* exhibited a higher infection rate among females compared to males, whereas *N. gonorrhea* demonstrated a higher infection rate among males compared to females. This finding was derived from the data presented in Table 1.

The outcomes of the current research, when comparing the sensitivity and specificity of both PCR and IC strips for detection of *C. albicans* [Figures 1 and 2], showed that the value of sensitivity was 91.76%, with confidence interval (95% CI: 83.77–96.62%), specificity 94.91% with confidence interval (95% CI: 85.85–98.94%) accuracy 93.06% with confidence interval (95% CI: 87.06–96.62%).

Of the 144 positive sexual clinical samples collected from female and male patients, there were 85 positive samples of Candidiasis fungus diagnosed by PCR technique.

The results of the current study, when comparing the sensitivity and specificity of both PCR and IC strip for detection of *T. vaginalis* [Figures 3 and 4], showed that the value of sensitivity was 87.23%, with confidence interval (95% CI: 74.26–95.17%) and specificity 97.93% with confidence interval (95% CI: 92.75–99.75%) accuracy 94.44% with confidence interval (95% CI: 89.35–97.57%).

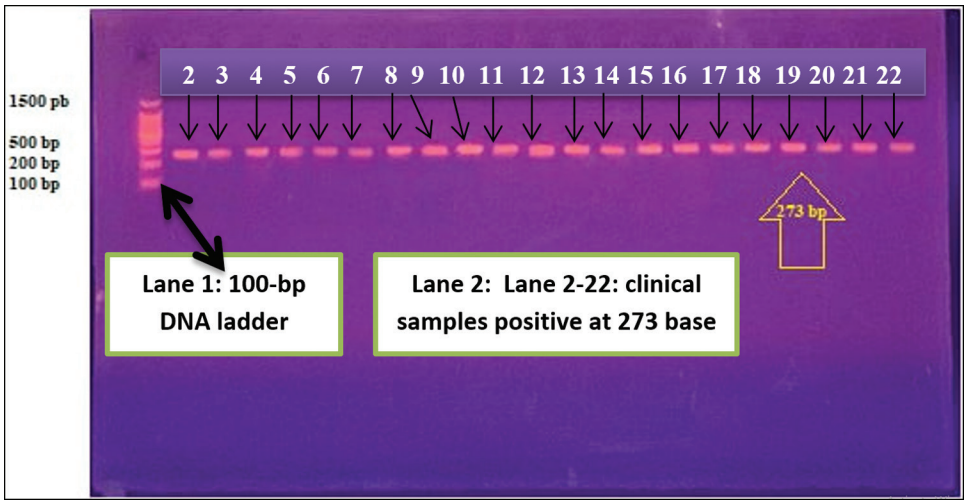

Figure 2: Agarose gel electrophoresis for detection of *Candida albicans* (internal transcribed spacer ITS gene) by polymerase chain reaction (PCR)

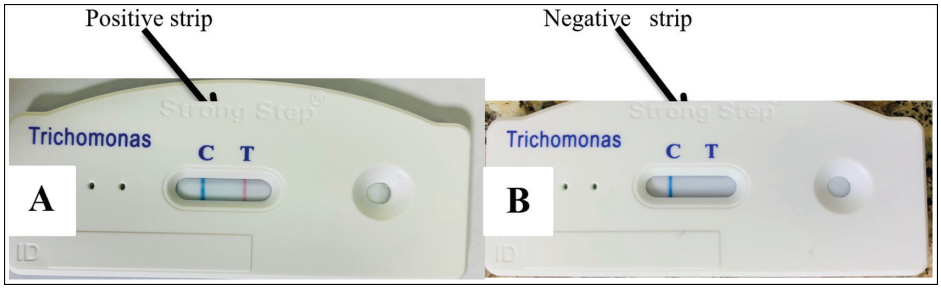

Figure 3: show the positive (A) and negative (B) IC strip for *Trichomonas vaginalas*

