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A comparative study between immunochromatographic strip by using PCR assay and routine diagnostic assay of urogenital patients in Karbala and Babylon governorates.

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Written by

Majed Hameed Jabur

B.Sc. in Clinical Laboratories /Applied Medical Sciences/University of Kerbala, 2018

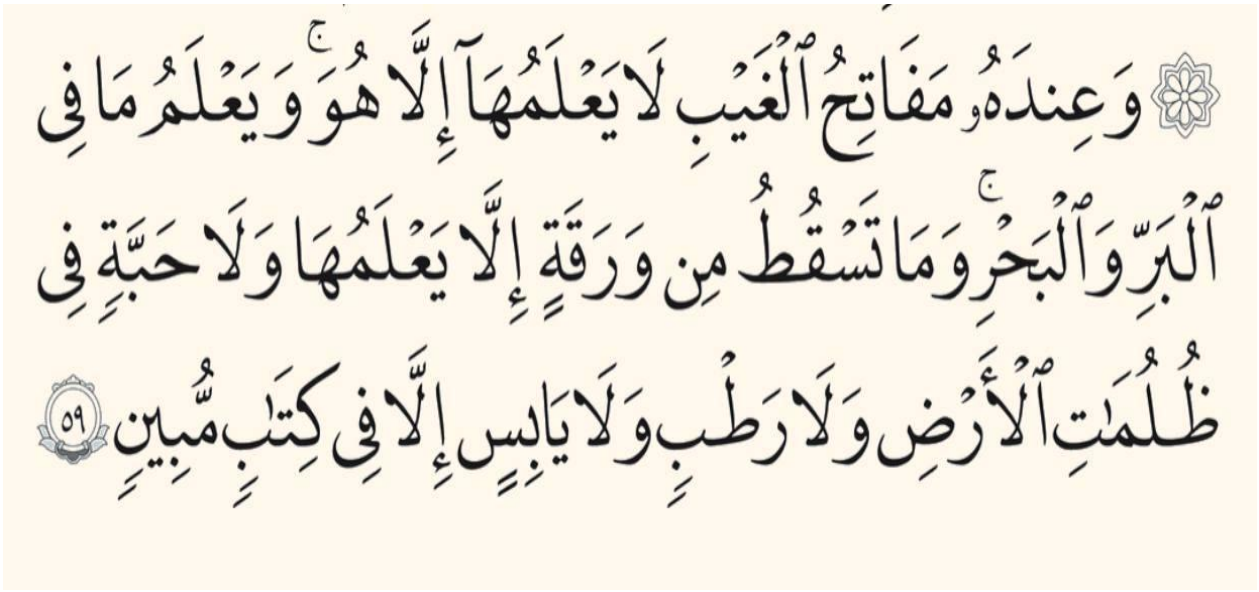
Supervised by

Prof.Dr. Hadi Rasool Hassan

Assist Prof. Dr. Hayder Ali Muhammed

(Second supervisor)

October (2023) **Rabie Alaker (1445)**



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Supervisor's certification

I certify the thesis entitled (**A comparative study between immunochromatographic strip by using PCR assay and routine diagnostic assay of urogenital patients in Karbala and Babylon governorates**)was prepared under my supervision by (***Prof. Dr. Hadi Rasool Hassan*** at the department of Clinical Laboratories\ College of Applied Medical Sciences and ***Assist. Prof. Dr. Hyder Ali Muhammed*** at College of Veterinary Medicine /University of Kerbala, in partial fulfillment of the requirements for the degree of Master in Clinical Laboratories.

Signature

Prof. Dr. Hadi Rasool Hassan

Supervisor

/ 11/ 2023

Signature

Assist. Prof. Dr. Hyder Ali Muhammed

Supervisor

8/ 11/ 2023

Head of Department Recommendation

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature

Assist. Prof. Dr. Linda Hammed Turki

Head of Clinical Laboratories Department

College of Applied Medical Sciences/ University of Kerbala

/11/2023

Approval Certification

We certify that the thesis entitled "**A comparative study between immunochromatographic strip by using PCR assay and routine diagnostic assay of urogenital patients in Karbala and Babylon governorates**" fulfills partial requirements of the degree of Master in Clinical Laboratories.

Signature

Head of Clinical Laboratories Department

Assist. Prof. Dr. Linda Hameed Turki

College of Applied Medical Sciences

University of Kerbala

\11\ 2023

Signature

Vice Dean Scientific Affairs

Assist. Prof. Dr. Huda Abdalreda Abdullah

College of Applied Medical Sciences

University of Kerbala

12 \11\ 2023

Examining Committee Certification

We, the examining committee, certify that we have read this thesis entitled" **A comparative study between immunochromatographic strip by using PCR assay and routine diagnostic assay of urogenital patients in Karbala and Babylon governorates** "and have examined the Student **(Majed Hameed Jabur)** in its content and that in our opinion it is accepted as a thesis for degree of Master of Clinical Laboratories.

Signature:

Prof. Dr. Zuhair Mohammed Ali Jaddoa

(Chairman)

8 /11/ 2023

Signature:

Assist. Prof. Dr. Zuhair Hameed Abboud

(Member)

/11/ 2023

Signature:

Lecture. Dr. Riyadh Hatim Haddawee

(Member)

8/11/ 2023

Signature:

Prof. Dr. Hadi Rasool Hassan

(Member / Supervisor)

/11/ 2023

Signature:

Assist. Prof. Dr. Hyder Ali Muhammed

(Member / Supervisor)

8 /11/ 2023

I have certified upon the discussion of the examining committee.

Signature:

Assist. Prof. Dr. Huda Abdalreda Abdullah

Dean of the College of Applied Medical Sciences/ University of Kerbala

12 /11/ 2023

Dedication

To the great Lord and creator who has help me get to where I am now.

To the Prophet of Mercy, Muhammad (peace and blessings be upon him), and his good and honorable family.

To the fruits of my efforts to the light of my eyes and the joy of my heart, who taught me to climb the ladder of life with wisdom and patience, whose prayers were his companion, pain, and excellence my dear mother.

To the one who gave me life, hope, and growth based on a passion for knowledge and learning, my dear father.

To whom God gave me their presence in my life my wife, uncle, brothers and my sisters.

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List of Abbreviations

Abbreviations	Discription
AIDS	Acquired immunodeficiency syndrome
ALS	Agglutinin-Like Sequence
API	Analytical profile index
CDC	Center for Diseases Control
CI	Confidence interval
DGI	Disseminated gonococcal infection
dsRNA	double-stranded Ribosome Nucleic Acid
GPI	glycosylphosphatidylinositol
HIV	Human Immunodeficiency Virus
Hsps	heat shock proteins
ICT	Immunochromatographic test
IMI	Institute of Microbiology and Immunology
IUD	Intrauterine Device
LOS	Lipooligosaccharide
NAAT	Nucleic Acid Amplification Tests
PCR	Polymerase Chain Reaction
PID	Pelvic inflammatory disease
RDTs	Rapid diagnostic tests
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SDA	Sabouraud Dextrose Agar
STDs	Sexually transmitted diseases
TBE	Tris Borate EDTA
TMA	Transcription-mediated amplification
VVC	Vulvovaginal Candidiasis

Summary

The study was beginning from November 2022 to end of June of 2023; participants were enrolled in the study. After taking a patient's history and performing a physical examination, clinicians swabbed to collect three specimens. One of these specimen was immediately placed in a plastic tube with phosphate buffer saline and sodium azide for determine the *Trichomonas vaginalis* (*T. vaginalis*) or *Candida albicans* (*C. albicans*) present by using immunochromatographic strip, also another swab was immediately inoculate in NaOH and HCl solution for determined *Neisseria gonorrhea* (*N. gonorrhea*) by using immunochromatographic strip. The remaining of specimen that used in immunochromatographic strip used for Polymerase Chain Reaction (PCR) assay and microscopic examination. Sometimes the clinicians take additional swab for culturing *C. albicans* and *N. gonorrhea*.

A cross-sectional investigation was conducted, and a total of 285 patients (146 males and 139 females) distributed as 271single isolation and 14 mixed isolation from patients older than 18 years (18-59) of age who presented with signs and symptoms of vaginitis, urethritis, prostatitis and pelvic inflammatory disease were enrolled in the study. In order to determine three microorganisms responsible for urogenital disease which include *T. vaginalis, N. gonorrhea* and *C. albicans.*

The study found 144 (50.52%) positive specimens of urogenital patients included 130 (90.27%) cases single isolation and 14(9.73%) cases mixed isolation, where 43 (15.08%) for *T. vaginalis*, 20 (7.02%) for *N. gonorrhea* and 81 (28.42%) for *C. albicans*. The remaining 141 (49.48%) negative strips may be due to other causative agents. Females had 11.93%, 2.11%, and 20.7% of *T. vaginalis*, *N. gonorrhea*, and *C. albicans* infections respectively in clinical specimen. Males had 3.15%,

4.91% and 7.72%, for *T. vaginalis*, *N. gonorrhea* and *C. albicans* respectively. There is a clear significant difference in microorganisms that transmitted by urogenital system for both sexes (X^2=16.74, P=0.0002), with *T. vaginalis* and *C. albicans* having the highest infection rates in females and *N. gonorrhea* in males.

The site of isolation plays a major role in the presence of microorganisms (X^2=80.94, P =0.001), as the vagina in females recorded more infection than other sites for both *T. vaginalis* and *C. albicans*; it was recorded as 18 (12.5%) of *T. vaginalis* and 52 (36.11%) *C. albicans* specimen, while *N. gonorrhea* found more prevalence in urethral of males which recorded 14(9.72%).

The results of the present study, when comparing the sensitivity and specificity of both PCR and Immunochromatography strip for detection of *T. vaginalis*, introduce that the value of sensitivity was 87.23% and specificity was 97.93%, while for *N. gonorrhea* presented that the value of sensitivity was 41.67%, and specificity 95.37% as well as *C. albicans*, presented that the value of sensitivity was 91.76%, and specificity was 94.91%.

The conclusion include the new immunochromatography strip have sensitivity and specificity more than routine diagnostic tests for *T. vaginalis N. gonorrhea*, and *C. albicans*, also Immunochromatographic strips given the results within few minutes in compare to microscopic and culture.

Chapter One
Introduction

1. Introduction

Sexually transmissible diseases (STDs) are the most prevalent acute illnesses worldwide. A group of illnesses made up of more than 30 types of bacterial, parasitic, and viral pathogens are spread through sexual contact. Even though some infections can spread by means other than sexual contact, epidemiologically speaking, sexual contact is the most essential way for them to do so. By facilitating the prevention of the spread of sexually transmitted infectious diseases and associated squeal, laboratory and point-of-care testing have the potential to be significant contributors to the management and control of STDs (Lipton *et al*., 2016).

The most common STDs in the world that are not caused by viruses are caused by *T. vaginalis*. The World Health Organization (WHO) reported about 276.4 million infected of *T. vaginalis* among adults worldwide in 2008. As well as compared to the combined cases of *Chlamydia trachomatis (C. trachomatis)* and *N. gonorrhea*, this constituted a significant increase in STD cases (Organization, 2012).

Despite the high frequency of *T. vaginalis* infection, this pathogen has historically received less attention in STD prevention efforts. *T. vaginalis* can cause abnormal vaginal discharge (trichomoniasis) in female, and it may be responsible for 10-12% of instances of non-gonococcal urethritis in male. However, at least 50% of female and 70% to 80% of male may have no symptoms of the infection (Sena *et al*., 2007;Wetmore *et al*., 2009).

The primary sexually transmitted illness is the old illness gonorrhea, which is brought on by *N. gonorrhea* (gonococcus), is nearly entirely spread by sexual intercourse. The WHO estimates that 106 million adult cases occurred worldwide in 2008. This designates *C. trachomatis* infection and gonorrhea as the most

common bacterial sexually transmitted diseases (STDs)(Organization, 2012). Therefore, gonorrhea—along with its serious complications—causes significant morbidity, incurs high financial expenses, and is still a major public health concern on a global scale. Due to the bacterium's current resistance to almost all antimicrobials used to treat gonorrhea, there is serious concern that the disease may eventually become incurable in some situations (Tapsall *et al*., 2009 ; Ohnishi *et al*., 2011).

Because it is fastidious, *N. gonorrhea* needs a sophisticated, nutritionally loaded culture media to develop *in vitro*. Only humans are infected with *N. gonorrhea*, which colonizes mucosal surfaces and is the cause of urethritis in males and cervicitis in female in lower urogenital tract infections. Asymptomatic urogenital infection affects fewer male than it does female, who are more likely to develop it (Ohnishi *et al*., 2011).

The most frequent infectious cause of genitourinary candidiasis is *C. albicans*. This commensal dimorphic yeast colonizes the gastrointestinal tract, skin, and reproductive systems. When observed longitudinally over a year, *C. albicans* can be isolated from the vaginal tracts of up to 70% of healthy, asymptomatic, non-pregnant female at any given time (Beigi *et al*., 2004).

Vulvovaginal candidiasis (VVC) in female, balanitis and balanoposthitis in males and candidurias in male and female are the most typical symptoms of genitourinary candidiasis. These illnesses are surprisingly widespread but affect both immunocompetent and immunocompromised groups. While candiduria is typically identified in immunocompromised patients or newborns, VVC primarily affects healthy females. Most females receive a diagnosis of VVC at least once while they are still capable of having children (Gracia Paul, 2019).

In the modern methods, humans required tests have high sensitivity, specificity, more reliable, short time and inexpensive assays, all this assays were present in serological tests (immunochromatographic) in contrast to microscopic test have high specificity but poor sensitivity, cultures required long time at least one day while the PCR test needed long time and have complexity in addition to cannot recognized the recent and last infection (Meyer & Buder, 2020).

The aim of study

The current research aimed to the investigation of the efficient diagnosis method for some urogenital disease.The research can be done according to the next steps:-

Comparison between modern immunochromatographic test, and routine diagnosis ways included Direct, stain smear, culture and serological test and evaluate by using Polymerase Chain Reaction (PCR) technique to detect **(*Trichomonas vaginalis, Neisseria gonorrhea* and *Candida albicans*)** in infected patients in Karbala and Babylon governorates.

Chapter Two
Literatures Review

2. Literatures Review

2.1. Urogenital diseases

Urogenital diseases are disorders that affect the reproductive organs (genitals) or urinary tract (urethra, ureters, bladder, and kidneys). According to WHO recommendations and the Centers for Disease Control and Prevention (CDC), the major STD signs and symptoms are genital ulcers, penile discharge, abdominal pain, vaginal discharge (leucorrhea), abnormal vaginal odor, redness, inflammation, and burning in the vaginal area should be managed syndromically. The parasite also causes female to go into labor early, have babies with low birth weight, get HIV, and be unable to have children (Burstein & Workowski, 2003 ;Harp & Chowdhury, 2011).

More than 1 million STDs are acquired every day. In 2020, WHO estimated 374 million new infections with 1 of 4 STDs: trichomoniasis (156 million), chlamydia (129 million), gonorrhea (82 million) and syphilis (7.1 million) in male and female aged (15–49) years (Organization, 2021b).

Sexually Transmitted diseases continue to spread rapidly throughout the world especially in developing countries ,and the importance of STDs lies in the fact that STDs often exist without symptoms, particularly in female, they are more than (70%) of gonococcal and chlamydial infections were asymptomatic. Both symptomatic and asymptomatic infections lead to development of serious complication in female and new born babies (cervical cancer, pelvic inflammatory disease (salpingitis), chronic pelvic pain, fetal wastage, ectopic pregnancy, infertility and related maternal mortality). In male, infection can spreads from urethra to epididymis and can outcome in urethral stricture and infertility (Al Jumaily, 2011).

Sexually transmissible conditions due to the fact that each day more than 1 million people get one of the four STDs that are most easily treatable, STDs are a significant worldwide health concern. *N. gonorrhea*, *C. trachomatis*, *Treponema pallidum*, and *T. vaginalis* (Organization, 2021a). Several studies have documented elevated rates of STDs among homosexuals (Bell *et al*., 2006). A Surveys in Western countries find, on average, that about 93% of male and 87% of female identify as completely heterosexual *(*Bailey *et al.*, 2016).

Lack of knowledge about STD prevention, having several sexual partners, being homosexual, and irregular condom use are some risk factors for STD acquisition. Many STD patients wait until their infections are advanced before seeking treatment, which may effects in problems that cannot be reversed. Due to stigma at medical institutions and the fact that many patients self-medicate at home, late diagnosis is caused by asymptomatic STDs in addition to other factors (Nyblade *et al*., 2019 ; Dagnew *et al*., 2020).

In resource-limited settings, the diagnosis and treatment of STDs are frequently impeded by a lack of symptoms, inadequate and restricted diagnostic availability, inaccessibility, and low treatment quality (Veldhuijzen *et al*., 2013).

In Iraq, despite being a Muslim country with a variety of bans and social norms, the prevalence of STDs has not been eliminated. Over 30,000 genital disorders were diagnosed in the Iraqi population in 2000, with gonorrhea (18% of all cases), bacterial vaginitis (9%), non-gonococcal urethritis (9%), and trichomoniasis (9%). In the same year, screening exams of pregnant females found that 0.01% of the females had a positive serological reaction in the course of syphilis. urogenital diseases are common and causes serious problem in Iraq; also there are many other types of organisms which can spread through sexual transmition (Korzeniewski, 2006).

2.2. Major causes of sexual transmitted diseases

Parasite, such as *T. vaginalis*.

Bacteria, including *N. gonorrhea, Treponema pallidum,* and *C. trachomatis*.

Viruses, including HIV/AIDS, herpes simplex virus, human papillomavirus, hepatitis B virus, cytomegalovirus (Sharifi-Rad *et al*., 2021) .

2.2.1. *Trichomonas vaginalis*

Is an anaerobic, flagellated protozoan parasite and the causative agent of a sexually transmitted disease called trichomoniasis. It is the most common pathogenic protozoan that infects humans in industrialized countries. The most prevalence of non-viral sexual transmitted illness in the world is *T. vaginalis*. In 2008, the World Health Organization (WHO) projected 276.4 million cases, with over 90% of these infections occurring among persons living in resource-limited situations (Organization, 2012). In 2020 the WHO estimated 156 million cases of *T. vaginalis* worldwide, and in the same year the WHO estimated the global prevalence of *T. vaginalis* to be 5 % in female and 0.6% in male and this ratio increased among the black population compared to among other groups (Organization, 2021b) .

Trichomonas vaginalis is more prevalent than *N. gonorrhea, C. trachomatis,* and *Treponema pallidum* combined. These findings may be overstated because they are based on research that employed microscopy rather than the more sensitive Nucleic Acid Amplification Tests (NAAT), and there are no official surveillance systems in place (Rowley *et al*., 2019).

Trichomonas vaginalis epidemiology is unknown due to the lack of surveillance programs. It varies widely depending on population and geography. In

the United States, two population-based studies using PCR testing discovered trichomoniasis rates of 2.3% among teenagers and 3.1% among female's aged 14-49. (Miller *et al*., 2005; Sutton *et al*., 2007). WHO describes that *T. vaginalis* in the African region occurs 10 times as often in female than in males. In 2017, the prevalence of *T. vaginalis* infections in the entire African region was estimated to be 42.8 million (De Waaij *et al*., 2017).

Trichomonas vaginalis prevalence varies substantially depending on the risk factor profile of the community. *T. vaginalis* is more common in Africans in general, as discover by greater rates in Sub-Saharan Africa, and between persons of Americans (Klinger et *al*., 2006 ; Paz-Bailey *et al*., 2009).

In the United States, the highest prevalence of *T. vaginalis* infection in US female is seen among African-Americans with rates ranging from 13–51 %. African American female have rates that are ten times higher than that for other racial/ethnic groups, also black female have trichomoniasis high compare to white female constitute a noticeable health disparity, this may association with black race may also reflect a decreased use of barrier protection in this population (Sutton *et al*., 2007).

Studies indicate that African-American males are less likely to use condoms than male of other racial groups because of a higher frequency of condom breakage and slippage, the rates of drug and alcohol use are higher in the African-American community, and this phenomenon could also contribute to the increased incidence and prevalence of *T. vaginalis* in the African-American community. In addition, compared with other racial and ethnic groups, a greater proportion of blacks are unmarried, divorced, or separated, and unmarried status is itself a risk marker for urogenital diseases (Beal *et al*., 1992).

Other risk factors for *T. vaginalis* include increased age, incarceration, intravenous drug use, commercial sex work and the presence of bacterial vaginitis (Rathod *et al*., 2011).

Trichomonas vaginalis is a rare cause of urethral and/or rectal infection among homosexual. One investigation of 678 homosexual males attending a Dutch sexually transmitted disease clinic (Van der Veer *et al*., 2016) and another longitudinal research of 600 homosexual found no cases of *T. vaginalis* infection using highly sensitive Nucleic acid amplification test (NAAT) tests (Kelley *et al*., 2012). This low prevalence is thought to be due to limited exposure to female with infected vaginal fluid, so routine screening of *T. vaginalis* among homosexual is not recommended (Muzny *et al*., 2016).

In Iraq, *T. vaginalis* infections among different governorates reveal highest percentage of infection by *T. vaginalis* in Baghdad city 525 (85.5%) from the total number 614 vaginal swab from urogenital patients during 2016, while lowest percentage of infection 89 (13.36%) of the 614 female vaginal swab from urogenital patients was recorded in Erbil city during 2015 (Nouraddin & Alsakee, 2015; Saheb *et al*., 2016).

This high rate of infection which was reported in Baghdad city and this is related to that the large population female in Baghdad city suffered with this parasite infection and act as a main sources for parasite transmission among multi-sexual partner female , lack health education and personal hygiene female , and lack of information on this parasite make a large problems in the parasite controlling (Saheb *et al*., 2016) . This lowest prevalence of infection was recorded in Erbil city this variation of infection rate among Iraqi provenance confirmed the

variation in the sample size, sample population, research site and mode of diagnosis (Nouraddin & Alsakee, 2015).

Also the prevalence of *T. vaginalis* in Baquba city was 54.1% of 146 female vaginal swab during 2017, where about 41/75 (45.66%) used contraception and 6/71 (8.45%) not used contraception (Salman , 2017), while the prevalence of trichomoniasis in Babylon city 2015 was (9 %) 72/797 cases in male and female in urine and vaginal swab ,which higher in female than male, while in Basra city 2013 there were 123/221 (55.65%) positive for trichomoniasis among married and unmarried female. The prevalence of infection among married female 13/176 (7.38%) higher than unmarried 5/119 (4.2%) ones (Al–Quraishi, 2015).

Also there was similarity in the outcome that recorded in 2013 in Kut and Kirkuk cities, in Kut there were 5/60 cases (8.33%) positive for *T. vaginalis* by used Wet-mount microscopy and 13/60 (21.67%) positive by used Real-time PCR *(*Rahi *et al*., 2014) ,while in Kirkuk city 33/161 (20.49%) of female were positive for trichomoniasis by used Real-time PCR (Salman & Kareem, 2013). In Najaf city 15/85 (17.64%) cases were positive (Al-Kafagy & Al-Hadraawy, 2014). In Sulaimaina city, there are 10/600 positive with *T. vaginalis*, where 450 pregnant female, 8/450 (1. 77%) appear positive effects, while 150 non-pregnant female, 2/150 (1.3%) were positive. The highest is found in female at the age group (26-35) years old (Kadir & Fattah, 2010).

2.2.1.1. Morphology and Pathogenesis of *T. vaginalis*

Trichomonas vaginals is a flagellated parasitic protozoan, typically pyriform but occasionally amoeboid in shape, extracellular to genitourinary track epithelium with a primarily anerobic lifestyle (Harp & Chowdhury, 2011). The individual organism is 10–20 μ m long and 2–14 μ m wide. Four flagella project from the

anterior portion of the cell and one flagellum extends backwards to the middle of the organism, forming an undulating membrane. An axostyle extends from the posterior aspect of the organism. *T. vaginalis* has a large genome (strain G3, 176,441,227 bp) with ~ 60,000 protein coding genes organized into six chromosomes (Carlton *et al*., 2007).

Trichomonas vaginals is an obligate parasite that phagocytoses bacteria, vaginal epithelial cells, and erythrocytes which is ingested by macrophages. *T. vaginalis* obtains its energy primarily from carbohydrates via fermentative metabolism in aerobic and anaerobic environments. The incubation period is usually between 4 and 28 days (Sethi, 2022).

Trichomonas vaginals infects the squamous epithelium of the genital tract and lives in the female lower genital tract, as well as the male urethra and prostate, where it replicates through binary fission. *T. vaginalis* is transmitted predominantly through sexual contact among humans, its sole known host. In females, infection can last for months or even years, but in males it usually lasts fewer than 10 days. The parasite does not appear to have a cyst form and does not live well in the external environment, but it can survive outside the human body for more than three hours in a damp environment (Kissinger, 2015).

Trichomonas vaginals thought to be rare of non-sexual transmission via fomites and possibly water (Crucitti *et al*., 2011). *T. vaginalis* can be infected with double-stranded RNA (dsRNA) viruses that may have important implication for trichomonal virulence and disease pathogensis (Goodman *et al*., 2011).

2.2.1.2. Clinical features of *T. vaginalis*

The majority of female (85 %) and male (77 %) with *T. vaginalis* are asymptomatic (Sutton *et al*., 2007 ; Sena *et al*., 2007). One third of asymptomatic

female become symptomatic within 6 months (Hay & Czeizel, 2007). Among those who do have symptoms, they include urethral discharge and dysuria. Among female, common sites of infection include the vagina, urethra and endocervix. Symptoms include vaginal discharge (which is often diffuse, malodorous, and yellow-green), dysuria, itching, vulvar irritation and abdominal pain, also vaginal pH increased more than 5 in compare with the normal vaginal pH is 4.5(Kissinger, 2015).

Colpitis macularis or strawberry cervix is seen in about 5 % of female, also this ratio rises with colposcopy to nearly 50 % (Núñez-Troconis, 2020). Other complications include infection of the adnexa, endometrium, and Skene and Bartholin glands. In male, it can cause epididymitis, prostatitis, and decreased sperm cell motility (Kissinger *et al*., 2021).

2.2.1.3. Laboratory diagnostic of *T. vaginalis*

There are many tests available for the laboratory diagnosis of trichomoniasis, ranging from straight forward microscopic to more intricate fast antigen and NAAT assays.

2.2.1.3.1. Microscopic

Microscopic investigation of a wet mount preparation of vaginal or urethral secretions is the conventional and most used approach for diagnosing trichomoniasis. About 100 pear-shaped *T. vaginalis* with recognizable jerky or trembling movement per milliliter of specimens are the detection limit for microscopy (Harp & Chowdhury, 2011). The approach is thought to be 100% specific, but due to the reduced parasite burden in male specimens, its sensitivity is weak (44 to 68%) and is lower (Hobbs & Seña, 2013).

To maintain the parasite motile, specimens should be examined 10 to 20 minutes after collection. Delays between specimen collection and microscopic examination, as well as less-than-ideal storage and transportation circumstances for the specimen, particularly at temperatures below 22 °C, can significantly reduce the sensitivity of microscopy (Hobbs & Seña, 2013). Although none of them are currently used for regular diagnosis of *T. vaginalis,* Trichomonas can be accidentally identified in traditional or liquid-based Papanicolaou (Pap) smears of cervical specimens (Aslan *et al*., 2005) .

2.2.1.3.2. Culture

Up until recently, the gold standard for diagnosing trichomoniasis was the parasite's culture in certain liquid media. The Centers for Disease Control and Prevention (CDC) advise that any female whose trichomoniasis is suspected but not verified by microscopy should have her vaginal secretions cultured for *T. vaginalis* (Skvarč *et a*l., 2014). The sensitivity of microscopic is lesser than that of culture, between 44 - 95% (Nathan *et al*., 2015) . Vaginal and urethral swabs, urine, and semen from males must all be properly collected, immediately inoculated into the medium (in less than an hour after collection), and correctly incubated at 37 °C (Garcia & Procop, 2016) .

When direct delivery of specimens to the diagnostic laboratory is not possible, specimens can first be injected into transport systems to maintain the vitality of the parasite for up to 24 hours at room temperature. The InPouch *T. vaginalis* culture system and Diamond's medium are the two media that are most frequently used to cultivate the parasite. In Pouch *T. vaginalis* can be stored at ambient temperature in contrast to Diamond's medium, which needs to be chilled to 4 °C before use. It can stay at room temperature after being vaccinated for up to 48 hours before being

incubated at 37 °C (Garcia & Procop, 2016). Culture can to be tested microscopically for 5 days until proven negative (Hobbs & Seña, 2013) .

2.2.1.3.3. Rapid diagnostic tests

Immunochromatographic test used to detects *T. vaginalis* membrane proteins within 10 minutes. The benefit of rapid diagnostic tests over microscopy and culture for finding *T. vaginalis* antigens or nucleic acids is that they are not constrained by immediate transportation or quick specimen processing. The Rapid test has a sensitivity range of 77 to 98% and a specificity range of 99 to 100% when applied to vaginal secretions or swabs; however it should not be applied to asymptomatic females or males. False positives could happen, especially in groups with low prevalence (Jones *et al*., 2013 ; Skvarč *et al.*, 2014).

Trichomonas rapid test is a point-of-care examination with an immunochromatography-based detection system using monoclonal-specific antibodies to detect *T. vaginalis* antigen. In a previous research in female with trichomoniasis, the sensitivity of the examination based on Trichomonas rapid test is 83%, culture 90%, and microscopic examination 56%. The Trichomonas Antigen Rapid test uses color immunochrommatograpgic, capillary flow technology. The test procedure requires the solubilisation of Trichomonas proteins from a vaginal swab by mixing the swab in specimen buffer, then the mixed specimen buffer was added to the test cassette specimen well and the mixture migrated along the membrane surface, if Trichomonas antibodies conjugated to colored particles (red) , the complex will then be bound by second anti-Trichomonas antibodies coated on the nitrocellulose membrane, so the appearance of a visible test line along with the control line will indicate a positive outcome (Achdiat *et al*., 2019).

2.2.1.3.4. Nucleic acid amplification tests (NAATs)

The development of highly sensitive and specific diagnostic tests based on amplification of *T. vaginalis* nucleic acid (e.g., polymerase chain reaction (PCR) and transcription-mediated amplification (TMA)) changed the diagnosis of trichomoniasis significantly. Because these tests are highly sensitive, they are suitable for screening (in epidemiological studies) and testing asymptomatic female and male patients. A variety of urogenital specimens can be used with NAATs, including endocervical swabs, urine, and self-collected vaginal swabs. As with rapid diagnostic tests, NAATs are not limited by immediate transportation at temperatures not lower than 22 °C and rapid specimen processing (Hobbs & Seña, 2013).

The specificity, sensitivity, performance complexity, and price points of the tests vary. The test operates effectively with specimen from males and has a 92 to 100% specificity and sensitivity range (Hobbs & Seña, 2013; Hathorn *et al*., 2015).

2.2.2. *Neisseria gonorrhea*

Neisseria gonorrhea is a strict human pathogen that causes the sexually transmitted infection termed gonorrhea. Importantly, gonorrhea is a major worldwide public health problem given its estimated yearly incidence of 87 million infections. In addition to causing a high incidence of infection and disease, the gonococcus is noted for its capacity to develop resistance to antibiotics used in therapy. Recently, the World Health Organization placed *N. gonorrhea* on the high priority pathogen list for developing new antibiotics resistance (Rowley *et al*., 2019).

Gonococci can be extracellularly and intracellularly survive in the body .The bacteria must adapt to pressures exerted by the host (Zughaier *et al*., 2014). Some studied reported that *N. gonorrhea* can survive in association with human monocytes and murine macrophages (Zughaier *et al*., 2015).

2.2.2.1. Female urogenital infections by *N. gonorrhea*

2.2.2.1.1. Cervicitis — female typically contract *N. gonorrhea* by mucosal infection of the cervix. Seventy percent or more of female who have a cervical gonococcal infection view no symptoms (Patel & Sheth, 2020). As a matter of course, less is known about the incubation time of gonorrhea in females than in males. Within 10 days of exposure, most female experience genital symptoms (Boiko *et al*., 2020). Infected female may experience vaginal itchiness and/or a mucopurulent discharge; in rare cases, they may also experience menorrhagia. In the loss of an upper respiratory tract infection, the pain is unusual. Indicators of a problem in the upper genital tract include abdominal pain and dyspareunia. The cervix may look normal or view evidence of open bleeding when examined (Humbert & Christodoulides, 2019).

2.2.2.1.2. Urethritis — Sixty percent of female with gonococcal cervicitis have *N. gonorrhea* isolated from their urethra. In addition, genital gonococcal infection most commonly occurs in the urethra of female who have had a hysterectomy (Katz *et al*., 2019 ; Abdallah *et al*., 2020).

Urethral involvement by gonococci is often asymptomatic, just like gonococcal cervicitis. When present, dysuria is the primary symptom, though urine urgency or frequency may also be present (Young *et al*., 2021) .

2.2.2.1.3. Pelvic inflammatory disease — Pelvic inflammatory illness is thought to be caused by *N. gonorrhea* in 30–40% of cases, and it occurs in about 10%–20% of female who have had cervical gonorrhea. Symptoms of pelvic inflammatory disease (PID) involved abdomen discomfort, abnormal vaginal wounded, and painful intercourse; this signs typically coincide in the begin of menstruation but can occur at any time. While female with gonorrhea-related PID may have a more severe acute illness and higher fever than those with nongonococcal salpingitis, it appears that inflammation and scarring of the tubes are similarly severe in both groups. Even in the absence of severe symptoms, PID can cause significant scarring and inflammation (Ross, 2019 ; Bittleston *et al*., 2021).

Examination findings for PID include discomfort in the abdomen, uterus, adnexa, or cervical motion; however, this findings don't differentiate among gonorrhea and non-gonorrhea causes (Xu & Gray-Owen, 2021).

2.2.2.1.4. Perihepatitis (Fitz-Hugh-Curtis syndrome) — Perihepatitis is a Glisson's capsule inflammation that surrounds the liver and is sometimes linked to PID. Since then, it has been discovered that *C. trachomatis* infection is most frequently linked to its relationship with gonococcal infection, which was first noted in 1934. It's unclear exactly how common this syndrome is when PID caused by *N. gonorrhea* is present (Gunasekaran, 2022).

Sharp pleuritic pain that is localized to the right upper quadrant is one of the symptoms, along with fever, nausea, and vomiting. Along the right anterior costal margin, there is a friction rub that can be heard. Frequently, liver function tests are normal or very slightly raised (De Boer *et al*., 2019).

2.2.2.1.5. Bartholinitis — about 6% of females with vaginal gonococcal disease may have symptomatic include the glands, which are located beneath the labia, while up to a third may have asymptomatic involvement. Signs and symptoms may involve edema of the labia, enlargement and soreness in the gland, and perilabial discomfort and discharge when symptoms are present (Rees, 1967).

2.2.2.1.6. Complications of pregnancy — Chorioamnionitis, early membrane rupture, premature birth, lower birth weight or little for gestation age newborns, and spontaneous miscarriages in pregnant female have all been linked to urogenital gonococcal infections (Heumann *et al*., 2017 ; Gao *et al*., 2021).

According to reports, there is a two to five times higher risk of serious consequences in those with gonococcal infections than infected controls (Liu *et al*., 2013) .

In addition, 30-50 % of mothers with untreated *N. gonorrhea* may pass the infection on their unborn child. Neonatal conjunctivitis, pharyngitis, arthritis, and gonococcemia can all affect infants born to infected moms (Wynn *et al*., 2022).

2.2.2.2. Male urogenital infections by *N. gonorrhea*

2.2.2.2.1. Urethritis — Due to high-risk sexual behavior (such as males engaging in penetrating anal sex without the use of a condom) and having several sexual partners, *N. gonorrhea* is frequent cause of urethritis, especially in urban settings (Mugalo *et al*., 2013). Studies conducted in urogenital clinics indicate that majority of male affected individuals have symptoms. Studies based on the general population, however, indicate that up to 60% of male may be asymptomatic or have just minor symptoms. The incubation period for *N. gonorrhea* in symptomatic males is often reported to range from 2 to 11 days (Fan *et al*., 2022) .

Any number of symptoms may be present in male with inflammation in urethra due to gonorrhea. Discharge was find in 82% of cases and dysuria in 53% of inflammation in urethra due to gonorrhea episodes described in one investigation, which included 1615 events. The discharge is frequently abundant, purulent or mucopurulent in color, and occurs spontaneously at the urethral meatus. However, the discharge may also resemble the more modest symptoms of nongonococcal urethritis, making them difficult to identify on the surface (Sherrard & Barlow, 1996).

Inflammation in urethra due to gonorrhea can cause rare complications like penile lymphangitis, edema, periurethral abscesses, and urethral strictures *(Fan et al*., 2022).

Characteristic symptoms on urethral swabs indicate inflammation in urethra is used to define urethritis. However, in an examination of male patients in an urban STD clinic, reach to 5% of those who were diagnosed with *N. gonorrhea* did not have any urethral irritation as seen by a Gram stain (Geisler *et al*., 2005).

2.2.2.2.2. Epididymitis— Inflammation of the epididymis is commonly seen in the outpatient setting. Etiology and treatment are based on patient age and the likely causative organisms. Epididymitis presents as the gradual onset of posterior scrotal pain that may be accompanied by urinary symptoms such as dysuria and urinary frequency. Physical findings include a swollen and tender epididymis with the testis in an anatomically normal position. *N. gonorrhea* and *C. trachomatis* are the most common pathogens in sexually active males in 14 to 35 years of age, epididymitis is usually caused by enteric bacteria transported by reflux of urine into the ejaculatory ducts secondary to bladder outlet obstruction (McConaghy & Panchal, 2016)

2.2.2.3. Extra urogenital infections

Infections of the pharynx and rectum by *N. gonorrhea* are uncommon and often asymptomatic. In extremely rare cases, disseminated infection can effect from bacteremic dissemination from a mucosal location (Dukers-Muijrers *et al*., 2015). In addition, *N. gonorrhea* can spread to adults and adolescents via nonsexual contact and cause an aggressive conjunctivitis (Lai & Ong, 2019).

2.2.2.3.1. Proctitis — Anorectal gonococcal infections are more likely in males who have sex only with females, and are more common in homosexuals who participate in anal receptive intercourse. The rate of rectal illness approximated from 0.2 to 24% between homosexuals in a meta-analysis of research on extragenital gonococcal infection, whereas it was between 0% and 5.7% (median 3%) (Chan *et al*., 2016). Ten percent of human who found themselves to an STD clinic testing positive for rectal gonorrhea according to a multicenter survey of more than 11,000. Up to 40% of homosexual male may exclusively be infected in the genital area, meaning that have anorectal gonorrhea (Moncada *et al*., 2009 ; Patton *et al.*, 2014). This is especially worrisome because studies have linked gonococcal proctitis in homosexual male to a risk of HIV infection that is around three times higher than in the general population (Kato *et al*., 2020).

The close proximity of the vagina to the anal canal allow for the transmission of *N. gonorrhea* from the vaginal tract to the anal canal in female, even in the absence of receptive anal intercourse. Infection rates may be significantly higher among female who report engaging in receptive anal interplay. The rate of rectal illness in female differ from 0.6% to 36.0%, according to studies assessing extra genital gonococcal infection in the body (Chan *et al.*, 2016). One research involving 2084 female who visited a urogenital clinic reported that 3% had rectal *N. gonorrhea*; of them, 30% didn't also have a vaginal illness (Javanbakht *et al*., 2012).

When present, symptoms and indicators of proctitis include tenesmus, anorectal pain, rectal fullness, constipation, anorectal bleeding, and mucopurulent discharge. Most cases of anorectal gonococcal infection are asymptomatic. Symptoms alone are insufficient to differentiate gonococcal proctitis from other infectious causes of proctitis (De Vries *et al.*, 2021).

2.2.2.3.2. Pharyngitis — Pharyngeal gonococcal infections are typically spread through oral sexual contact. The prevalence of pharyngeal infections has been found to vary by demographic, and infections have been found even when no risk behaviors were recorded (such as receptive oral intercourse) in studies investigating extragenital gonococcal infections (Wiesner *et al*., 1973 ; Chan *et al*., 2016). Between female about 0% and 30% (with a median of 2%) and among homosexuals, estimates range from 5% to 17% also males who have sexual relations only with female have a range of 0.45-15.55% (median 2%). Oropharyngeal infections caused by *N. gonorrhea* are mostly asymptomatic, while some patients may experience a redness of throat, cervical lymphadenitis, and pharyngeal exudate (Perry, 2021) .

Horizontal transfer of gonococcal antimicrobial resistance genes is likely to occur in the throat, despite lower bacterial densities there than in the rectum and genitals (Deguchi *et al*., 2012) .

2.2.2.3.3. Disseminated gonococcal infection — it is estimated that between 0.5 and 3% of infected patients would experience bacteremic dissemination of *N. gonorrhea* from the site of initial infection. Most disseminating gonorrhoea strains don’t produce inflammation in urethra, and injury at a mucosal location typically precede cases of DGI, however host and microbial variables may both play a role in transmission (Crew *et al*., 2019).

Both purulent arthritis and the triad of tenosynovitis, dermatitis, and polyarthralgias are common clinical disorders caused by dissemination (Zhou *et al.*, 2019).

2.2.2.3.4. Conjunctivitis — Gonococcal conjunctivitis mainly affects infants born to untreated mothers. In adults and adolescents, sporadic cases can occur as a matter of course of autoinoculation from an anogenital source (Mak *et al.*, 2001).

Gonococcal conjunctivitis can range from a mild, symptomatic illness to an aggressive, invasive infection with conjunctiva illness, purulent drainage, and edoema around orbital that, if left without treated , can lead to corneal ulceration, perforation, and blindness (Wan *et al.*, 1986 ; National Center for STD Control *et al.*, 2020).