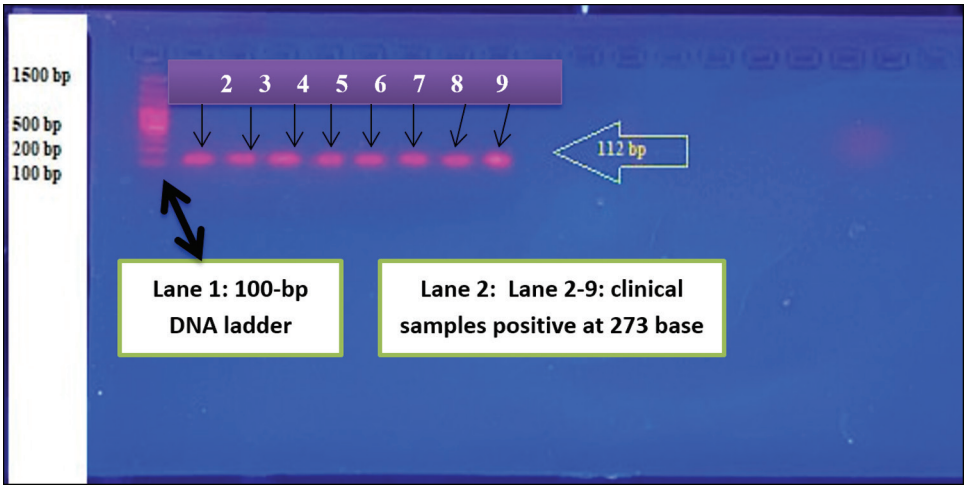

Figure 4: Agarose gel electrophoresis for detection of *Trichomonas vaginalis* (β-Tubulin gene) by polymerase chain reaction (PCR)

Out of a total of 144 positive sexual clinical samples collected from females and males from private hospitals, 47 samples were positive for *T. vaginalis* using the PCR technique.

The results of the current study, when comparing the sensitivity and specificity of both PCR and IC strip for detection of *N. gonorrhea* [Figure 5], showed that the value of sensitivity was 41.67%, with confidence interval (95% CI: 25.51–59.24%) and specificity 95.37% with confidence interval (95% CI: 89.53–98.48%) accuracy 81.94% with confidence interval (95% CI: 74.67–87.85%).

On the other hand, A PCR assay was performed on 14 gonorrhea strains from males and 6 from females to evaluate the specificity of the chosen beta-tubulin gene primers [Figure 6].

Of a total of 144 sexual clinical samples collected from females and males from private hospitals, 36 were positive for *N. gonorrhea* using the PCR technique.

Discussions

Depending on the findings of the current investigation in Table 2, the new IC strips have more sensitivity and specificity (100%) compared to culture which was sensitive to 96% and specialized to 93%, because the culture method is characterized by several mistakes, this method is very sensitive, the sample can be contaminated making it useless in diagnosis. It also can identify the cause of the injury zone through phenotypic diagnosis (color, shape, and growth method of the colonies in agar medium). *Candida* spp. colonies appear on medium within 24 to 72 h. Some species may require more than 3 days to appear on culture medium, while the new IC strip does not take a long time, it needs 10–20 min to get a result and there is no contamination. These results agree with studies.[10]

The new IC strips are more sensitive and specialized (100%) compared with the germ tube test which has a sensitivity (98%) and specificity (95%). This is a rapid method for identifying *C. albicans* and *C. dubliniensis* by its ability to produce short, slender, tube-like structures called germ tubes when it is incubated in serum at 37°C for 2 h. Due to the time required to prepare human serum and the safety problems concerned with its use, many clinical microbiological laboratories have started using non-human serum media for testing germ tube production. These include egg white, saliva, tissue culture medium, sheep serum, and various media. It needs to be accurate in the time and temperature, for example, the incubating period for more than 3 h may produce pseudo-germ tubes. The observer must be able to differentiate between the germ tube and the pseudohyphae, any observer must be experienced in diagnosis, while the new IC strip dissent needs any factors of the above. These results agree with studies.[10]

The new IC strips are more sensitive and specific (100%) compared with the API20C test to the sensitivity (100%) and specificity (97%), due to the convergence of results ratios the API20C test has less subjective errors in the interpretation of results, it is a costly commercial system, they have several advantages like rapid identification, require no or less supplemental tests, while the new IC strip is low cost commercially. These results agree with studies.[10]

Some researchers reported that the rapid test equipment gave faster and better effects than conventional microscopy and culture for the diagnosis of vaginitis. These findings were consistent with the findings of the current research, which discovered 31 (19.4%) of 160 patients of candidiasis. This straightforward diagnostic test will be helpful to medical professionals who are treating females who exhibit signs of fungal vaginitis in patients who have been admitted to the hospital in Mosul city.[11]