2.2.2.4. Laboratory diagnostic of *N. gonorrhea*

2.2.2.4.1. Grams stain (microscopy) — are mostly used to diagnose urethritis in a male who is experiencing symptoms. Polymorphonuclear leukocytes that found within intracellular gram-negative diplococcus in a Gram stain of a male urethral specimen is highly specific for detecting *N. gonorrhea* infection in male (Meyer & Buder, 2020).

In symptomatic males, 94% of cases were diagnosed by Gram stain, but in asymptomatic patients, the sensitivity reduced to 81%. Gram stain in a male has been proven to have similar performance to culture, with sensitivity from89 to 94% and specificity from 94 to 97%, according to older publications. Therefore, Gram stain of urethral specimen is sensitive, specific, and cost effective in male with symptoms. However, if gonorrhea is suspected due to risk factors and Gram stains are negative in an asymptomatic male, further testing is warranted (Goodhart *et al.*, 1982 ; Sherrard & Barlow, 1996).

Due to the presence of additional nonpathogenic gram-negative diplococci, Gram staining of pharyngeal or rectal specimens is not suggested for the reliable diagnosis of extra genital infections. Gram stain is helpful in diagnosing conjunctival infections, however it is best to have a culture to double check (Ghanem, 2020).

Methylene blue/gentian violet stain is a faster alternative to Gram stain for identifying WBC and gonococcal forms on urethral swab specimens. While the outcomes are consistent with a Gram stain, *N. gonorrhea* discover up as a deep purple on the methylene blue/gentian violet dye *(*Taylor *et al*., 2011) .

2.2.2.4.2. Culture — the first specimens should be inoculated onto selective agar containing antimicrobial compounds that prevent the development of commensal bacteria and fungi, as well as onto nonselective chocolate agar. The antibiotics vancomycin, colistin, and trimethoprim lactate and the fungicides nystatin, anisomycin, or amphotericin B can be found in modified Thayer-Martin, Martin Lewis, and New York City medium, respectively. The concentrations of vancomycin or trimethoprim used in the selective media are optimized for some fastidious strains, such as the arginine, hypoxanthine-, and uracil-requiring strains.

When supplementation in selective media inhibits an isolate, it is best to grow it in a less selective medium. Atypical isolates, such as vancomycin-susceptible organisms, should be sent to reference laboratories for further testing. It is preferable to compare isolation rates on selective and nonselective medium on a regular basis as part of a quality assessment program (Ng & Martin, 2005).

In the United States, the Centers for Disease Control and Prevention (CDC) advise that if treatment failure is suspected, a gonococcal culture should be obtained and antimicrobial susceptibility testing should be performed. Large

commercial and public health laboratories maintain gonorrhea culture capacity (Workowski *et al*., 2021).

Neisseria gonorrhea cultures are processed on Thayer-Martin agar to prevent overgrowth, using rayon, Dacron, or calcium alginate tips. Sensitivity ranges from 72-95%, depending on comparison assay, site, and patient population. (Papp *et al*., 2014).

2.2.2.4.3.1. Types of culture specimen

Using a male **urethral** cotton swab, insert it 2–3 cm into the urethral meatus and rotate it 360˚ twice or three times to obtain a urethral swab. Swabs are used to collect **cervical** specimens by inserting their tips a centimeter or two into the cervical of and then spinning the swab 360 ˚twice or three times. Swabbing 3–4 cm into the **rectal** vault can collect rectal specimens. Swabs are used to collect **pharyngeal** specimens from the back of the throat.

Urine is one of the specimen types suitable for nucleic acid tests for diagnosing *N. gonorrhea* infections in males and females. Leak-proof containers should be provided to patients for the collection of urine specimens(Ghanem, 2020). Diagnosis of gonococcal ophthalmia neonatorum and adult conjunctivitis is made using the purulent discharge from the **conjunctiva**. **Body fluids**: Blood and fluid from arthritic joints is acceptable specimen for culture when patients exhibit signs of systemic or widespread illnesses (Ng & Martin, 2005).

2.2.2.4.4. Nucleic acid amplification — For the most accurate diagnosis of gonorrhea (and chlamydia, a common co-pathogen) in both symptomatic and asymptomatic people, nucleic acid amplification testing (NAAT) is highly recommended (Workowski *et al*., 2021 ; Papp *et al*., 2014). When compared to traditional procedures such as culture, that call for a pelvic exam in female and a swab of urethral in males, the possibility to employ NAAT for self-collect

specimen or urine specimens represents a significant improved. One major limitation of NAAT methodology is that it cannot be used to determine antimicrobial susceptibility, which is critical in cases of possible infection that are resistant to antibiotics (Knox *et al*., 2002), while NAAT tests may be more expensive per test than culture or other methods, high-throughput labs may use "pooling" techniques to identify a positive specimen (made up of aliquots from several clinical specimens); a "positive" pooled specimen is then parsed to find the single positive clinical specimen (Ghanem, 2020).

Amplification of Nucleic Acids Vaginal swab testing with NAAT is equally reliable as cervical swab testing. The sensitivity of urine test in females appears to be little compared with vaginal specimens, which is the main reason why vaginal swabs are used instead of urine specimens (Knox *et al*., 2002). Amplification of Nucleic Acids Urinary and urethral specimens from both sexes, as well as end cervical and vaginal specimens, can all be processed by NAAT (Ghanem, 2020).

Urine collected first thing in the morning is best for male, while a vaginal swab is best for female. If the patient is getting a speculum exam for another reason, an end cervical swab is a good idea (Papp *et al*., 2014).

Amplification of Nucleic Acids When compared to other methods, NAAT is more sensitive at detecting *N. gonorrhea* in non-genital locations such the oropharynx and rectum. Pharyngeal and rectal swabs from patient have been demonstrated to perform similarly to clinician-collected swabs, just as has been established for urine and vaginal swabs (Freeman *et al*., 2011 ; Levy *et al*., 2012).

2.2.2.4.5. Rapid tests — A qualitative lateral flow immunoassay, the Gonorrhea Rapid Test Cassette (Cervical/Urethral swab) can identify Gonorrhea antigen from either the female cervical or the male urethra. The test line region is coated with antibodies specific to the Gonorrhea antigen. The extracted antigen solution is tested by reacting with a Gonorrhea antibody that has been immobilized on particles. A color line is produced in the test area as the mixture migrates upward to react with the Gonorrhea antibody on the membrane. If this colored line is visible in the vicinity of the test line, the outcome is positive; otherwise, the matter of course is negative. The presence of a colored line in the control line region serves as a procedural check, demonstrating that the correct volume of specimen has been introduced and membrane wicking has taken place (Murray, 2015).

The immunochromatographic test used for detection of *N. gonorrhea*. These immunoassays include the interaction of an antigen with a colorimetric particle, which is facilitated by capillary action flow down a nitrocellulose membrane. Sensitivities varied from 60% to 94% and specificities from 89% to 97%, depending on the test (Smith *et al*., 2013).

Point-of-care rapid diagnostic tests (RDTs) may yield matter of course within the patient's acceptable time frame, allowing for the start of antibiotic therapy and the starting of partner notification all in the same visit. Other *N. gonorrhea* RDTs is based on antigen-detection by immunochromatography or optical immunoassays, suggesting that early identification and treatment of *N. gonorrhea* infection with RDTs may potentially limit ongoing transmission. Some investigations used culture or an old PCR test with inadequate sensitivity as the reference method, suggesting that sensitivity was significantly lower than reported (Nuñez-Forero *et al*., 2016).

2.2.2.4.6. Nucleic acid hybridization (nucleic acid probe) tests — Current genetic probe approaches necessitate invasive test with direct swab from the end cervix or urethra since they do not employ amplification of genetic targets (Ghanem, 2020) .

The major benefit of this test is low price; however they are not as commonly used as they once were because their sensitivity is too lower than NAAT and because NAAT have become more cost competitive. Nucleic acid hybridization test uses chemiluminescent DNA probes to determined nucleic acid sequence of the organism. Probe tests have a potential benefit in that specimen preservation and transportation are less important than in culture. The material must be delivered to the lab within 24 hours for culture, but can sit out at room temperature for up to seven days for DNA probe assays (Vlaspolder *et al*., 1993 ;Ghanem, 2020).

2.2.2.5. Transmission

Neisseria gonorrhea could even survive outside the host, and sexual person are only means by which the pathogen can spread from the core, massive population, where the majority of infections occurs medium-risk group, which then spread back to the main group and to the members' partners. High-risk populations include the indigent and the homeless (Papp *et al*. 2014). Another common occurrence is that people are unaware that they are connected to bigger sexual persons. There is an attachment of *N. gonorrhea* to sperm (Harvey *et al*., 2000). Moreover, the fact that ejaculates harbor a large number of bacteria makes it possible for males to pass the infection to their partners. How female maintain transmission efficiency to their spouses is unclear. Bacterial sialidases, released by female cervicovaginal microbiota, first must desialylate *N. gonorrhea* lipooligosaccharide (LOS) to facilitate efficient transfer from female to male *(*Ketterer *et al*., 2016).

2.3. *Candida albicans*

Candida albicans is the main opportunistic yeast infection in the world, which continues to be the most common. Although this yeast is responsible for approximately 50-90% of human candidiasis, *C. albicans* a part of the commensal flora of more than half of the healthy population. Colonization by this yeast is beneficial to the host, because not only limits the growth of other opportunistic pathogenic fungi, but also promotes the functioning of the immune system (Vázquez-González *et al*., 2013) .

Fungal pathogens are responsible for at least 13 million infections and 1.5 million deaths globally per year, primarily in those with some compromised immune function (Bongomin *et al*., 2017)

In united states, during 2013–2017 the average incidence (rate of new infections) was approximately 9 per 100,000 people; however, this number varies substantially by geographic location and patient population (Tsay *et al*., 2018).

Vulvovaginal candidiasis (VVC) is the second most prevalent vaginal infection in female of reproductive age, primarily affecting the vulva and vagina. It is anticipated that 70-75% of females of reproductive age will experience at least one episode of VVC during their lifetime, with 40-50% experiencing a recurrence. *C. albicans* causes over 80-90% of VVC, with other species accounting for only a handful of cases (10-20%). *C. albicans* is a natural element of the vaginal microbiome. When the body lacks protective immunity and is resistant to clearance, it develops into a strong opportunistic fungal pathogen and becomes the major cause of VVC. Furthermore, the anus is anatomically close to the vagina, making migration much easier (Zeng *et al*., 2018).

In male, balanitis, which usually appear after sexual contact, is characterized by the appearance of a rash, more or less prickly, followed by small pustules on rocking groove-preputial discharge, more or less abundant. Although this kind of injury is well defined, in particular cases it can extend to the groin and perianal region (Vázquez-González *et al*., 2013).

The risk factors for VVC included antibiotic use, hormonal fluctuations, diabetes, HIV/AIDS infection, immunocompromised patients, smoking, black race, obesity, pregnancy and not usually considered sexually transmitted (Venugopal *et al*., 2021).

In the last two decades, it has been observed a considerable increase in the incidence of deep fungal infections, not only in immunocompromised patients, but also related to nosocomial infections, and even in healthy population (Tsai *et al*., 2013). Thus, with the increased incidence of deep fungal infections (chronic candidiasis), the primitive idea that they were related to a restrict number of pathogenic fungi and specific geographical area was completely changed. Furthermore, with the rapid increase of candidiasis incidence (Lott *et al*., 2005).

In Iraq research, which involved 100 suspected female with *C. albicans*, It was found that 25/30 (83.3%) of pregnant female had *C. albicans* infection, 8/54 (14.5%) of non-pregnant married female (menstruating); 3/15 (20%) of lactating female and 2/10 (20%) of postmenopausal female had *C. albicans* infection. The finding that a high infection rates among married female than the other groups may be attributed to role of husband in transmission of the infection as *C. albicans* infection is urogenital microorganisms. behavior practices and sexual practices may affect the infection rate of candidiasis, which could not be explored in Iraq (Al-Obadi & Al-Abidi, 2000).

2.3.1. Clinical feature of Candidiasis in vagina

Candida albicans can cause infection if conditions change inside the vagina to encourage its growth. Things like hormones, medicines, or changes in the immune system can make infection more likely. The common term for candidiasis in the vagina i vaginal yeast infection. Other names for this infection are vaginal candidiasis, vulvovaginal candidiasis, or candidal vaginitis. The symptoms of vaginal candidiasis include vaginal itching or soreness, pain during sexual intercourse, pain or discomfort when urinating and abnormal vaginal discharge. Vaginal candidiasis is often mild, but some female can develop severe infections involving redness, swelling, and cracks in the wall of the vagina (Gonçalves *et al*., 2016).

2.3.2. Pathogenesis of *C. albicans*

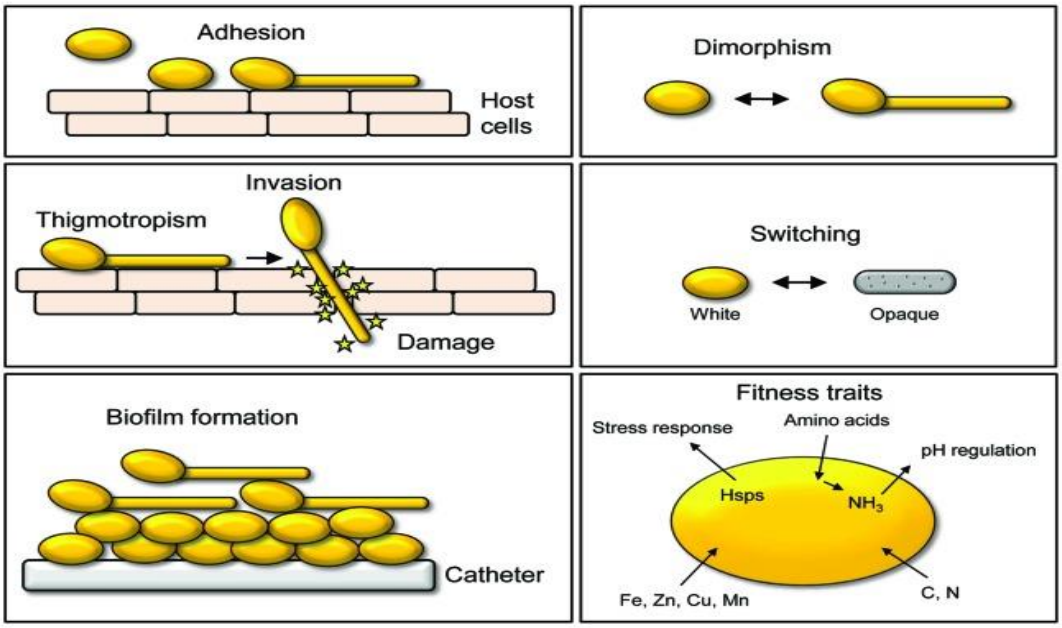

Figure (2-1) pathogenesis mechanisms of *C. albicans* (Nicholls *et al*., 2011)

Candida albicans pathogenesis mechanisms involved Yeast cells adhere to host cell surfaces by the expression of adhesins. Contact to host cells triggers the yeast-to-hypha transition and directed growth via thigmotropism. The expression of invasins mediates uptake of the fungus by the host cell through induced endocytosis. Adhesion, physical forces and secretion of fungal hydrolases has been proposed to facilitate the second mechanism of invasion, i.e., fungal-driven active penetration into host cells by breaking down barriers (Mayer *et al.*, 2013).

The attachment of yeast cells to abiotic (e.g., catheters) or biotic (host cells) surfaces can give rise to the formation of biofilms with yeast cells in the lower part and hyphal cells in the upper part of the biofilm. Phenotypic plasticity (switching) has been proposed to influence antigenicity and biofilm formation of *C. albicans*. In addition to these virulence factors, several fitness traits influence fungal pathogenicity. They include a robust stress response mediated by heat shock proteins (Hsps); auto-induction of hyphal formation through uptake of amino acids, excretion of ammonia (NH_3) and concomitant extracellular alkalinization; metabolic flexibility and uptake of different compounds as carbon (C) and nitrogen (N) sources; and uptake of essential trace metals, e.g., iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) (Mayer *et al*., 2013).

2.3.3. Virulence factors

2.3.3.1. Polymorphism

Candida albicans is a polymorphic fungus that can grow in several different forms, primarily yeast, pseudohyphae, and hyphae. For its pathogenicity, its ovoid-shaped budding yeast and parallel-walled true hyphae forms are the most important. The hyphae form is more prevalent for an infection, while the yeast form is believed to be important in the spread of *C. albicans* (Sudbery *et al*., 2004).

The role of pseudohyphae is not very well understood, other than being an intermediate form between yeast and hyphae. Several factors can cause a change in morphology, such as pH differences, temperature changes, carbon dioxide levels, starvation, and quorum-sensing molecules (farnesol, tyrosol, and dodecanol) (Mayer *et al*., 2013).

2.3.3.2. Adhesion

Candida albicans have special sets of glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins that allow it to adhere to the surfaces of microorganisms. These glycoproteins are encoded by 8 sets of Agglutinin-Like Sequence (ALS) genes, ranging from Als1-7 and Als9. For adhesion, the Als3 gene appears to the most important as it is upregulated during an infection of oral and vaginal epithelial cells. Also, it helps with biofilm formation by helping with adhesion to each other (Murciano *et al*., 2012).

2.3.3.3. Invasions

Along with adhesion, Als3 proteins can function as invasins that help with the invasion of *C. albicans* into host epithelial and endothelial cells. Another important invasin gene is Ssa1, which normally codes for heat-shock proteins. Basically, these specialized proteins on the pathogen's surface mediate binding to host ligands, such as E-cadherin on epithelial cells and N-cadherin on endothelial cells, and it induces host cells to engulf the fungal pathogen. Another method of invasion is the active penetration of *C. albicans* into host cells by an unknown mechanism involving hyphae (Wächtler *et al*., 2011).

2.3.3.4. Biofilm formation

Candida albicans has the ability to form biofilms on living and non-living surfaces, such as mucosal membranes and catheters, respectively. After the adherence of yeast cells to the surface, there is development of hyphae cells in the upper part of the biofilm. Eventually, this leads to a more resistant, mature biofilm and the dispersion of yeast cells – both contributing to the pathogen's virulence. In the process of biofilm formation, Bcr1, Tec1 and Efg1 function as important transcriptional factors (Fanning & Mitchell, 2012). Recent studies discover that biofilms protect *C. albicans* colonization from neutrophil attack and deter the formation of reactive oxygen species (Xie *et al*., 2012).

2.3.3.5. Secreted hydrolases

Candida albicans secrete 3 main classes of hydrolases: proteases, phospholipases and lipases. It is proposed that these hydrolases help facilitate the pathogen's active penetration into host cells and the uptake of extracellular nutrients from the environment. There are about 10 known secreted aspartic proteases (Sap1-10), and their exact contribution to pathogenicity is controversial. For phospholipases, there are 4 major classes (A, B, C, and D), and all 5 members of the B class are involved with the disruption of a host cell surface. Thirdly, lipases are consisted of 10 members (LIP1-10), and studies discover that there is decreased virulence in their absent (Wächtler *et al*., 2012).

2.3.3.6. Metabolic adaption

Candida albicans are usually found in the gastrointestinal microbiome of healthy individuals, and in this environment, nutrient levels are relatively high. However, during niche changes in the course of an infection, available nutrient levels will also change. Consequently, the fungus can quickly undergo metabolic adaption, such as their glycolysis, gluconeogenesis, and starvation responses. For example, in the case of candidemia, *C. albicans* infect the bloodstream, which is typically rich in glucose. Nevertheless, it might be phagocytosed into a macrophage or neutrophil, where it's surrounded by ROS, RNS, and AMPs. In response, *C. albicans* quickly switch from its glycolysis to starvation response with the activation of the glyoxylate cycle. Due to this flexibility, *C. albicans* can infect almost every organ in a human host through the bloodstream, providing candidemia's higher mortality rate (Brock, 2009).

2.3.4. Laboratory diagnostic of *C. albicans*

Approximately 50% of patients view positive microscopy of a wet mount or saline preparation, revealing yeast cells and hyphal components. In recognising yeast cells or hyphae, a 10% potassium hydroxide (KOH) preparation is more sensitive than a saline preparation. The pH of the vaginal cavity is frequently evaluated to rule out other infections such as bacterial vaginosis or trichomoniasis, where it is elevated (>4.5), whereas it is normal (4.0 to 4.5) in VVC. If microscopy is negative but VVC is suspected, vaginal culture is the most accurate method for diagnosing VVC. There appears to be no difference between Sabouraud agar, Nickerson's medium, and Microstix-candida medium among the many culture methods. Antigen detection, serologic testing, and PCR-based diagnostics are all available (Gracia Paul, 2019).

2.3.4.1. Direct examination

Direct microscopic examination is a rapid method for diagnosis of candidiasis. It requires less expertise (Deorukhkar & Santosh, 2014). The swab (specimen) with KOH (10%) solution is examined and fixed on the slide. The solution analyzes the epithelial cells accompanying the specimen, leaving the yeast cells, allowing seeing the false filaments and the oval shape of the yeast. For Candida, this method is commonly used in laboratories for its speed of giving the outcome, but is inaccurate (Newlands & Kerawala, 2020).

2.3.4.2. Culture

The specimen is taken from the affected area by candidiasis, and the planning survey is carried out on a container planting plate on the appropriate medium for its growth (Sabouraud Dextrose Agar, Blood agar medium, Candida Chrome agar medium), the incubated in the incubator at a temperature of 37°C for 48 hours. And through the color, shape and growth method of the colonies can identify the cause of the injury zone. This method is less useful than direct examination using a microscope because it takes a long time and is very sensitive. The specimen can be contaminated making it useless in diagnosis (Purkait, 2011).

2.3.4.3. Germ tube test

In diagnostic mycology the basic work up for yeast identification starts with a germ tube test. Germ tube formation was first reported by Reynolds and Braude and hence the germ tube test is also known as Reynolds-Braude Phenomenon (Deorukhkar *et al*., 2012). This is a rapid method for identifying *C. albicans* and *C. dubliniensis* by its ability to produce short, slender, tube like structures which is called the germ tubes when it is incubated in serum at 37°C. Distinguish between

species belonging to the genus Candida; If *C. albicans* isolates produce the germ tube when incubated with the human serum at a temperature of 37°C for three hours (Moya-Salazar & Rojas, 2018).

2.3.4.4. Analytical profile index (API) Yeast Identification System

The API Candida system consists of a single-use disposable plastic strip with 10 wells to perform 12 colorimetric biochemical tests: five sugar assimilation tests (for glucose, galactose, sucrose, trehalose, and raffinose) and seven enzymatic tests (for β-maltosidase, α-amylase, β-xylosidase, β-glucuronidase, urea hydrolysis, N-acetylb-glucosaminidase, and β-galactosidase). Inoculation of the wells was performed by adding a yeast suspension to the dehydrated substrates. The outcomes were read after incubation for 18 to 24 h at 35°C.A four-digit numerical profile was generated for each isolate depending upon the reactions it produced. Identifications were made by referring to the list of numerical profiles and a computer program provided by the manufacturer (Campbell *et al*., 1999).

2.3.4.5. Vitek Yeast identification system [specific biochemical reaction]

Vitek system was used in order to diagnose the *C. albicans, C. glabrata, C. tropicalis and C. krusei* isolates, which included several steps as follows: Preparation of fungus suspension , Inoculation of identification card and Card sealing and incubation (Makwana *et al*., 2012).

2.3.4.6. *Candida albicans* Rapid Test (Vaginal Swab)

Is a rapid chromatographic immunoassay for the qualitative detection of *C. albicans* antigens from vaginal swabs, the rapid test was more sensitive than wet mount (87.9 % versus 64.5 % respectively) and had the specificity of (98.4 %

versus 96.9 % for culture). The negative predictive value was 96.9 % and the positive predictive value of 93.1%, the efficient diagnosis of Vulvovaginal Candidiasis by using a New Rapid Immunochromatography Test (ICT), was found that the test had a significantly higher sensitivity (96.6 %) than microscopic examination (61.6%) and a higher specificity (98.6%) than fungal culture, they found that the sensitivities of microscopic examination, culture, and ICT for the diagnosis of VVC were 61%, 100% and 96.6%, respectively, while the specificities of the three methods were 100%, 82%, and 98.6%, respectively and found that ICT had a negative predictive value of 98.6%, a positive predictive value of 96.6%.

The limitation of the *C. albicans* Rapid Test is that this test does not differentiate between viable and non-viable organisms and between individuals that are carriers and individuals that have an acute infection (Alghnam & Y AL-Dabbagh, 2012).

2.3.4.7. Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is an enzymatic process that allows for the detection of specific genes within an environmental DNA specimen. PCR utilizes short, user defined DNA sequences called oligonucleotide primers, the sequence of which are complementary to target regions of genes known to encode for specific microbial functions (e.g. contaminant degradation) (White *et al*., 2006).

In brief, the DNA specimen is denatured to produce single stranded DNA, called template DNA, to which the oligonucleotide primers can bind. The enzyme DNA polymerase then adds nucleotide bases to the end of each primer, using the template DNA as a guide to extend the primer thereby producing new double stranded DNA. This process is repeated for a number of cycles to enrich the DNA

specimen for the desired genes targeted by the oligonucleotide primers. Since each cycle of PCR involves creating two new double stranded DNAs from each DNA molecule present, the amount of DNA theoretically doubles with every cycle of PCR. Therefore, after two cycles the concentration of DNA increases by 2^2 -fold, after 3 cycles a 2^3 -fold increase, etc. After N cycles, PCR generates a 2^N-fold increase in the target DNA (Mothershed & Whitney, 2006).

Chapter Three
Materials and Methods

Materials and Methods

3.1. Materials

3.1.1. The equipment's

Table (3-1): The equipment's and their country.

No.	Equipment's	Company/ country
1	Beaker	Iwaki glass/Japan
2	Bunsen burner	Shndon/England
3	Conical flask	Marienfeld/Germany
4	Cotton Swabs	Nanjing liming bio-products / china
5	Disposable gloves without powder	Bioneer/Korea
6	Disposable speculum	Foshan suncare / china
7	Disposable syringe	Changazhou medical appliances/China
8	Disposable tips	CAPP/Denmark
9	Eppendrof rack	Eppendrof/Germany
10	Eppendrof tube (1.5)ml	Heittch/Germany
11	Loop	Shndon/England
12	Micropipettes	Slamid/Germany
13	Petri dish	Sterilin/England
14	Rapid test cassette for *C. albican*s	Hangzhou tongzhou Biotechnology/ china
15	Rapid test cassette for *N. gonorrhea*	Gufeng Trading limited/ china
16	Rapid test cassette for *T. vaginalis*	Nanjing liming bio-products / china
17	Slide	Sail Brand/China
18	Test tubes	Arth Al-Rafidain/China

3.1.2. Biological and Chemical Materials

Table (3-2): Biomedical and Chemical Substance and their country

No.	Biomedical and Chemical Substance	Company/ country
1	Absolute ethanol	Scharlau/spain
2	Agarose	Pronadisa/spain
3	DNA ladder (100) bp	KAPA/South Africa
4	Ethidium bromide	BDH / England
5	Gram's Stain	Drugs and medical appliances/Iraq
6	Hydrogen peroxide (H2O2) 30%	SDI / Iraq
7	Normal saline	Haidylena/Egypt
8	Oil immersion	BDH/UK

3.1.3. Culture Media:

The following cultures media using in the research:-

Table (3-3): The Culture media employed in the study.

No.	Medium	Company/ country
1	Blood agar	Biolab / Hungary
2	Sabouraud Dextrose Agar (SDA)	HiMedia /India

3.1.4 Instruments

The instruments used through this research were listed in table (3-4).

Table (3-4): The instruments and their country

No.	Instruments	Company/ country
1	Autoclave	Hirayamy/Japan
2	Centrifuge	Hettich/Germany
3	Digital camera	Canon/ Japan
4	Distiller	LabTech/ Korea
5	Electrophoresis apparatus	Bio-Rad/Italy
6	Hood	LabTech/ Korea
7	Incubator	Memmert/ Germany
8	Light microscope	Human/Germany
9	Oven	Hirayama /Japan
10	pH meter	Radiometer /Denmark
11	Refrigerator	LG /Korea
12	Sensitive balance	Sartorius /Germany
13	Thermo cycler	Syngene/ England
14	UV- Trans illuminator	Stuart/UK
15	Vortex mixer	Memmert /Germany
16	Water bath	Techen/ England

3.1.5. Stains, Solutions and Media Preparation:

3.1.5. 1.Grams Stain Solution according to (Coico, 2006).

Gram stain components included crystal violet stain, ethanol, safranin stain, and iodide solution.

Counterstain:-Two and half g of safranin mixed in one hundred ml of 95% ethanol.

Crystal violet specimens:-Twenty gram of crystal violet dyes (85% dyes) resolved in one hundred ml of 95% ethanol and storage at room temperature (25 ◦C) for approximately 1 year.

Decolorizing solution Acetone and similar volume of 95% ethanol had combined.

Gram's iodine solution:-One gram iodine crystals and two grams potassium iodide diluted in five milliliters D.W. then 240 milliliters D.W. and 60 milliliters of a five percent (w/v) sodium bicarbonate solution were added, mixed thoroughly, and stored for six months at room temperature (25 °C) in a fully covered bottle.

3.1.5.2. Catalase Reagent

This reagent was made with 3% H_2O_2 and was used to determine bacterial ability to manufacture catalase enzyme (MacFaddin, 2000)

3.1.5.3. Oxidase Reagent

The reagent prepared by dissolved 0.1g of tetramethyl-ρ-paraphenylene diamine dihydrochloride in 10 ml of D.W, and then saved in dark bottle (MacFaddin, 2000).

3.1.5.4. Chocolate agar

The agar prepared by dissolved the components casein/animal tissue digest 15g, cornstarch 1g, sodium chloride 5g, dipotassium phosphate 4g, monopotassium phosphate 1g, blood 2%, koenzyme enrichment 10ml and agar 10g (Wilson & Zinnemann, 1979).

3.1.5.5. Sabouraud Dextrose Agar (SDA)

The agar prepared by dissolved the components Dextrose (Glucose) 40gm, Peptone 10gm, agar 15gm, and Distilled Water 1000ml, where all components combine in ~900 ml of deionized water, adjust to pH 5.6 with HCI and adjust final volume to 1 liter, heat to boiling to dissolve the medium completely, autoclave at 121ºC for 15 minutes, Cool to ~45 to 50°C and pour into petri dishes (Agar, 2015).

3.1.5.6. Tris Borate EDTA (TBE) Buffer (1X):

This buffer was prepared by dissolved 100ml of the 10X concentrated TBE in 900ml of D.W (Sambrook & Russell, 2001).

3.1.6. PCR Primers assay

Table (3-5): The Primers employed in the study. F=Forward**, R=R**everse

Microorganism	**Target genes**	**Primer Sequence 5´-3´**	**Product Size (bp)**	Reference
Candida albicans	Internal transcribed spacer	F:TTTATCAACTTGTCACACCAGA R:ATCCCGCCTTACCACTACCG	273	(Tamai *et al*., 2021)
Neisseria gonorrhea	parC (topoisomerase IV subunit C)	F:GTTTCAGACGGCCAAAAGCC R:GGCATAAAATCCACCGTCCCC	331	(Rostami *et al*., 2017)
Trichomonas vaginalis	β-Tubulin gene	F:CATTGATAACGAAGCTCTTTACGAT R:GCATGTTGTGCCGGACATAACCAT	112	(Ozuna & Tadeo, 2016)

3.1.7. Preparation of primers (According to Manufacture Company).

According to the manufacturer's instructions, each primer used in this investigation (table 3-5) was made by dissolved a lyophilized primer in the necessary amount of nuclease free water to produce a stock solution containing 100 pmol /µl. By using dilution techniques, a work solution with a final concentration from 10 pmol/µl was created.

3.1.8. Molecular Kits

3.1.8.1. Addprep Genomic DNA Extractions kit

Table (3-6) kit DNA Extractions components

Solution & Material	Size	Country
Binding Buffer	25 ml	**Addbio/Korea**
Elution	25 ml	
Lysis Buffer	30 ml	
Precipitation	20 ml	
Proteinase K (20 mg/ml)	1.2 ml X 2 tubes	
Spin column	100 ml	
Washing 1	30 ml (Add Ethanol 22.5 ml)	
Washing 2	12 ml (Add Ethanol 48 ml)	

3.2. Methods

3.2.1. Study Designs

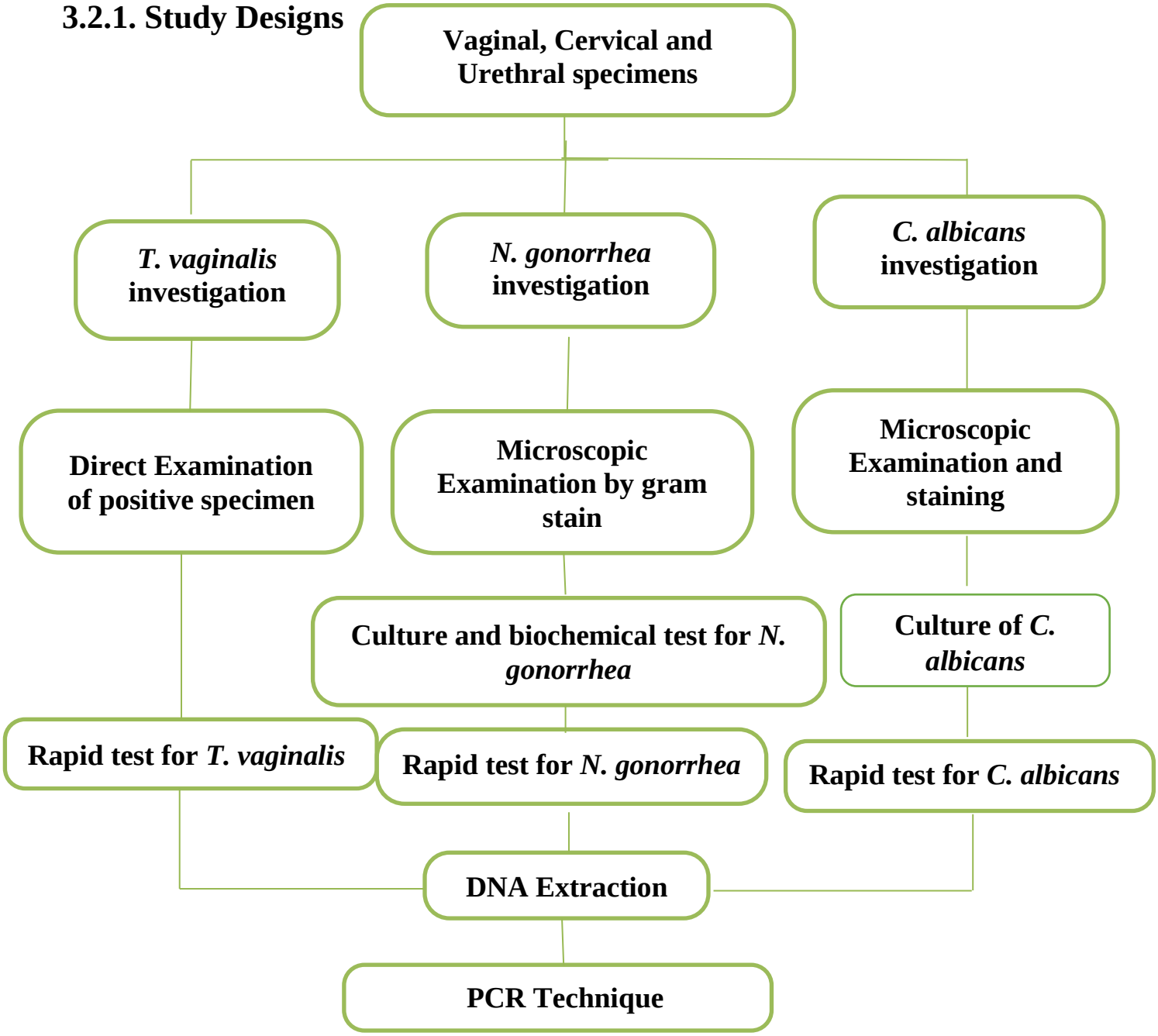

Figure (3-1): Study Design

- Clinicians have taken 285 swabs (males 146, females 139) distributed 271single and 14 mixed isolation from vaginal, cervical and urethral of patients attending to the hospitals or clinical private sites after informed consent was obtained.

3.2.2. Specimen collection and storage for *T. vaginalis* (According to Manufacture Company).

1- The swab was inserted in the vagina and rotated for 20 second, and then the swab was pulled out slowly.

2- The swab was putted to the extraction tube

3- The swabs can be stored for 24 hours at room temperature, and the specimens should be reached to room temperature before testing.

4- To run a culture as well as the immunochromatographic test, separated swabs must be collected because the specimen buffer will kill *T. vaginalis*.

3.2.3. Procedure of detection *T. vaginalis* by using direct microscopy (Hassan *et al*., 2019).

Each patient's swabs inoculated with vaginal discharge were gently stirred in one drop of normal saline on a clean slide before being covered with a coverslip. The presence of motile *T. vaginalis* was determined by the characteristic undulating motility of the wet mount using 40 objectives.

3.2.4. Procedure of detection *T. vaginalis* by using immunochromatographic strip (According to Manufacture Company).

1-Twenty drops of extraction buffer was added to the extraction tube.

2- The specimen swab was put into tube, and then mixed well by rotating the swab vigorously on the side of the tube for at least ten times. Best effects are obtained when the specimen mixed well in the solution.

3- The swab was allowed to saturate in the extraction buffer for at least one minute prior to next step.

4- Squeezed a lot of liquid as possible from the swab by pinching the side of the flexible extraction tube as the swab is removed. The specimen buffer solution must remain at least half in the tube for capillary migration to occur.

5- The tip was put onto the extracted tube.

6- The contaminated swab was discarded in a bio-hazard waste container.

7- The test was removed from its sealed pouch, and placed it on a level surface and clean. The device was labeled with patient or control identification. The best outcome can be obtained by examined in one hour.

8- Approximately 100µl of extracted specimen was added on the test cassette.

9- Waited the colored band(s) to discover. The outcome should be read within15 minutes.

10- The test tubes used and Test Cassette was discarded in suitable biohazardous waste container.

3.2.5. Specimen collection and preparation of *N. gonorrhea* (According to Manufacture Company).

1-The Gonorrhea Rapid Test Cassette (Cervical / Urethral swab) can be done by used male urethral swab and female cervical swab.

2-The quality of specimens acquired is very importance. Detection of Gonorrhea antigen needed a strong and overall collection technique that provides enough amount of antigen.

Female Cervical Swab specimen collection:

A) Used the swab provided in the kit. Alternatively, any plastic - shaft swab may be used.

B) Before specimen collection, removed excess mucus from the endocervical area with a cotton ball and discard. The swab should be inserted into the endocervical canal, past the squamocolumnar junction until most of the tip is no longer visible. This will permit acquisition of columnar or cuboidal epithelial cells, which are the main reservoir of the Gonorrhea organism. Firmly rotate the swab 360 in one direction (clockwise or counterclockwise), allowed to stand for 15 seconds, and then withdraw the swab.

C) If the test is to be conducted immediately, put the swab into the extraction tube.

It is recommended that specimens be processed as soon as possible after collection.

D) If immediately testing is not possible, the patient swab specimens should be placed in a dry transport tube for storage or transport. The swab may be stored for 4-6 hours at room temperature (15-30C). All the specimens should be allowed to reach the room temperature (15-30 ° C) before testing.

3.2.6. Procedure of detection *N. gonorrhea* by using immunochromatographic strip *(*According to Manufacture Company).

The test, reagents and swab was allowed to reach at room temperature (15-30°C) before testing.

1. The test cassette was removed from the seal pouch and used it within one hour.

2. The Gonorrhea antigen was extracted according to the type of specimen. Approximately 300ul from the first reagent bottle was added to the extraction tube vertically where reagent 1 is colorless, then the swab was inserted immediately in

the extracted tube and rotated the swab for 15 times. The extracted tube allowed standing for at least 2 minutes.

Approximately 200ul from the second reagent bottle was added to the extraction tube vertically where the specimens become turbid then rotated the swab 15 times until the solution become clear with a few green or blue color. The extracted tube allowed standing for at least 1 minute.

Pressed the swab against the side of tube and then pulled the swab squeezing the tube. The tube was kept much liquid as possible. The dropper tip was suited on the top of extraction tube.

3. The rapid test was placed on a level surface and clean. Approximately 100ul of the extracted solution was added to the specimen well of the rapid test, and then waited the outcome.

4. The effect was read at 10 minutes; the effect was not interpreted after 30 minutes.

3.2.7. Specimen collection and preparation of *C. albicans* (according to Manufacture Company)

The swab was inserted into the vagina, and rotated for 10 seconds. The swab was pulled out carefully.

2- The swab was putted to the extraction tube and the specimen should be reached to room temperature of 15-30°C before testing.

3- About 100 µl drops was added of the solution to the specimen well directly.

4- Separated swabs must be used for culture as well as *candida* rapid test, because the buffer will affect *C. albicans*.

3.2.8. Procedure of detection *C. albicans* by using immunochromatographic strip (According to Manufacture Company)

The test, swab, buffer, was allowed to reached to room temperature (15-30 °C) prior to testing.

1. Approximately 450µl of extraction buffer was added to new extraction tube that fixed in rack.

2. The swab was putted in the extraction tube and mixed well the swab with solution by rotating the swab 15 times against the side of tube.

3. Let the swab in extraction buffer for 1 minute then squeezed out the swab to extract as much liquid. At least half of buffers stay in the tube.

4. Removed the swab in bio-hazard waste container, and then putted the tip on the top of extraction tube.

5. Removed the rapid test from its sealed pouch, and putted it on plane surface and clean. The best effect of test can be seen during one hour.