These outcomes were consistent with what was explained by one research which comparing IC strips and *C. albicans* culture, it was found that the sensitivity, specificity, positive predictive value, and negative predictive value of the immunochromatography method were calculated to be 80.3% (49/61), 99.3% (138/139), 98% (49/50), and 92% (138/150), respectively.[12]

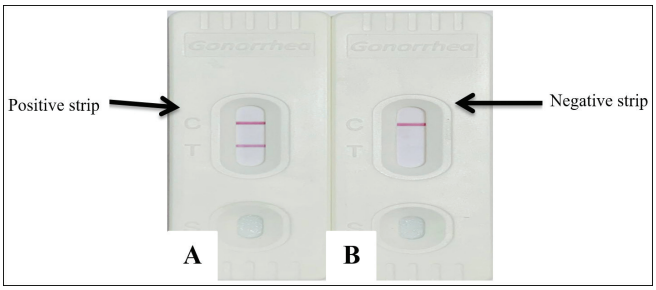

Figure 5: Positive (A) and negative (B) immunochromatographic strip for *N. gonorrhea*

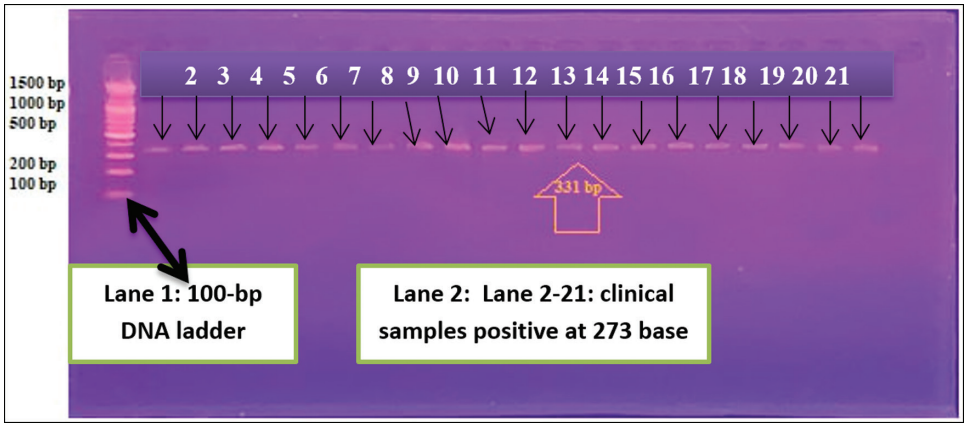

Figure 6: Agarose gel electrophoresis for detection of *N. gonorrhea* (topoisomerase IV subunit C) by polymerase chain reaction (PCR)

Table 2: Sensitivity, specificity, and accuracy of *Candida albicans*

Candida albicans		PCR			
		+	−	Total	
Immunochromatographic strip	+	78	3	81	Sensitivity = 91.76% 95% CI = 83.77–96.62% Specificity = 94.91% 95% CI = 85.85–98.94% Accuracy = 93.06% 95% CI = 87.60–96.62% Confidence interval (CI)
	−	7	56	63	
	Total	85	59	144	

Table 3: Sensitivity, specificity, and accuracy of *Trichomonas vaginalis*

Trichomonas vaginalis		PCR			
		+	−	Total	
Immunochromatographic strip	+	41	2	43	Sensitivity = 87.23% 95% CI = 74.26–95.17% Specificity = 97.93% 95% CI = 92.75–99.75% Accuracy = 94.44% 95% CI = 89.35–97.57% Confidence interval (CI)
	−	6	95	101	
	Total	47	97	144	

Table 4: Sensitivity, specificity, and accuracy of *Neisseria gonorrhea*

Neisseria gonorrhea		PCR			
		+	−	Total	
Immunochromatographic strip	+	15	5	20	Sensitivity = 41.67% 95% CI: 25.51–59.24% Specificity = 95.37% 95% CI: 89.53–98.48% Accuracy = 81.94% 95% CI: 74.67–87.85%
	−	21	103	124	
	Total	36	108	144	

Also, the outcome was agreed with another research was found the sensitivity, specificity, and accuracy of the IC strips were 89.8%, 90.9%, and 90.7%, respectively. When comparing the IC strip with culture and decided that IC strip evaluated could be made readily available for clinical use in detecting *C. albicans*.[13]