6. Approximately 100µl of extracted specimens was added on the rapid test.

7. The effect can be read within 10- 20 minutes.

3.2.9. The procedure of extraction DNA from (*T. vaginalis, N. gonorrhea and C. albicans)* (According to Manufacture Company).

1. The specimens were transferred into a 1.5 ml micro-centrifuge tube (not provided).
2. Two hundred µl of Lysis Solution was added, and resuspend the cell pellet by pipetting.

3. Twenty µl of Proteinase K solution (20 mg/ml) was added to the specimen tube, mixed by vortex, and incubated at 56℃ until the tissue is completely lysed

4. The tube was Spin down briefly to remove any drops form inside of specimen tube lid.

5. Two hundred µl of Binding Solution was added to the specimens tube, and mixed well by pulse-vortex for 15 sec.

6. Incubated the specimen tube at 56℃ for 10 min.

7. Absolute ethanol was added (200 µl) then mixed vigorously by pulse-vortex for 15 sec.

8. Carefully transferred the lysate to the spin column collection tube 2.0 ml.

9. Centrifuged for 1 minute at 13,000 rpm: poured off the flow-through and assembled the spin column with the collection tube 2.0 ml.

10. Putted 500 µl of Washing 1 Solution to the spin column with collection tube then centrifuged for 1 minute at 13,000 rpm: poured off the flow-through and assembled the spin column with the collection tube 2.0 ml.

11. Putted 500µl of Washing 2 Solution to the spin column with collection tube and centrifuged for 1 minute at 13,000 rpm: poured off the flow-through and assembled the spin column with the collection tube 2.0 ml.

12. Additional dried centrifugation for 1 min at 13,000 rpm to spin column to remove the remaining ethanol in spin column.

13. Transferred the spin column to the new 1.5 ml micro-centrifuge tube.

14. Elution Solution 100 ~ 200 µl was added to the spin column with new 1.5 micro-centrifuge tube, then allow to settled for at least 1 minute.

15. Centrifuged the tube for 1 minute at 13,000 rpm, where the genomic DNA eluted.

3.2.10. The status of PCR Thermocycler

The status of PCR thermo cycler at genes of *T. vaginalis*, *N. gonorrhea and C. albicans* review in table (3-7), (3-8) and (3-9)

Table(3-7):The status of PCR Thermocycler for *T. vaginalis*(Ozuna & Tadeo, 2016).

PCR Steps	Temp.	Time	Repeat
Initial Denaturation	95 ºC	5min	1
Denaturation	95 ºC	30sec	30cycles
Annealing	60ºC	30sec	
Extension	72 ºC	30sec	
Final extension	72 ºC	5min	1

Table (3-8): PCR Thermocycler status for *N. gonorrhea* (Rostami *et al*., 2017).

PCR Steps	Temp.	Time	Repeat
Initial Denaturation	95 ºC	5min	1
Denaturation	95 ºC	30sec	30cycles
Annealing	63ºC	30sec	
Extension	72 ºC	30sec	
Final extension	72 ºC	5min	1

Table (3-9): The status of PCR Thermocycler for *C. albicans* (Tamai *et al*., 2021)**.**

PCR Steps	Temp.	Time	Repeat
Initial Denaturation	95 ºC	5min	1
Denaturation	95 ºC	30sec	30cycles
Annealing	61ºC	30sec	
Extension	72 ºC	30sec	
Final extension	72 ºC	5min	1

3.2.11. PCR Product analysis

These following steps for PCR produced resolved by using agarose gel electrophoresis:-

One hundred ml of 1X TBE buffer added to 1.5 gram agarose powder then at temperature 100 ° C boiling in water bath for 15 minutes to its completely melted then cooled at 45° C.

Ethidium bromide dye five µl was added into agarose gel solution. Fixed the comb in right place and the added the agarose gel solution in the tray after that, stay the gel at 25˚ to become solid, then ejected the comb from the tray carefully.

Added DNA marker (100 bp) to the first well as stander then added 4-5 µl of DNA specimens for other comb wells.

Electrophoresis chamber filled with 1XTBE buffer then put the gel tray then electric current applied for one hour at 50 volt (5V/cm between electrodes)

Using UV Transilluminator to seen the PCR products.

3.3. Exclusion criteria: The research was exclude the followed :- not active sexual partner, less than 18 years and above 59 years, urine specimens for both sexes, patient taken antibiotic and specimen drying or delayed.

3.4. Results Interpretation

Positive result: Clear **reddish** colored bar found on test line part (T) for three microorganisms, as well as a **red** colored line on the control line part (C) for *N. gonorrhea* and *C. albicans* while **blue** colored in control part (C) for *T. vaginalis*.

Negative result: Only one line (red) appears in control region (C) for *N. gonorrhea and C. albicans,* while only blue line appears in control region (C) in the state of *T. vaginalis.*

Invalid: There are no lines seen in both regions (control, test) after added the specimens during 10-20 minutes.

3.5. Statistical analysis

All data were arrangement in Microsoft excel (2010) sheet, variable data was calculated by using student Chi-square (χ^2) test. Sensitivity and Specificity between two assays Immunochromatography and Polymerase Chain Reaction (PCR) was determined according to the following formula:- True positive X 100/total positive was used to measure **sensitivity** and the formula for determining **specificity** was true negative X 100/total negative (Armitage *et al*., 2008).

- **The colors in each tables represented the highest values.**
- **All data calculated at statistical level (*P*-value > 0.05)**

3.6. Ethical Consideration

Ethical approval was recorded according to Ethical Committee at the College of Applied Medical Science/University of Kerbala, and all specimens collection was taken from patient after oral consultation.

Chapter Four

Results and Discussion

4.1. Results and Discussion

According to current information, more than 30 different bacteria, viruses and parasites are transmitted through sexual contact, including vaginal, anal intercourse. Some urogenital diseases can also be transmitted from mother to child during pregnancy, childbirth and breastfeeding. Eight are the pathogens linked to the highest incidence of urogenital diseases. Four of those infections can be cured: trichomoniasis, gonorrhea, syphilis and *chlamydia*. All of them portend growing challenges in providing adequate services for urogenital diseases prevention and control (Organization, 2019).

Table (4-1):-The Percentage of urogenital microorganisms among sex.

Types of microorganisms	**Female** **No. (%)**	**Male** **No. (%)**	**Total** **(%)**
Positive *C. albicans*	59 (20.7)	22 (7.72)	81(28.42)
Positive *T. vaginalis*	34 (11.93)	9 (3.15)	43 (15.08)
Positive *N. gonorrhea*	6 (2.11)	14 (4.91)	20 (7.02)
Negative specimens	40 (14.04)	101(35.44)	141 (49.48)
Total	139	146	285 (100)
Statistical analysis	X^2= 16.74 , DF= 2 P= 0.0002		

Table (4-2):- The urogenital microorganisms according to the single isolation.

Types of Microorganism	Vaginal swabs (Female)(%)	Cervical swabs (Female)(%)	Urethral swabs (Male)(%)	Total (%)
C. albicans	52 (36.11)	3 (2.08)	20 (13.89)	75 (52.08)
T. vaginalis	18 (12.5)	12 (8.33)	7 (4.86)	37(25.69)
N. gonorrhea	4 (2.78)	0 (0.0)	14 (9.72)	18 (12.5)
Total	74 (51.39)	15 (10.41)	41(28.47)	130 (90.27)
Statistical analysis	X^2= 80.94, DF= 6 P= 0.0 01			

Out of a total 285 patients distributed as 146 males and 139 females, where 144 (50.52%) positive cases, from its 130 cases single isolation and 14 cases mixed isolation. The positive specimens of urogenital diseases distributed as 81(28.42%) positive specimens for *C. albicans*, 43 (15.08%) positive specimens for *T. vaginalis*, and 20 (7.02%) specimens for *N. gonorrhea*, while the remaining 141 (49.48%) negative specimens, it may be refer to other causative agents table (4-1). The infection was distributed in the clinical specimens according to sexes, as it recorded 20.7%, 11.93% and 2.11% in females for *C. albicans, T. vaginalis and N. gonorrhea*, respectively. Moreover, the specimens also recorded 7.72%, 3.15% and 4.91% in males for *C. albicans, T. vaginalis and N. gonorrhea*, respectively. The present research noticed through table (4-1) there is a clear significant difference (X^2= 16.74, P= 0.0002) in some urogenital disease for both sexes, where the *T.*

vaginalis and *C. albicans* recorded highest infection in females compared to males, while the *N. gonorrhea* recorded the highest infection in males compared to females.

The outcomes are agree with what was explained by similar studies, where it was found that the infections of candidiasis in female are more than in male, as they recorded 66.63% in female and 33.37% in male (Loster *et al*., 2016).

The prevalence of *C. albicans* was found to be 46 (38%) of 121 vaginal specimens in Babylon City, 75 (29.66%) out of 253 vaginal specimens in Basra city, and 73 (36.5%) out of 200 vaginal specimens in the Najaf city. In 2017 rates of infection with *C. albicans* in the cities of Baghdad, Kirkuk, and Ramadi; in Baghdad city, 41% of vaginal specimens tested positive for the *C. albicans* while the rate of infection was 20 (4.7%) out of 425 vaginal isolation in the Kirkuk city, and it was recorded 6% in the city of Ramadi. In the 2018 infection rate was 36.5% out of 23 specimens in the Najaf city, while 12 (30%) out of 40 specimens in the Dahuk city were positive for *C. albicans* (Mohmmed *et al*., 2022).

It is estimated that 30–70% of healthy females harbor this pathogen as part of the natural flora of their vaginal mucosa, skin, and gut. When the immune system is weakened, the immunity balance shifts, allowing for the spread of *C. albicans* and subsequent harmful infections (Chen *et al*., 2006).

Also (table 4-2) introduced the site of isolation play a major role with the found of microorganisms, as the vagina in female recorded more infection rather than other sites for both *T. vaginalis* and *C. albicans*, where that the specimens of the *C. albicans* were more present and isolated from the vaginal genital tract, its recorded 52 (36.11%) positive specimen as single, followed by the *T. vaginalis*, it was recorded 18 (12.5%) from vaginal specimen. If vaginal conditions are altered in

a way that favors *C. albicans* growth, an infection can outcome (Gonçalves *et al*., 2016).

Some local studies introduced the presence of infections in pregnant with candidiasis up to 29% of female who visit hospitals in Tikrit city. This high frequency was attributed to suppression in the immunity of body as a result of pregnancy which lead to disturbance in the balance between yeast and *lactobacillus* bacteria and the end result proliferation of yeast leading to occurrence of the disease (Alsharifi, 2017).

Vaginal candidiasis it affects among 75% of female at some point in their lives; 40%-45% of these female will experience it more than once (Conte *et al*., 2023).

The presence of abnormal microorganisms (bacterial or candida vaginosis) has been associated with elevated concentrations of selected bacteria, a high pH, and elevated cytokine levels in the vagina and cervix. The high pH, favors the growth of *T. vaginalis*. It appears that the way in which the cytokines react to microbial colonization is different from the way in which they respond to the absence of vaginal lactobacillary, though being one of the lives of vaginal defense. was found to be enhance the attachment of *T. vaginalis* at initial steps of infection but it's effect was deletion there after the absence of *Lactobacillus* in most cases of the present might explain the reasons for the increase in the colonization of *T. vaginalis* . The decrease in the *Lactobacillus* as normal flora in the vaginal environment is found at the end of menstrual cycle and during menopause. In Najaf city, a total of 64 females were examined and of those, 9 (14.06%) of 64pateints were discovered to be infected with *T. vaginalis* in vaginal swabs (Taher *et al*., 2018).

The investigation was agree with what was found in other studies such as an overall, 525 (85.5%) among 614 was screened positive for *T. vaginalis* of females

who was admitted to AL-Liqa'a Hospital in Baghdad city. Other Iraqi studies in Mosul city reported (25.86%) of *T. vaginalis* infection , and (22.6%) in Baghdad city (Saheb *et al*., 2016).

The research view useful information in table (4-1) about the spread of *N. gonorrhea* and identifies the symptoms of the infection in Karbala and Babylon cities, which can be used to plan ways to avoid it. The prevalence of *N. gonorrhea* was 20 /285(7.02 %) in patients who went to the hospitals and some private medical clinics in Karbala and Babylon cities, the research was agree with was found in different regions of Iraq.

Neisseria gonorrhea was recorded to be more present in the urethral of male compared to vaginal and cervical sites of female which recorded 14 (9.72%) out of 18 cases. Purulent urethral discharge and dysuria are characteristic symptoms of gonococcal urethritis in males (Buder & Lautenschlager, 2022).

Other Iraqi studies found infection with *N. gonorrhea* as the following 3/96 (3.13%) in female admitted to Al-Yarmook Teaching Hospital in Baghdad city (Hoom *et al*., 2008), 28/312 (8.97%) in male who admitted to some private medical clinics in Erbil city (Mansoor, 2014), and 33/180 (18.3%) of in females attending to Babylon Hospital for Maternity and Children in Babylon city and suffering from cervicitis (AL-Janabi, 2011).

The rate of infection with *N. gonorrhea* in this region seems to be lower compared to that reported for the advanced and neighboring countries. The prevalence of *C. trachomatis* and *N. gonorrhea* infections among male with urethritis in Kuwait found that the rate of infection was 23.9%. In another study carried out in Saudi Arabia, found that the rate of infection with *N. gonorrhea* was 14.2%. However the high incidence of gonorrhea infection in Saudi Arabia and

Kuwait compared to this region might be due to the presence in large number of foreign workers since no marital sex and homosexuality are prohibited by Muslim religion (Mansoor, 2014).

Cervical swabs in female recorded less infection with each of *C. albicans*, *T. vaginalis* and *N. gonorrhea*, compared to vaginal and urethral swabs and this due to purity of the uterine lining in female. Both the vagina and uterine mucosa have host unique microbial communities (Agostinis *et al*., 2019).

Table (4-3):-urogenital microorganisms according to the mixed isolation.

Types of Microorganism	**Vaginal swabs (Female)(%)**	**Cervical swabs (Female)(%)**	**Urethral swabs (Male)(%)**	**Total**
C. albicans +T. vaginalis	6 (4.17)	1 (0.69)	2 (1.40)	9(6.26)
C. albicans + N. gonorrhea	2 (1.40)	0 (0)	1 (0.69)	3(2.09)
T. vaginalis + N. gonorrhea	0 (0)	0 (0)	1 (0.69)	1(.069)
Three mixed infection	1 (0.69)	0 (0)	0 (0)	1(0.69)
Total	9	1	4	14(9.73)
Statistical analysis	X^2= 3.63, DF=6 P=.0 73			

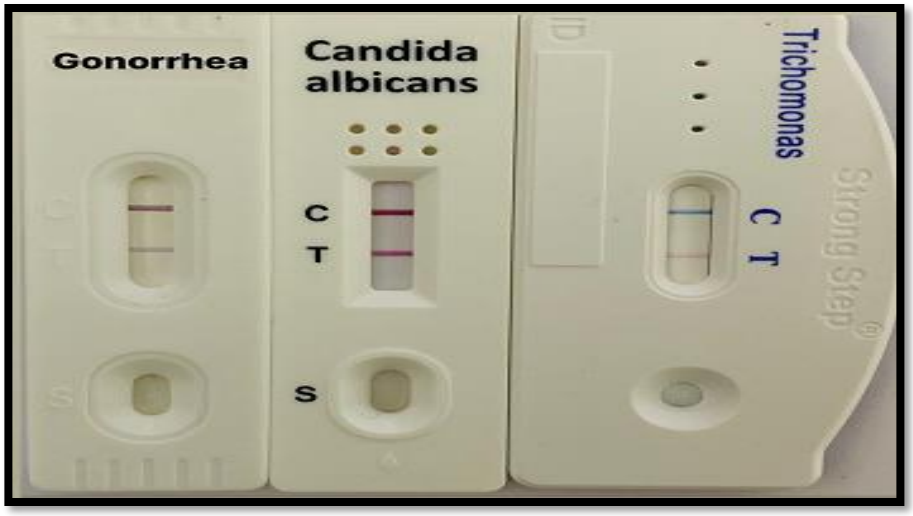

Figure (4-1) Rapid Immunochromatography Test for detection of urogenital diseases.

The positive result represents a clear reddish colored bar found on test line part (T) for three microorganisms, as well as a red colored line on the control line part (C) for *N. gonorrhea* and *C. albicans* while blue colored in control part (C) for *T. vaginalis* as well as negative result only one line (red) appears in control region (C) for *N. gonorrhea* and *C. albicans*, while only blue line appears in control region (C) in the state of *T. vaginalis* while invalid there are no lines seen in both regions (control, test) after added the specimens during 10-20 minutes.

The outcome of the current research introduced the relationship among the type of specimen isolated from patients of both sexes with the mixture of microorganisms. *C. albicans* and *T. vaginalis* were considered the most abundant microorganisms, recording 6.26% compared to the total mixture of other microorganisms.

An investigation found that double infections in female hospitalized in AL-Liqa'a Hospital in Baghdad city included the *T. vaginalis* with *C. albicans*, with a rate of 1.14% (Saheb *et al*., 2016).

These outcomes were consistent with what the researcher explained when he found a double infection in only 3 female out of a total of 193 female suffering from genital infections (Glehn *et al*., 2016) .

One research that was carried out in PHC found a mixed infection rate of 0.23% between *C. albicans* and *T. vaginalis* , but another research that was carried out in PHC also reported a co-infection rate of 14% (Levi *et al*., 2011; López-Monteon *et al*., 2013).

Likewise, other researches recorded a co-infection of both *T. vaginalis* and *C. albicans* in 3 female out of 249, with a rate of 1% (Nsagha *et al*., 2015).

In the current research, it's found that is a co-infection of both *C. albicans* and *N. gonorrhea*. This is consistent with the findings of an Italian research in which *N. gonorrhea* and *C. albicans* were simultaneously isolated from 27 clinical specimens. All 27 *C. albicans* were able to limit the growth of standard forms of *N. gonorrhea*, although 44 percent of the gonococcal isolates were resistant to inhibition (Hipp *et al*., 1975).

Table (4-3) indicates that there is a co-infection of both *T. vaginalis* and *N. gonorrhea* in Iraqi female, with a rate of 0.69% of cases.

In this examination there is only one case that recorded three infections in female **(figure 4-1)**, and this research came in agree with the researcher who worked on the research of the three infection in the patients of the admitted of London hospitals (Gunther *et al*., 2014).

The research was agree with the findings of other investigation which discovered that the overall prevalence of *N. gonorrhea*, *T. vaginalis* and *C. trachomatis* infections were 1.7%, 6.7% and 8.7%, correspondingly. The rates of co-

infection was low, 1.3% of subjects were co-infected with *C. trachomatis* and *T. vaginalis* ; 0.61% were co-infected with *N. gonorrhea* and *T. vaginalis* ,while co-infected with *N. gonorrhea* , *T. vaginalis* and *C. trachomatis* were 0.24% of subjects had a triple co-infection (Kalichman *et al*., 2011).

Table (4-4):- The Percentage of urogenital microorganisms according to clinical finding.

Sex	Clinical finding	*C. albicans*	*T. vaginalis*	*N. gonorrhea*
Female No. 99	Vaginal discharge	25 (17.36%)	23 (15.97%)	5 (3.47%)
	Pelvic Inflammatory Disease	6 (4.17%)	5 (3.47%)	1 (0.70%)
	Vaginitis	19 (13.19%)	6 (4.17%)	0 (0)
	Vaginal itching	9 (6.25%)	0 (0)	0 (0)
Statistical analysis	$X^2 = 13.6$, DF= 6 P= 0.034			
Male No. 45	Dysuria	3 (2.08%)	2 (1.39%)	1 (0.70%)
	Epididymitis	3 (2.08%)	0	0
	Prostatitis	0	0	4 (2.78%)
	Urethritis	16 (11.11%)	7 (4.86%)	9 (6.25%)
Statistical analysis	$X^2 = 13.08$, DF= 6 P= 0.04			

Table (4-4) introduced the most cases of urogenital diseases infection was vaginal discharge where recorded as 25 (17.36%), 23 (15.97%) and 5 (3.47%) positive specimens in *C. albicans*, *T. vaginalis* and *N. gonorrhea* respectively in females followed by vaginitis, vaginal itching and the last PID, while in male the

highest clinical finding was urethritis which recorded 16 (11.11%), 7 (4.86%) 9 (6.25%) for *C. albicans*, *T. vaginalis* and *N. gonorrhea* respectively, followed by prostatitis, dysuria and epididymitis.

Regarding vaginal discharge the study showed that pathological vaginal discharge was the most common symptom in non –IUDs and is significantly higher than in IUDs user and this is due to higher incidence of infection in this group of patients. Pelvic pain was the most common symptom in the IUDs user and the type was either chronic pain (most patients) or few of them complain of dyspareunia and this is complication of IUDs that the pain either due to genital tract infection or because of uterine contraction. The study showed that about half of non – IUDs user patients with genital tract infection were asymptomatic, this may reflect that those with IUDs may be instructed about any complain that they developed at early time and they have a good looking forward about symptom of genital tract infection so they are aware of the symptoms (AL-Janabi, 2011).

Pelvic inflammatory disease the clinical syndrome that encompasses infections of the female upper genital tract. It must be kept in mind in the differential diagnosis of female of childbearing age that come to the emergency room with PID, becoming the most frequent cause of gynecological admission, according to some series. Most are diagnosed in nulliparous female between the ages of 15-24 (Curry *et al*., 2019).

One of the most common issues that arise in a gynecologist's everyday clinical practice is vulvovaginitis. Infections of the female genital tract are included in the scope of pelvic inflammatory disease. Due to difficulties in the acute phase and the squeal, which might include persistent discomfort and infertility, early diagnosis and effective treatment are crucial. The most frequent symptoms are lower abdominal pain, vaginal bleeding, or excessive vaginal discharge. The last symptom will be one

of the main ones in the vulvovaginitis and vaginitis (Esim Buyukbayrak *et al*., 2010).

One research explained that the characteristic of vulvovaginitis was the intense itching accompanied by whitish leucorrhoea in the form of lumps and not malodorous. In turn, it produces erythema, vulvar edema, and dyspareunia (Fakhim *et al*., 2020).

The highest isolation rate of *T. vaginalis* was from vaginal discharge. Since the vagina is the normal habitat of *T. vaginalis* than cervix, also the vaginitis recorded high rate of infections, this may due to low levels of estrogen present to act upon the vagina, resulting in a thin, easily abraded epithelium. In addition the pH of vagina is alkaline, so vaginitis during childhood is a common (Mahdi *et al*., 2001).

The clinical findings of trichomniasis was characterized by the existence of a profuse, abundant, grey-yellowish-greenish, fluid, frothy and malodorous vaginal discharge. The picture usually presents with genital itching, dysuria and dyspareunia, although it can be asymptomatic in 10-50% of cases (Gupta *et al*., 2021).

Genital itching represents another reason for emergency care, mainly in young sexually active patients. The frequency and etiology differ according to the geographical area, although *T. vaginalis* is the most frequent cause of this, followed by syphilis and *C. albicans* (Squire *et al*., 2019 ; Abd Ellah *et al*., 2021).

Urethritis is the most infection of urogenital diseases microorganisms among male who admitted to private clinic; it was recorded as 16, 7 and 9 for *C. albicans*, *T. vaginalis* and *N. gonorrhea* respectively.

In urban sexual health clinic, a research has been carried out to determine the prevalence of *T. vaginalis* and *N. gonorrhea* in male with urethritis. The prevalence of *T. vaginalis* and *N. gonorrhea* was 3 (3.6%) , 14 (16.8%) respectively from 83 participant (Khatib *et al*., 2015).

Only a minority of male who have nongonococcal urethritis (NGU) may develop persistent or recurrent urethritis; it is likely that *T. vaginalis* is responsible for only a subset of these occurrences. Trichomoniasis is more difficult to diagnose clinically and in the laboratory in male patients (Kaydos-Daniels *et al*., 2004).

Sexual dysfunction may accompany the voiding symptoms and genitourinary pain associated with prostatitis. 25% of male will be diagnosed with prostatitis at some point in their lives, although only about 10 percent will actually have a bacterial infection. Infection with uropathogens, mainly gram-negative bacilli, is the cause of bacterial prostatitis, but the origins and management of nonbacterial prostatitis are mostly unclear (Su *et al*., 2020).

- Sensitivity= TP/ (TP+FN) **- Specificity= TN/ (TN+FP)**

-Accuracy= (TP+TN)/ (TP+FP+TN+FN)

- (TP) The number of specimens infected with urogenital microorganisms and has a positive result.

- (FN) The number of specimens non-infected by urogenital microorganisms and has a negative result.

- (TN) The number of specimens non-infected by urogenital microorganisms and has a negative result (other fungi and bacteria).

- (FP) The number of specimens non- infected with urogenital microorganisms and has a positive result.

Table (4-5):- The Sensitivity, Specificity and Accuracy of *C. albicans.*

C. albicans		PCR			
		+	_	Total	**Sensitivity = 91.76 %**
IC strip	+	78	3	81	**95% CI=83.77% to 96.62%**
	_	7	56	63	**Specificity =94.91 %**
					95% CI=85.85% to 98.94%
	Total	85	59	144	**Accuracy=93.06%**
					95% CI = 87.60% to 96.62%

The outcomes of the current research , when comparing the sensitivity and specificity of both PCR and Immunochromatographic strips for *C. albicans*, presented that the value of sensitivity was 91.76%, with confidence interval (95% CI; 83.77% to 96.62%), specificity 94.91% with confidence interval (95% CI; 85.85% to 98.94%) accuracy 93.06% with confidence interval (95% CI; 87.06% to 96.62%). **Figure (4-2) (4-3)**

The number 78 in the table (4-5) represented the positive specimens for both IC strips and PCR technique, the number 3 represented positive specimens by IC strips but negative by PCR technique, the number 7 represented negative specimens by IC strips but positive by PCR, the last number 56 represented the negative specimens for both IC strips and PCR technique.

The new Immunochromatographic strips is more sensitivity and specificity (100%) compared to culture Which was sensitive to (96%) and specialized (93%), because of the culture method characterizes 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 hours. Some species may require more than 3 days to appear on culture medium, while the new immunochromatographic strip does not take a long time, it need 10-20 minutes to get a result and there is no contamination. These results agree with studies (Hassan *et al*., 2019).

The new Immunochromatographic 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 hours. Due to the time required to prepare human serum and 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. Its need to be accurate in the time and temperature for example, incubating period for more than 3 hours 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 immunochromatographic strip dissent need any factors of the above. These results agree with studies (Hassan *et al*., 2019).

The new Immunochromatographic strips is more sensitive and specificity (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 immunochromatographic strip is low cost commercially. These results agree with studies (Hassan *et al*., 2019).

Some of researches 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%) from 160 patient of candidiasis. This straightforward diagnostic test will be helpful to medical professionals who are treating female who exhibit signs of fungal vaginitis on patients who have been admitted to the hospital in Mosul city (Alghnam & Y AL-Dabbagh, 2012).

These outcomes were consistent with what 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 (Matsui *et al*., 2009).

Also the outcome was agree with another research was found the sensitivity, specificity, and accuracy of the IC strips were 89.8%, 90.9%, and 90.7%, respectively. When compared IC strip with culture and decided that IC strip evaluated can be made readily available for clinical use in detecting *C. albicans* (Matsui *et al*., 2020).

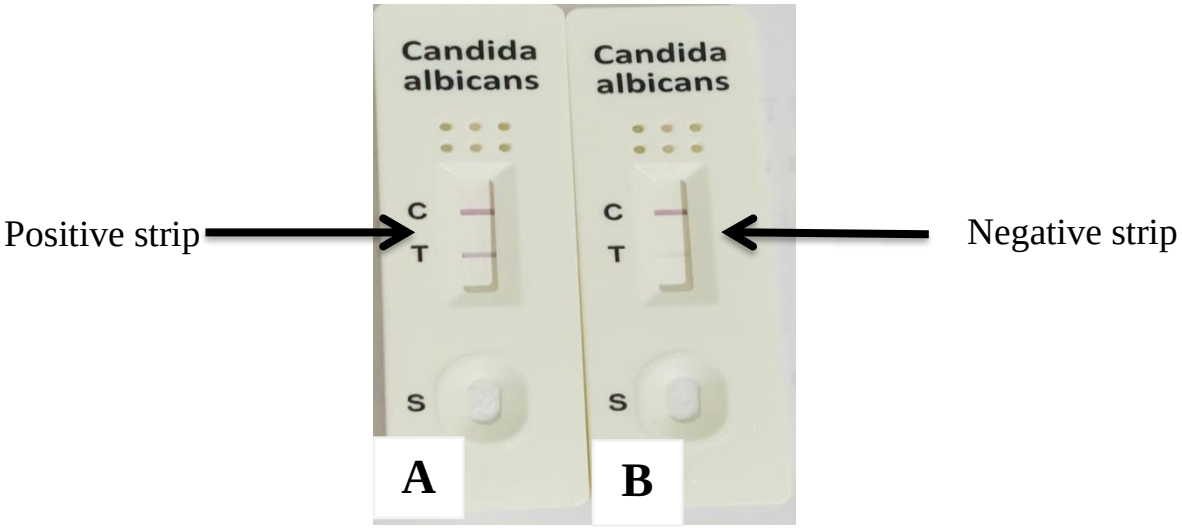

Figure (4-2) show the positive (A) and negative (B) IC strip for *C. albicans.*

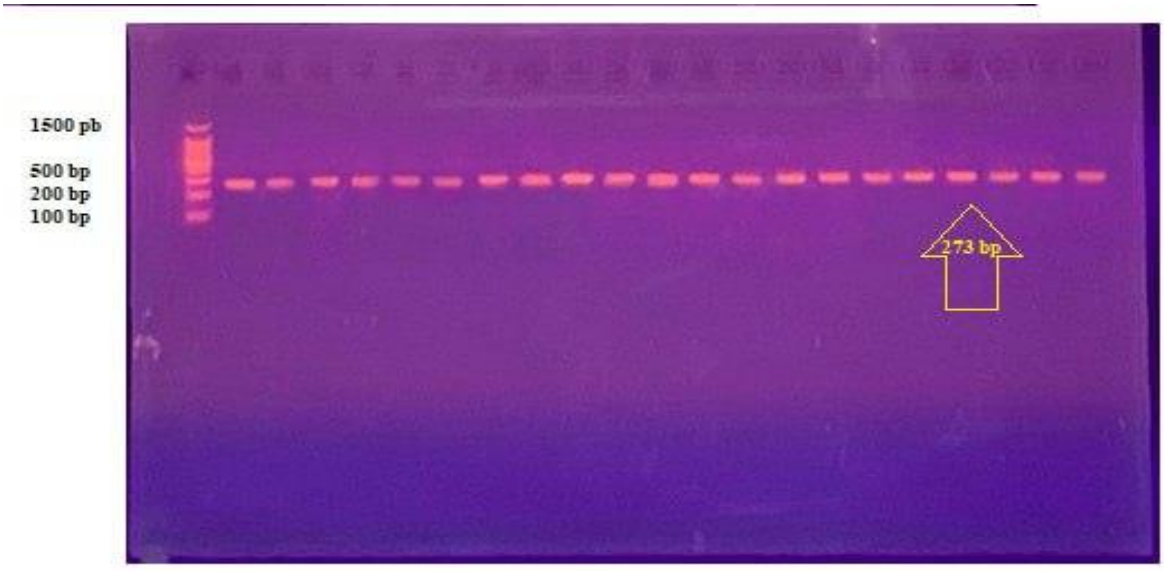

Figure (4-3) Agarose gel electrophoresis for detection of *C. albicans* (Internal transcribed spacer ITS gene) by polymerase chain reaction (PCR). Lane 1: 100-bp DNA ladder; Lane 2: Lane 2-21: clinical specimens positive at 273 base pair.

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

Table (4-6):- The Sensitivity, Specificity and Accuracy of *T. vaginalis*.

T. vaginalis		PCR +	PCR –	PCR Total	
IC Strip	+	41	2	43	**Sensitivity = 87.23%** **95% CI = 74.26% to 95.17%** **Specificity = 97.93%** **95% CI = 92.75% to 99.75%** **Accuracy = 94.44%** **95% C I= 89.35% to 97.57%**
	–	6	95	101	
	Total	47	97	144	

The outcomes of the current research, when comparing the sensitivity and specificity of both PCR and IC strip for detection of *T. vaginalis*, presented that the value of sensitivity was 87.23%,with confidence interval (95% CI; 74.26% to 95.17%) and specificity 97.93% with confidence interval (95% CI = 92.75% to 99.75%) accuracy 94.44% with confidence interval (95% CI; 89.35% to 97.57 %).

The number 41 in the table (4-6) represented the positive specimens for both IC strips and PCR technique, the number 2 represented positive specimens by IC strips but negative by PCR technique, the number 6 represented negative specimens by IC strips but positive by PCR, the last number 95 represented the negative specimens for both IC strips and PCR technique.

Some of examination presented that the highest rate of infection by this parasite was in Baghdad city 162 (85.3%) from 190 during 2016 while lowest percentage of infection was 9 (3.1%) from 290 in Erbil city during 2015. It had concluded that geographical location of Iraqi governorates with specificity 93% and sensitivity 90.5% (Al-Marjan & Sadeq, 2022).

The outcome was agree 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 (Herath *et al*., 2021).

Several authors have view that the ICT is 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 test 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 technicians 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 for 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 (Huppert *et al*., 2005;Pattullo *et al*., 2009).

The Institute of Microbiology and Immunology (IMI), Faculty of Medicine, University of Ljubljana, conducted a 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 results was the real-time PCR have sensitivity and specificity more than wet mount microscopy and culture (Šoba *et al*., 2015).

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. **Figure (4- 4)**

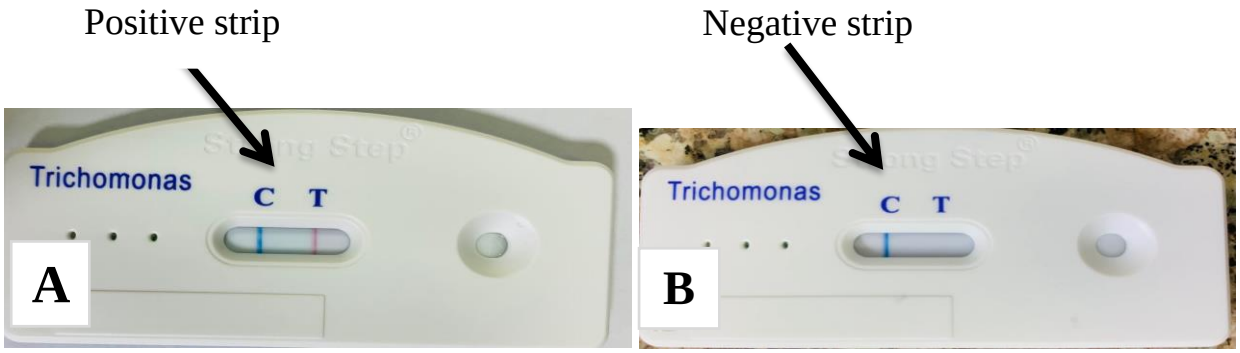

Figure (4-4) show the positive (A) and negative (B) IC strip for *T. vaginalas.*

The majority of clinicians who presently test female for *T. vaginalis* infection rely upon insensitive diagnostic methods, such as wet mount, and asymptomatic female are seldom tested at all. Although wet mount is the standard of care, it is only 60% sensitive compared to culture. In many health care 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 microscopist 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 (Huppert *et al*., 2005).

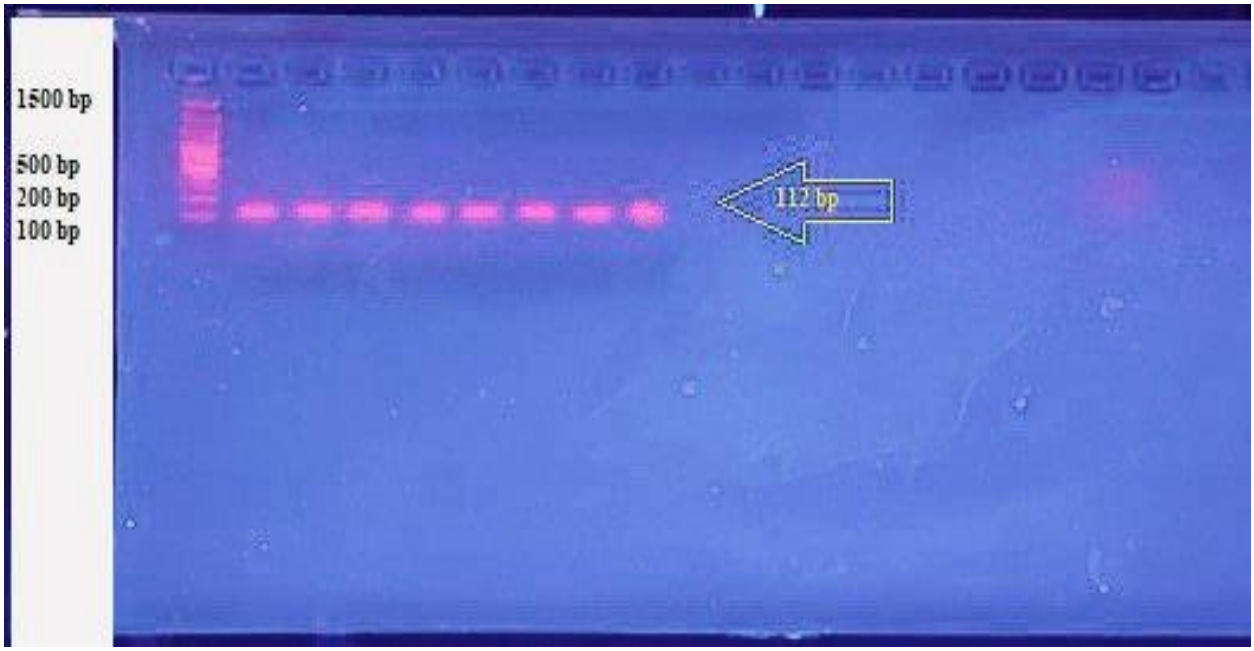

Figure (4-5) Agarose gel electrophoresis for detection of *T. vaginalis* (β-Tubulin gene) by polymerase chain reaction (PCR). Lane 1: 100-bp DNA ladder; Lane 2: Lane 2-8: clinical specimens positive at 112 base pair.

Out of a total of 144 positive sexual clinical specimens collected from female and male from private hospitals, 47 specimens were positive for *T. vaginalis* parasite using the PCR technique.

Table (4-7):- The Sensitivity, Specificity and Accuracy of *N. gonorrhea.*

N. gonorrhea		PCR			
		+	_	Total	**Sensitivity = 41.67%**
IC Strip	+	15	5	20	**95% CI= 25.51% to 59.24%**
	_	21	103	124	**Specificity = 95.37%**
	Total	36	108	144	**95% CI= 89.53% to 98.48%**
					Accuracy= 81.94%
					95% CI= 74.67% to 87.85%

The outcome of the current investigation, when comparing the sensitivity and specificity of both PCR and Immunochromatographic strip for detection of *N. gonorrhea*, presented that the value of sensitivity was 41.67%, with confidence interval (95% CI; 25.51% to 59.24%) and specificity 95.37% with confidence interval (95% CI; 89.53% to 98.48 %) accuracy 81.94% with confidence interval (95% CI; 74.67% to 87.85%). **Figure (4-6)**

The number 15 in the table (4-5) represented the positive specimens for both IC strips and PCR technique, the number 5 represented positive specimens by IC strips but negative by PCR technique, the number 21 represented negative specimens by IC strips but positive by PCR, the last number 103 represented the negative specimens for both IC strips and PCR technique.
The outcome were close with some studies, in which it was indicated that the infection rate was 30 (30%) from 100 using immunochromatograpic strip in patient who were admitted to Baghdad hospitals (Ali & Shia, 2018).

Some research found the sensitivity and specificity of the *N. gonorrhea* strip compared with the outcome of standard culture were 94.1% (32/34) and 95.8% (23/24), respectively. When an evaluation of a ICT for *N. gonorrhea* as a diagnostic tool for gonococcal urethritis in male patients (Suzuki *et al*., 2004).

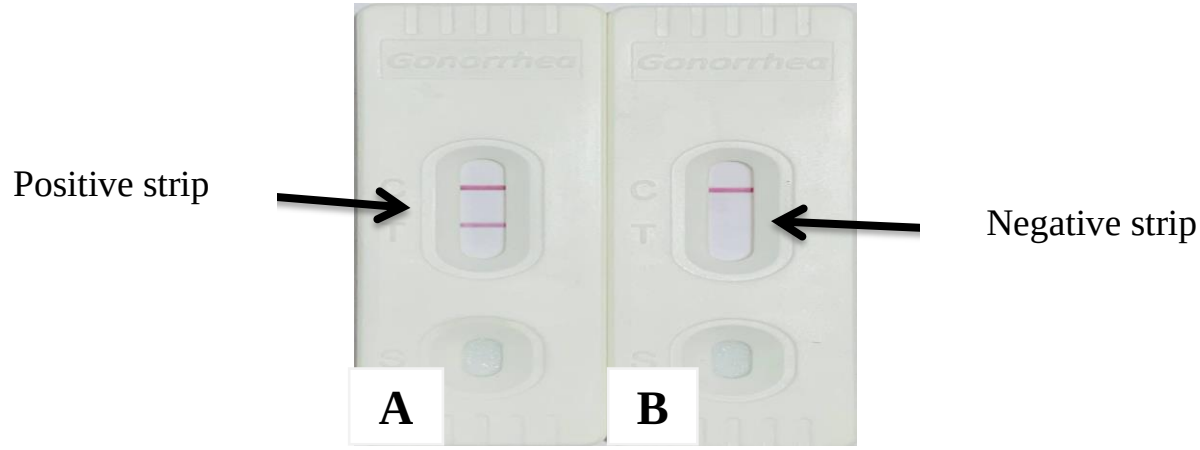

Figure (4-6) Positive (A) and negative (B) IC strip for *N. gonorrhea*.