According to the outcomes in Table 3, some of the examinations presented that the highest rate of infection by this parasite was in Baghdad city 162 (85.3%) from 190 during 2016 while the lowest percentage of infection was 9 (3.1%) from 290 in Erbil city during 2015. It had concluded that the geographical location of Iraqi governorates with specificity of 93% and sensitivity of 90.5%.[14]

The outcome agreed with one research which was found the sensitivity of microscopic methods 35.30 with 95% CI (15.26–61.38), and the sensitivity of culture was 41.20 with 95% CI (19.43–66.55) while the sensitivity of PCR assay more than both microscopic and culture associated with *T. vaginalis* infection in Sri Lanka.[15]

Several authors have viewed the ICT as a lateral-flow, point-of-care device that can identify *T. vaginalis* membrane proteins. It has been claimed to have a sensitivity of 85%–90% and a specificity of 100%, the ICT can be used on saline solution after a traditional wet mount is performed and can identify *T. vaginalis* not detected by wet mount. Immunochromatographic tests could have an important impact on individual, as well as societal, consequences of untreated STDs. In addition, this rapid test is projected to cost significantly less than culture and nucleic acid amplification methods and approximately the same as wet mount when cost estimates are based on a technician time; also ICT is more sensitive and specific than wet mount for detecting *T. vaginalis* in a research setting with expert microscopic. It requires less technical expertise and time than *T. vaginalis* culture. Test performance was not affected by the presence of other pathogens. ICT can detect *T. vaginalis* in samples that have a lower organism load and that require longer incubation time in culture before being classified as positive, also ICT can be delayed until after the wet mount is read, and

it can be performed on the used wet mount swab with no loss of sensitivity.[16]

The Institute of Microbiology and Immunology (IMI), Faculty of Medicine, University of Ljubljana, conducted research to compare the effectiveness of three methods for detecting *T. vaginalis* in urogenital swabs: wet mount microscopy, culture, and real-time PCR. This research used real-time PCR for detecting *T. vaginalis* infection. Specimens were taken from 75 male and 80 female patients who had urogenital symptoms, engaged in risky sexual conduct, or had a partner who had a diagnosed urogenital. The result was that real-time PCR has more sensitivity and specificity than wet-mount microscopy and culture.[5]

The *T. vaginalis* antigen rapid test makes use of capillary flow technology based on dyed latex immunochromatography. *Trichomonas* proteins from vaginal, cervical, and urethral swabs must be solubilized for the test to proceed. Primary anti-*Trichomonas* antibody conjugated to dye latex particles (red) will bind to *Trichomonas* in the specimen if it is present. A second anti-*Trichomonas* antibody placed on the nitrocellulose membrane will then bind the complex.[17]

The majority of clinicians who presently test females for *T. vaginalis* infection rely upon insensitive diagnostic methods, such as wet mount, and asymptomatic females are seldom tested at all. Although wet mount is the standard of care, it is only 60% sensitive compared to culture. In many healthcare settings, the lack of an experienced microscopist precludes accurate detection of *T. vaginalis*. In some settings, the wet mount is transported to the microbiology laboratory and read by technicians after significant time delay. The sensitivity of wet mount microscopists for detecting *T. vaginalis* declines substantially with even relatively short time intervals between collection and examination. Where wet mount is available, the appropriate *T. vaginalis* test for wet mount-negative subjects has not been delineated.[18]

The result of Table 4 was close to some studies, in which it was indicated that the infection rate was 30 (30%) from 100 using immunochromatography in patients who were admitted to Baghdad hospitals.[19]

Some studies found the sensitivity and specificity of the *N. gonorrhea* strip compared with the results of standard culture were 94.1% (32/34) and 95.8% (23/24), respectively. Researchers found that when they evaluated a fast antigen detection test for *N. gonorrheae* in urine sediment as a diagnostic tool for gonococcal urethritis in male patients.[20,21]

Conclusions

The new IC has sensitivity and specificity more than routine diagnostic tests for *C. albicans*, *T. vaginalis,* and *N. gonorrhea,* also IC strips give the results within a few minutes in comparison to conventional tests.

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Nil.

Conflict of interest

There are no conflicts of interest.

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