On the other hand, a PCR assay was performed on 14 gonorrhea specimens from male and 6 specimens from female to evaluate the sensitivity and specificity of the chosen topoisomerase lV subunit C gene primers. **Figure (4-7)**

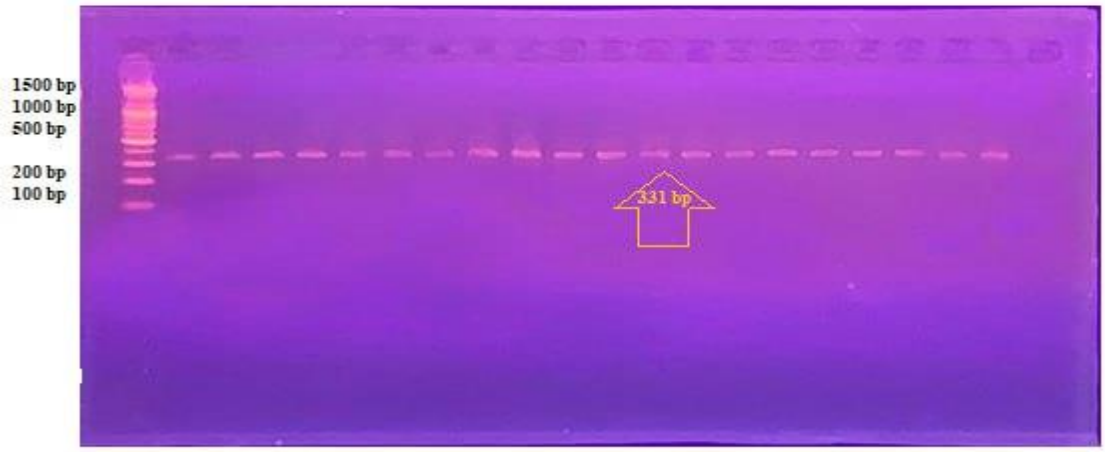

Figure (4-7) Agarose gel electrophoresis for detection of *N. gonorrhea* (topoisomerase lV subunit C) by polymerase chain reaction (PCR). Lane 1: 100-bp DNA ladder; Lane 2: Lane 2-20: clinical specimens positive at 331 base pair.

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

Table (4-8):- The Percentage of urogenital microorganisms according to residence location.

Types of microorganisms	NO.	Karbala / Babylon	
		Urban No. (%)	Rural No. (%)
Positive *C. albicans*	81	43 (29.86)	38 (26.39)
Positive *T. vaginalis*	43	31 (21.53)	12 (8.33)
Positive *N. gonorrhea*	20	15 (10.42)	5 (3.47)
Statistical analysis	$X^2 = 6.011$, DF = 2 P = .0049		

The research found a significantly increased urogenital diseases microorganism in patient lived in urban area in Karbala and Babylon cities than rural area, which recorded 43 (29.86%) from 81 cases in urban while in rural recorded 38 (26.39%) from 81cases with *C. albicans*.

These researches agree with was explained by another researchers in Karbala city which found the prevalence of *T. vaginalis* in urban recorded (10%) 11/111 more than in rural was (7.43%) 9/121cases, this understanding how transmission may vary between rural (or sparsely populated) and urban (or densely populated) areas (Alhusseini & Alquraishi, 2021).

Some of author found that the infection rate of trichomoniais was much greater for rural residents than those who lived in urban areas in Najaf city which disagree to the present examination (Taher *et al*., 2018).

The research was disagree with the explained by another study that found the infection of *T. vaginalis* and *C. albicans* more prevalence in rural than urban in both sex, the reasons for this high prevalence in rural females could be lack of awareness, poor literacy rates, low socioeconomic status, poor personal hygiene and most importantly poor treatment seeking behavior (Arora *et al*., 2014).

The prevalence of *T. vaginalis* in Vanuatu female significantly higher in comparison with developed countries. Female in rural settings have less likely to have access to prevention and treatment programs for urogenital diseases and this disagree with the current study, the United Nations predicts that nearly 70% of the global population will live in urban areas by 2050. Population size is the most significant driver of epidemic dynamics (Fotinatos *et al*., 2008).

Table (4-9):- The Percentage of urogenital microorganisms according to the education levels.

Types of microorganisms	N0.	No Education	Primary Education	Secondary Education	Higher Education
Positive *C. albicans*	81	27 (18.75)	20 (13.89)	18 (12.5)	16 (11.11)
Positive *T. vaginaliss*	43	14 (9.72)	11 (7.64)	10 (6.94)	8 (5.56)
Positive *N. gonorrhea*	20	9 (6.25)	7 (4.86)	2 (1.39)	2 (1.39)
Statistical analysis	$X^2 = 3.551$, DF=6 P= 0.737				

Table (4-9) introduce the relationship of microbial infections with the level of education, where the research found that patients with a low level of education are more infected at a non-significant level with the patients who have a primary, intermediate or higher level of education.

This research was agree with which was explained by another research that view illiterate and secondary education more prevalence with *T. vaginalis* than other stages which recorded 6 (14.63%) from 41 cases in Karbala city, this may due to poor health care and lack of females awareness programs, loss of immunity as a result of malnutrition, a lack of knowledge, and a failure to seek adequate treatment all this put the female at the risk of infection (Alhusseini & Alquraishi, 2021).

The present research was agree with was revealed by another researches presented that females with low education have the highest percentage (18.7%) , also studies in Baghdad and Al-Najaf cities view that uneducated female illiterates were more associated with the disease that other age classes (Taher *et al*., 2018).

This outcome were consistent with other local research who was found low education more prevalence with *T. vaginalis* than other educational levels, it was recorded 87 (77.67%) from 112 from participant in Maysan city (Alhusseini & Alquraishi, 2021).

The investigation was agree with some local studies that explained candidiasis more prevalence in low education than other education level in Tikrit city, which recorded 68 (59%) from 115 (Alsharifi, 2017).

Although that education and counseling are the main strategies for the prevention and control of urogenital diseases, as well as the development of structured educational programs that must include the generation adequate knowledge, in order to change risk attitudes and practices, However, some urogenital diseases have been recorded in patients who have a higher education level. The WHO reports that half of the new cases occur in the population adolescent and young adults (Cordero & Montero, 2018).

The research was disagree with other studies that explained studied female with vaginal delivery recorded 155 (65%) from 239 with secondary education, 76 (31.7%) from 239 were low education level, and 8 (3.3%) from 239 cases with university degrees infected with *N. gonorrhea* (Pourabbas *et al*., 2018).

The incidence of trichomoniasis has declined sharply in developed countries in the recent past probably due to early diagnosis, use of better diagnostic techniques, proper management and emphasis on behavioral change. In contrast, in undeveloping countries, and amongst disadvantaged groups, the infection appears to be widespread (Gillespie & Pearson, 2001).

Table (4-10):-The Percentage of urogenital microorganisms according to economic levels.

Types of microorganisms	NO.	Low economic condition	Middle economic condition	Highly economic condition
Positive *C. albicans*	81	39 (27.08)	22 (15.28)	20 (13.90)
Positive *T. vaginalis*	43	19 (13.19)	14 (9.72)	10 (6.94)
Positive *N. gonorrhea*	20	9 (6.25)	7 (4.86)	4 (2.78)
Statistical analysis	X^2 = 0.739 , DF= 4 P = 0.946			

Table (4-10) introduce the most infection of urogenital diseases in the patient who they have low economic condition; it was recorded as 27.08%, 13.19 % and 6.25% for *C. albicans* and *T. vaginalis* and *N. gonorrhea* respectively.

The research was agree with other local studies that presented *T. vaginalis* more prevalence in low economic level than other levels, which recorded 267(76%) from 352 with vaginal discharge in Basra city (Mahdi *et al*., 2001).

The result was agree with the finding in another studies that explained 18 (45%) of 40 *C. albicans* occurs in low economic in Iran (Esmailzadeh *et al*., 2018).

In low-middle-income countries, laboratory tests are not usually used to diagnose urogenital diseases, but attempts are made to identify clusters indicative of easily recognize sign and symptom to write treatment(Yang *et al*., 2020; Lu *et al*., 2022).

The knowledge that the population has about urogenital diseases is limited and mainly concentrated in population groups with better economic resources, better accessibility, people located in the upper stratum have better services and facilities. For example, a higher level of knowledge favors the constant use of condoms. On the other hand, the socioeconomic factor directly influences the accessibility of protection methods in terms of cost, young people with high incomes have more opportunity to buy protection methods (Flórez, 2005).

Table (4-11):- The Percentage of urogenital microorganisms according to contraception.

Types of microorganisms	NO.	Intrauterine Devices	Condom	Oral Contraceptive	None
Positive *C. albicans*	81	25 (17.36)	8 (5.56)	28 (19.44)	20 (13.89)
Positive *T. vaginalis*	43	11 (7.64)	5(3.47)	14 (9.72)	13 (9.03)
Positive *N. gonorrhea*	20	2 (1.39)	3 (2.08)	4 (2.78)	11(7.64)
Statistical analysis	$X^2 = 8.952$, DF= 6 P= 0.176				

Table (4-11) noted that the most cases of infection in females using the oral contraceptive were Candidiasis fungus, with a rate of 19.44% followed by intrauterine device that recorded 17.36%, also *T. vaginalis* recorded the highest infection in female that used oral contraceptive which have 9.72%, while in *N. gonorrhea* the highest percent occur due to other causes than oral contraceptive, intrauterine device (IUD) and condom which recorded 7.64% of cases.

The research was agree with a research occurs in Al Basra city that observed highest incidence of *C. albicans* occur in females used oral contraceptive. The more frequent cases among females was attributed that there is more sexual activity, more using antibiotic, more using of oral contraceptive pills in addition to pregnancy as a risk factor (Sharief, 1998).

An investigation occur in AL-Batool hospital in Baquba city observed the highest rate of infection with *C. albicans* occurs in females used IUDs which recorded (62%), while the lowest rate of infection occur in females that husbands used condoms which recorded 3 (6%) of 50 females (Ahmed & Aial, 2016).

This research was disagree with a research occur in Karbala city that explained decreased the rate of *T. vaginalis* in females that used oral contraceptive which recorded 5 (7.24%) from 64 patients. In addition its recorded 15 (9.2%) from 148 patients not used oral contraceptive (Alhusseini & Alquraishi, 2021).

The investigation was agree with a local research that done in the female Hospital in Babylon city presented that the percentage of female infected with *N. gonorrhea* was reduced when they used an Intrauterine Contraceptive Device. This result may be attributed to that females with IUDs attend regular visit for gynecological clinic and hospital for checking of IUDs so can treat infection earlier than non-user The distribution of microorganisms among IUCDs user and non-users

showed that the presence of IUDs associated with absent of *N. gonorrhea* while in non-user incidence is 18.2% and this may be explained by the fact that the copper containing loop with the time part of copper covering will dissolute in the secretion of uterus, and this copper containing fluid in the uterus and vagina act as germicidal solution to gonorrhea and as the cell membrane of *N. gonorrhea* is permeable to copper ion yet it is a lethal intracellular ion to *N. gonorrhea* cells (AL-Janabi, 2011).

The research was agree with other researches that introduced at least 65% of female who used oral contraceptives acquired *T. vaginalis* and *N. gonorrhea,* while condom user had 34% and 30% decreased rates, respectively (Rosenberg *et al*., 1992).

This outcome was agree with other research which found an increase of *C. albicans* infection in female who have taken oral contraceptive drugs. This examination disagree with another research that explained *T. vaginalis* decreased in female who they taken oral contraceptive drugs (Bramley & kinghorn, 1979).

This research was agree with another research that discovered oral contraceptive drugs are one of the risk factors that play a role in the increased infection of *T. vaginalis* in females (Bouchemal *et al*., 2017).

(Table 4-11) presented that the number of urogenital diseases decreased in female and male who use condoms, as the number of infections decreased, where it was recorded 8(5.56 %), 5 (3.47 %) and 3 (2.08 %) for *C. albicans*, *T. vaginalis* and *N. gonorrhea* respectively.

The research was agree with other studies that presented and consistent the correct condom use reduces the risk of that *N. gonorrhea* 90% of cases, barrier protection (condom) should be used during intercourse until the infection is eradicated in both partners. The population must be educated about this disease and

the means of transmission, since education on sexual behavior and genital hygiene may help in its prevention and control (Crosby *et al*., 2003).

The current study was agree with other research that explained during sexual activity, people frequently use barrier products like condoms to avoid pregnancy and the transmission of urogenital diseases. The use of condoms is strongly advised as a means of preventing urogenital diseases. It has been proven that they are effective in reducing infection rates in both sexes. A lot of sexually transmitted diseases are being reduced by condoms among them is gonorrhea. The use of condoms and IUD is recommended when birth control and preventing sexually transmitted diseases are priorities (Kandler *et al*., 2014; Mashaphu *et al*., 2022).

Table (4-12):- The Percentage of urogenital microorganisms according to history of urogenital diseases.

Types of microorganisms	NO.	Self-reported history of urogenital diseases	No reported history of urogenital diseases
Positive *C. albicans*	81	52 (36.11)	29 (20.14)
Positive *T. vaginalis*	43	35 (24.31)	8 (5.55)
Positive *N. gonorrhea*	20	17 (11.81)	3 (2.08)
Statistical analysis	X^2= 6.03 , DF=2 P=0.04		

The research found significant differences in the self-history of urogenital diseases in all microorganisms in the table (4-12), these outcomes was agree with (Birhane *et al*., 2021).

Some urogenital diseases can be detected and treated, but reinfection may occur despite this. Reinfection is common due to several factors, including not completing treatment, a partner not getting treated, and unsafe sexual behaviors (Mehta *et al*., 2003).

Both male and female have higher chance of reinfection with *N. gonorrhea*. The used of IUD was linked to a lower risk of reinfection in males, however, further investigation presented that the number of sexual partners was a confounding factor in the connection between condom use and reinfection (Hosenfeld *et al*., 2009).

Chapter Five

Conclusions and Recommendations

5.1. Conclusions

1- The new immunochromatographic strip have sensitivity, specificity and accuracy more than routine diagnostic(microscopic and culture) tests for *T. vaginalis*, *N. gonorrhea* and *C. albicans* and given the result within short time .
2- Immunochromatographic strips given the results within few minutes in compare to conventional tests.
3- Molecular diagnostic tests perform can be used in innovative screening and testing programmers for urogenital microorganisms.
4- Most prevalence of urogenital diseases was in urban regions in Karbala and Babylon cities.
5- The most common causes of urogenital diseases was *C. albicans.*

5.2. Recommendations

1- Immunocromatographic tests advise to be as a screening assay in laboratories of hospitals, for detect urogenital microorganisms especially *T. vaginalis*.
2- Molecular and genotyping for *N. gonorrhea* know the types of strain in Iraq.
3- Study the period of infection , if its early or lated.
4- Study of some virulence factors responsible for systemic candidiasis.
5- Using new culture media to diagnostic *T. vaginalis.*

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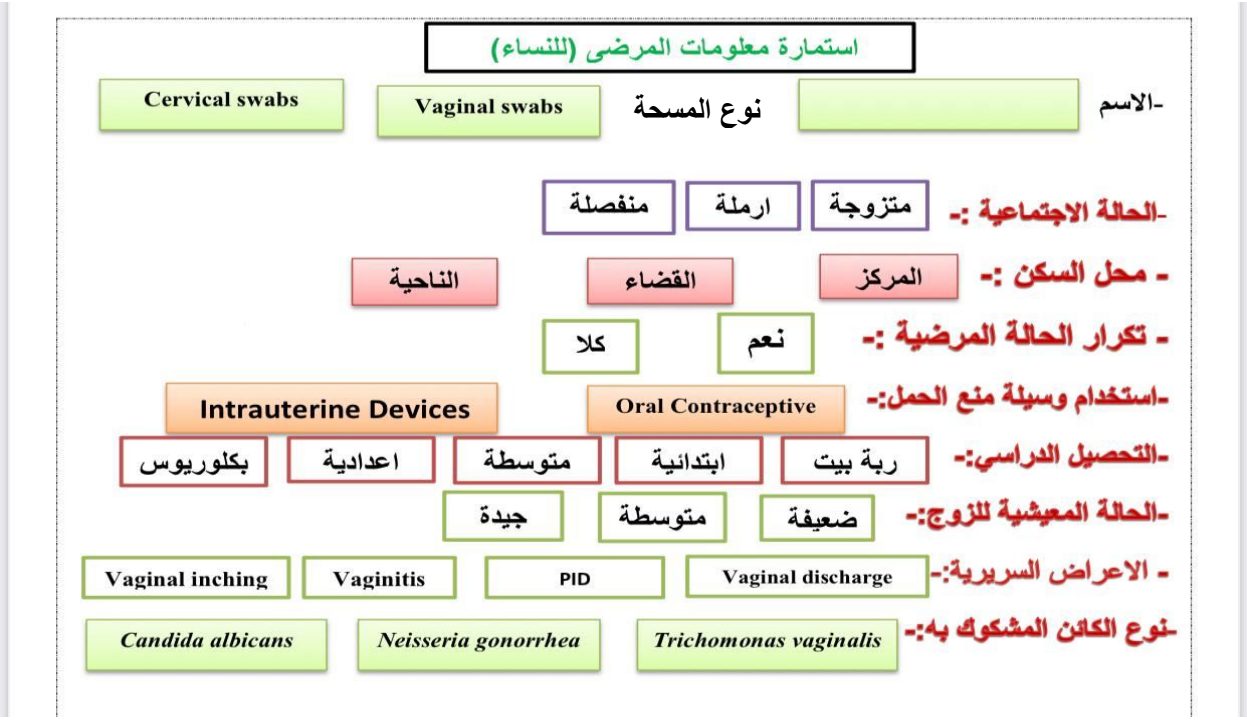
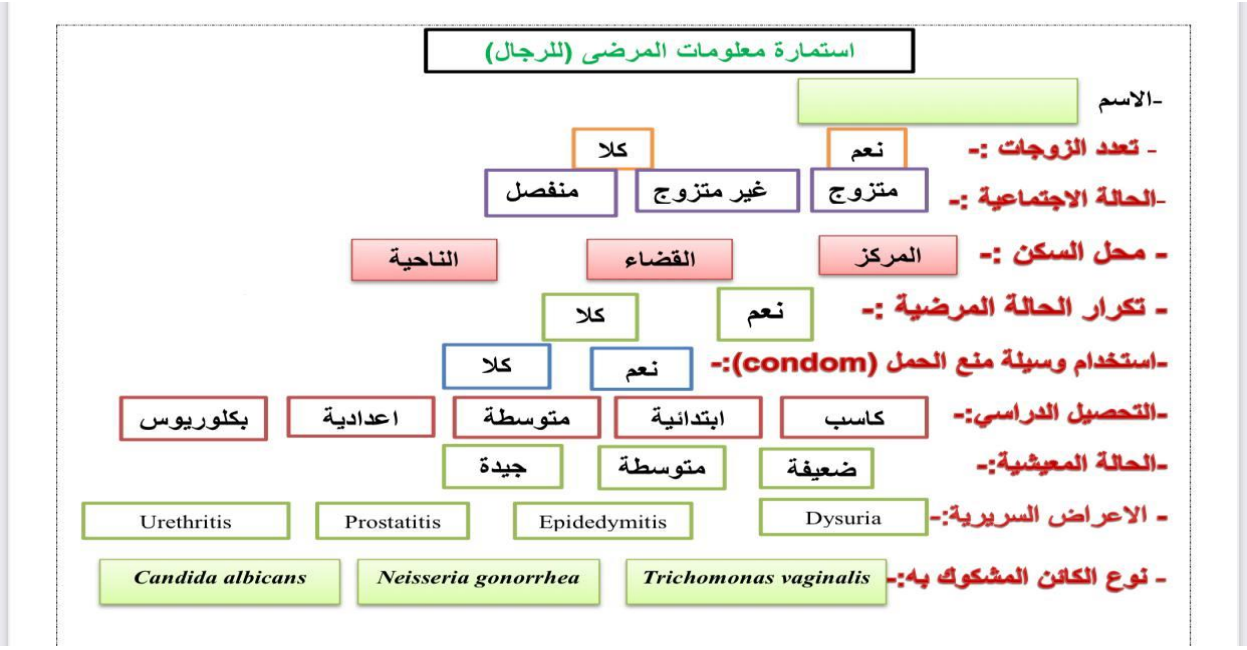
The Appendices

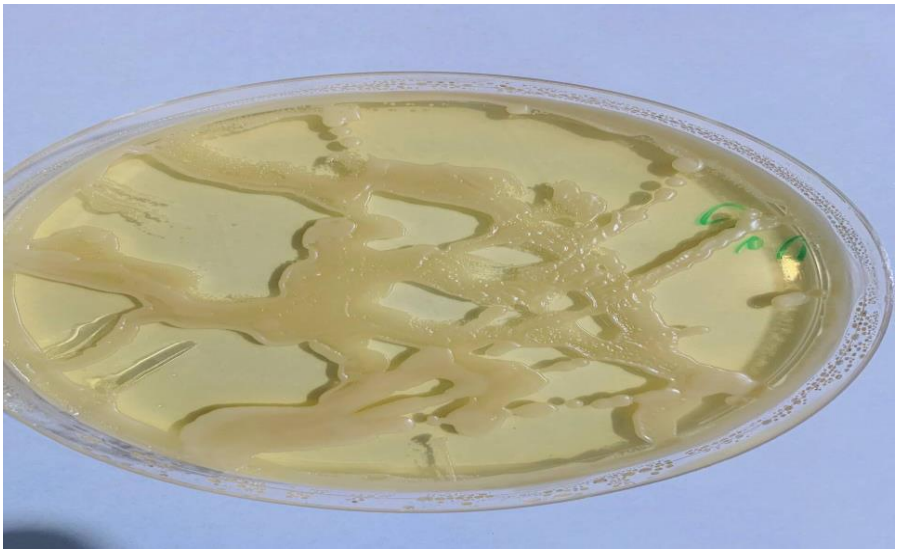
Apendix 1: Questionnaire of patients



Master sheet for female questionnaire

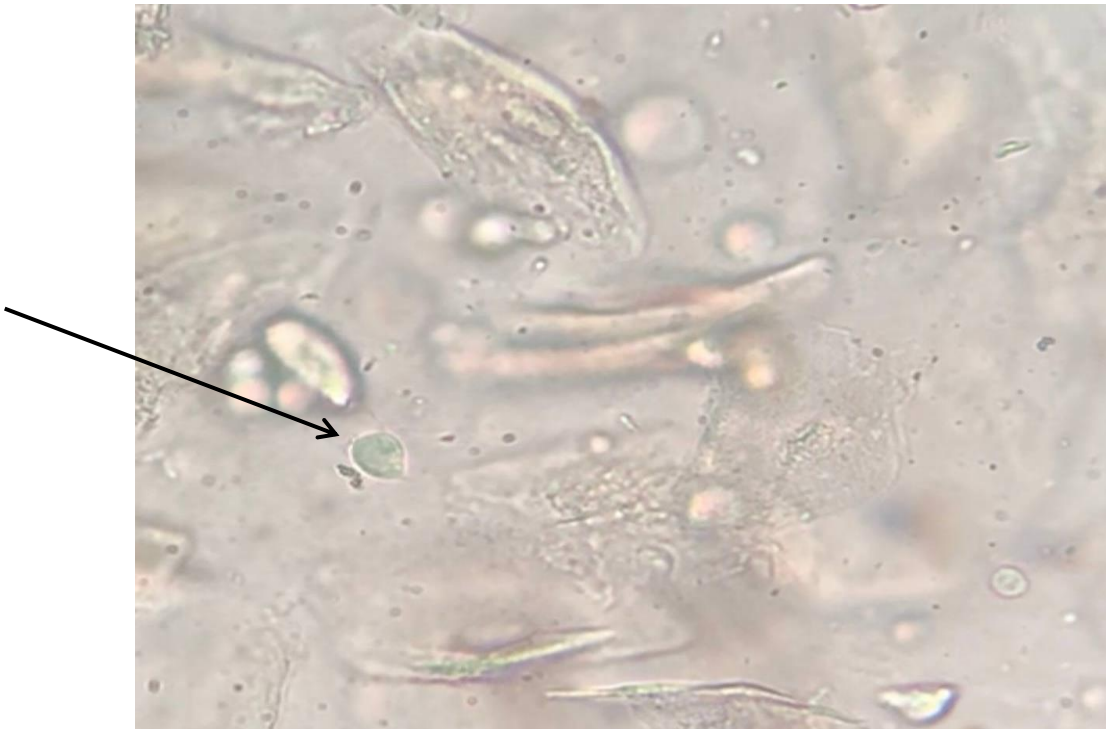


Master sheet for male questionnaire

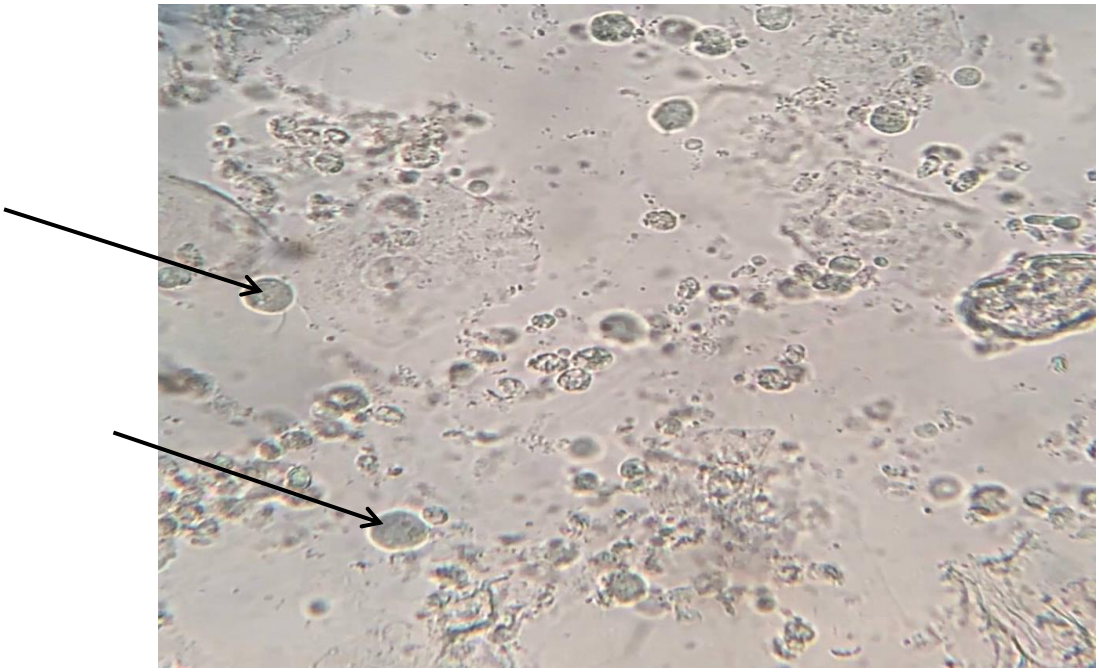


C. albicans **on sabrouaud dextrose agar**

C. albicans **on blood agar**



***Trichomonas vaginalis* from vaginal swab**



***Trichomonas vaginalis* from vaginal swab**

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

- Place a clean extraction tube in the designated area of the workstation. Add 20 drops of extraction buffer to the extraction tube.
- Put the specimen swab into the tube. Vigorously mix the solution by rotating the swab forcefully against the side of the tube for at least fifteen times (while submerged). Best results are obtained when the specimen is vigorously mixed in the solution.
  Allow the swab to soak in the extraction buffer for one minute prior to the next step.
- Squeeze out as much liquid as possible from the swab by pinching the side of the flexible extraction tube as the swab is removed. At least 1/2 of the sample buffer solution must remain in the tube for adequate capillary migration to occur. Put the cap onto the extracted tube.
  Discard the swab in a suitable biohazardous waste container.
- The specimens extracted can retain at room temperature for 60 minutes without affecting the result of the test.
- Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.
- Add 3 drops (approximately 100 µl) of extracted sample from the extraction tube to the sample well on the test cassette.
  Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
  As the test begins to work, you will see color move across the membrane.
- Wait for the colored band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.

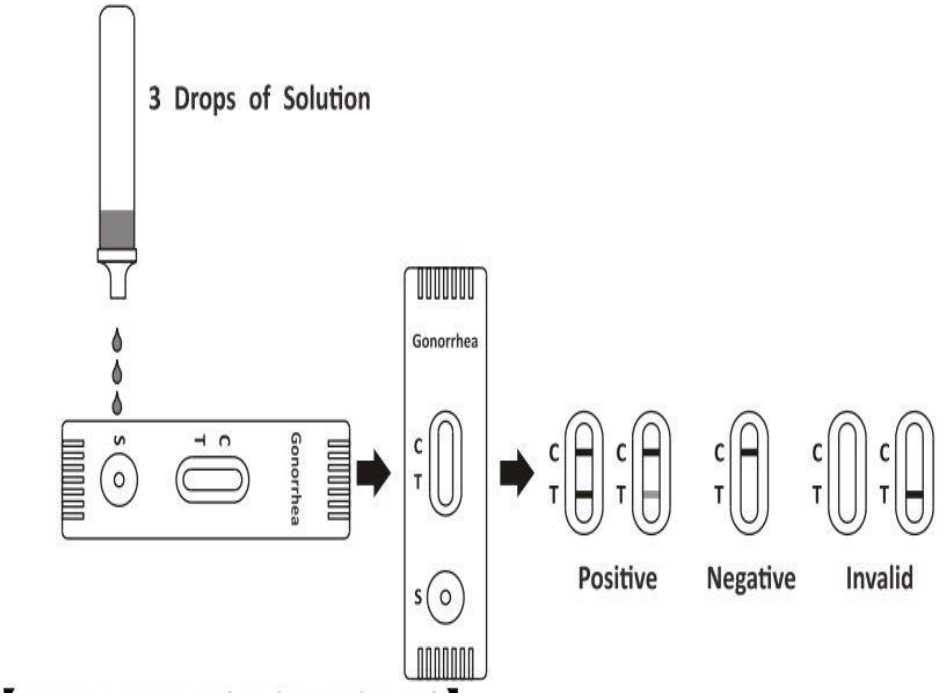
Discard used test tubes and Test Cassettes in suitable biohazardous waste container.

Procedure of detection *T. vaginalis* according to Manufacture Company

【DIRECTIONS FOR USE】

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

1. Remove the test cassette from the seal pouch and use it within one hour. Best result will be obtained if the test is performed immediately after opening the foil pouch.
2. Extract the Gonorrhea antigen according to the specimen type.
   - Hold the reagent 1 bottle vertically and add **5 drops of reagent 1** (approx. 300ul) to the extraction tube. Reagent 1 is colorless. Immediately insert the swab, compress the bottom of tube and rotate swab 15 times. Let stand for 2 minutes.
   - Hold the reagent 2 bottle vertically add **4 drops of reagent 2** (approx. 200ul) to the extraction tube. The solution would turn turbid. Compress the bottle of tube and rotate the swab 15 times until the solution turn clear with a slight green or blue tint. If the swab is bloody, the color will turn yellow or brown. Let stand 1 minute.
   - Press the swab against the side of tube and withdraw the swab while squeezing the tube. Keep as much liquid in the tube as possible. Fit the dropper tip on top of extraction tube.
3. Place the test cassette on a clean and level surface. Add 3 full drops of the extracted solution (approx. 120ul) to the specimen well of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well.
4. Wait for the color to appear. Read the result at 10 minutes; do not interpret the result after 30 minutes.



Procedure of detection *N. gonorrhea* according to Manufacture Company

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

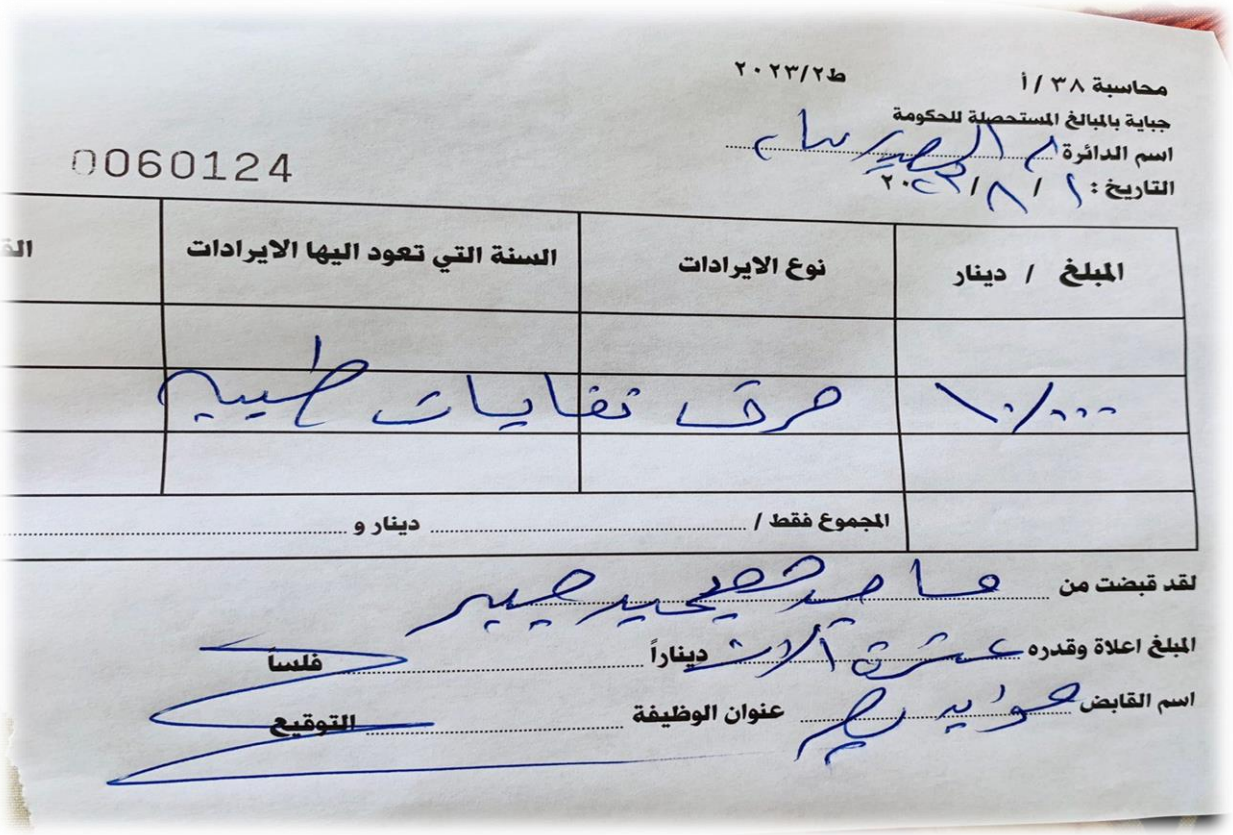
- Place a clean extraction tube in the designated area of the workstation. Add 20 drops of extraction buffer into the extraction tube.
- Put the specimen swab into the tube. Vigorously mix the solution by rotating the swab forcefully against the side of the tube for at least fifteen times (while submerged). Best results are obtained when the specimen is vigorously mixed in the solution.
  Allow the swab to soak in the extraction buffer for one minute prior to the next step.
- Squeeze out as much liquid as possible from the swab by pinching the side of the flexible extraction tube as the swab is removed. At least 1/2 of the sample buffer solution must remain in the tube for adequate capillary migration to occur.
  Put the cap onto the extracted tube.
  Discard the swab in a suitable biohazardous waste container.
- The specimens extracted can retain at room temperature for 60 minutes without affecting the result of the test.
- Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.
- Add 3 drops (approximately 100 µl) of extracted sample from the extraction tube to the sample well on the test cassette.
  Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.

As the test begins to work, you will see color move across the membrane.

- Wait for the colored band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.

Discard used test tubes and Test Cassettes in suitable biohazardous waste container.

Procedure of detection *C. albicans* according to Manufacture Company



محاسبة ٣٨ / أ
ط٢/٢٠٢٣
جباية بالمبالغ المستحصلة للحكومة
اسم الدائرة
التاريخ : ١ / ٨ / ٢٠٢٣
0060124

المبلغ / دينار	نوع الايرادات	السنة التي تعود اليها الايرادات	الـ
١٠/٠٠٠	عرف نفايات طبية		
المجموع فقط / ..... دينار و			

لقد قبضت من
المبلغ اعلاة وقدره ..... ديناراً ..... فلساً
اسم القابض ..... عنوان الوظيفة ..... التوقيع

Medical wastes incineration master sheet

الخلاصة

بدات الدراسة بجمع العينات من تشرين الثاني 2022 وحتى نهاية حزيران 2023 ، تم تسجيل المشاركين في الدراسة. بعد أخذ تاريخ المريض وإجراء الفحص العياني ، حيث يقوم الطبيب باجراء مسحة لجمع ثلاث عينات. تم وضع إحدى هذه العينات على الفور في أنبوب بلاستيكي به محلول الفوسفات الملحي وأزيد الصوديوم لتحديد وجود المشعرات المهبلية أو المبيضات البيضاء باستخدام شريط الامتزاز المناعي، ثم وضعت مسحة أخرى في محلول هيدروكسيد الصوديوم (NaOH) وكلوريد الهايدروجين(HCl) للكشف عن وجود النيسريا البُنِية ايضا باستخدام شريط الامتزاز المناعي.اما المتبقي من العينة المستخدمة في شريط الامتزاز المناعي فاتستخدم في اختبار تفاعلات السلسلة المتبلمرة(PCR). بعض الاحيان ياخذ الطبيب مسحة اضافية لغرض زراعة النيسريا البنية او المبيضات البيضاء.

تم إجراء دراسة cross-sectional، اذ كان إجمالي العينات 285 مريض (146 رجال و 139 اناث) توزعت 271 عينة عزلت بشكل مفرد و 14 عينة كعزلت بشكل مشترك co-infections كانت اعمارهم اكبر من 18 سنة تتراوح بين (18-59) سنة حيث ظهرت عليهم علامات وأعراض التهاب المهبل ، والتهاب الإحليل ، والتهاب البروستات ، ومرض التهاب الحوض . من أجل الكشف عن ثلاث كائنات دقيقة مسؤولة عن الأمراض المنقولة جنسياً والتي تشمل المشعرات المهبلية ،النيسريا البُنِية والمبيضات البيضاء.

وجدت الدراسة هناك 144(50.52%) حالة موجبة من الامراض التناسلية البولية تتضمن 130(90.27%) عينة مفردة و 14(9.73%)عينة مشتركة، حيث كان هنالك 43 (15.08%) للمشعرات المهبلية ، 20 (7.02%) للنيسريا البنية و 81 (28.42%) للمبيضات البيضاء. اما العينات السلبية المتبقية 141 (49.48%) ربما ناتجة عن مسببات أخرى. كان لدى الإناث % 11.93 ، 2.11% و 20.7% من عدوى المشعرات المهبلية والنيسريا البنية و المبيضات البيضاء في العينات السريرية تواليا. كان لدى الذكور 3.15% ،4.91 % و 7.72% من المشعرات المهبلية والنيسريا البنية والمبيضات البيضاء تواليا. هناك فرق واضح معنوي في الكائنات الحية المنقولة بالجهاز البولي التناسلي لكلا الجنسين(X^2=16.74,P=0.0002) ، حيث سجلت المشعرات المهبلية والمبيضات البيضاء أعلى معدلات الإصابة في الإناث و نيسريا البنية عند الذكور.

يلعب موقع العزلة دورًا رئيسيًا في وجود الكائنات الحية الدقيقة (X^2=80.94, P=0.001)، حيث سجل المهبل عند الإناث عدوى أكثر من المواقع الأخرى لكل من كائنات المشعرات والمبيضات ؛ تم تسجيل 52 عينة (36.11%) من المبيضات و 18 (12.5%) عينة من المشعرات، بينما النيسريا البنية كانت اكثر انتشارا في الاحليل عندة الرجال حيث سجلت 14 (9.72%).

أظهرت نتائج الدراسة الحالية ، عند مقارنة التحسسية والتخصصية لكل من اختبار الامتزاز المناعي وتفاعلات السلسلة المتبلمرة (PCR) للكشف عن المبيضات البيضاء، أن قيمة التحسسية كانت 91.76٪ و التخصصيية 94.91٪ بينما للمشعرات المهبلية اظهرت أن قيمة التحسسية كانت 87.23٪ والتخصصية 97.93٪، كذلك النيسريا البنية اظهرت أن قيمة التحسسية كانت 41.67٪ والتخصصية 95.37٪. الاستنتاجات تشير إلى أن شريط الامتزاز المناعي الجديد يتمتع بتحسسية و تخصصية أكثر من الاختبارات التشخيصية الروتينية لمرض المشعرات المهبلية والمبيضات البيضاء والنيسريا البنية، كما أن شرائط الامتزاز المناعي تعطي النتائج خلال دقائق قليلة مقارنة بالاختبارات التقليدية(المجهري والزرع).

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

دراسة مقارنة بين اختبار الامتزاز المناعي باستخدام تفاعلات السلسلة المتبلمرة و الفحوصات الروتينية لمرضى الجهاز البولي التناسلي في محافظتي كربلاء و بابل.

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في التحليلات المرضية

كتبت من قبل

ماجد حميد جبر

بكالوريوس تحليلات مرضية/ كلية العلوم الطبية التطبيقية – جامعة كربلاء /2018

بأشراف

ا.د هادي رسول حسن

ا.م.د حيدر علي محمد

(مشرف ثاني)

ربيع الآخر (١٤٤٥)

تشرين الاول (٢٠٢٣)