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New Immunological Technique for Diagnosis of *Candida albicans* Infection

A Thesis

Submitted to the Council of the College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Master in Science of Biology /Immunology

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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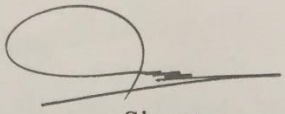

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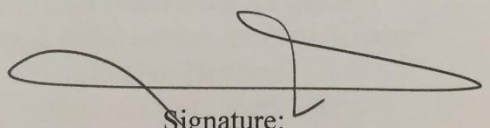

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DEDICATION

TO THE BIG HEART IN UNIVERSE AND THE SMILE OF MY LIFE MY MOTHER AND FATHER.

TO THOSE MY HEART ALWAYS REMEMBERS THEM, TO MY BROTHER AND MY SISTERS.

TO THE TWO PERSON WHO GUIDES MY WAY IN SCIENCE AND KNOWLEDGE DR. HAZIMA MOSSA AL-ABASSI AND DR. ALI ABDUL HUSSAIN MAHDI

RUSSUL

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Abstract

In this study, included produce a new technique for diagnosis of *Candida albicans* (skin, mouth, and vagina) infection. This study included 100 samples of the patient (immunocompromised women with average age (55-57) year and babies with average age (1-18 month), patients infected with various (fungi, bacteria) infection, in addition to 20 samples for an apparently healthy individual (a control). Samples were collected from AL- Dowaly Private Hospital in Baghdad during the period January 2019 to June 2019. All infections were diagnosed by consultant medical staff at the hospital by using several tests including (germ tube, culture, Vitek and API20C). Cases enrolled in this study were as follow:

- Thirty- Samples from patients with *C. albicans* infection (skin scales).
- Fifteen-Samples from patients with *Aspergillus* spp. infection (skin scales).
- Fifteen-Samples from patients with *Tricophactone spp*. (infection (skin scales).

  {Skin scales were isolated from fingers foot (intertriginous)}
- Ten-Samples from patients with *C. albicans* infection (skin swabs).
- Ten-Samples from patient's bacteria with *Staphylococcus aureus* infection (skin swabs).
- Ten-Samples from patients (1-18 month) infected with *C. albicans* (mouth swabs).
- Ten-Samples from patients with *C. albicans* infection (vagina swabs).

The Samples were examined using the new technique compared to the routine methods of diagnosis, we found the new technique gave a positive result for samples infected with *C. albicans*, while the rest of the samples non - *C. albicans* infection (fungal, bacteria) gave a negative result.

It was shown that the Vitek test was more sensitivity (100%) and specific (100%) for diagnosis of *C. albicans* than other methods (API20C sensitivity (100%) and specific (97%), Colonial morphology "culture" sensitivity (96%) and specific (93%) and germ tube test sensitivity (98%) and specific (95%). The results of the new technique test in this study for the diagnosis of *C. albicans* was similar to the result of Vitek test in sensitivity (100%) and specificity (100%), the benefits of this new technique are to provide information with high accuracy, economical (low-cost) and reduced time consumption. for example, physicians can use through the attending visit of the patient without having to be sent to the laboratory.

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List of abbreviations

C. albicans	*Candida albicans*
Ag-Ab interaction	Antigen-Antibody interaction
IgG	Immunoglobulin Gamma
FITC	fluorescein isothiocyanate
FC-assay	Flow cytometry assay
RIA	Radioimmunoassay
ELISA	Enzyme Linked Immuno-Sorbent assay
VBC	Vitek biochemical card
IL-17	Interleukin -17
IL-4	Interleukin -4
IFN-γ	Interferon Gamma
UV-Light	Ultraviolet-Light
UV-Stain	Ultraviolet-Stain
DC	Disseminated Candidiasis
PCR	Polymerase Chain Reaction

Chapter one
INTRODUCTION

1.1 Introduction

Immunological techniques are a wide varieties of methods and specialized experimental protocols devised by immunologists for inducing, measuring, and characterizing immune responses (Van Emon.,2016). They allow the immunologists to alter the immune system through cellular, molecular and genetic manipulation. It's used usually to diagnosis human diseases. Laboratory tests vary widely in clinical immunology; some are essential for diagnosis while others are useful in sub classifying disorders. Some are of research interest only, but may add to our immunological armamentarium in the future. In this regard, it is important to understand that these tests do vary in their sensitivity and specificity. The sensitivity of a test is defined as the number of diseased individuals that are positive for the test compared with those who are negative. These techniques have developed and used in the medical and biotechnology fields. And the immunological techniques used in the diagnosis, which relied on the principle of antigen reaction with antibodies (e.g. ELISA, Immune Fluorescent and Radioimmunoassay) (Carpenter.,1975; Schultz.,2009; Zabriskie.,2009& Dunbbar.,2012).

Among species of genus Candida, *C. albicans* is the pathogen most frequently isolated from the human body, including the oral cavity and gastrointestinal and able of causing life-threatening opportunistic fungal infections. *C. albicans* is considered opportunistic pathogen can cause harm under abnormal conditions (Vilela et al., 2015; Strijbis et al., 2014). The included ratio infection of candidiasis in Iraq from (2017 - 2018) was (15-14.8) / 105 aura of the population respectively (Minster of health).

When the immune system is suppressed, this yeast can multiply rapidly, penetrate the intestinal lining and move into the blood stream, Yeast population is controlled by probiotic or beneficial bacteria (Mohamed et al., 2010).

The immune mechanisms of defence against fungal infections are numerous, and range from protective mechanisms that were present early in evolution (innate immunity) to sophisticated adaptive mechanisms that are induced specifically during infection and disease (Abood, 2014).

Innate immunity is the first line of unspecific host defense against pathogens carried out by macrophages, neutrophils, and dendritic cells (Kiyoura and Tamai, 2015). In adaptive immunity both CD4+ (T helper cells) and CD8+ (cytotoxic T cells) have been reported a play role in antifungal immunity, the nature of the T cell response is established by the cytokine of the T cells during encounter activation: IL-12/IFNγ for Th1 cells, IL-4 for Th2 cells (Naglik, 2014 and Sadeq.,2017).

1.2. Aims of the study

The laboratory diagnosis of candidiasis depends on the infection caused by it. Prompt and accurate identification of Candida species is very essential for effective therapeutic outcome. Conventional methods for the diagnosis of candidiasis are less sensitive and time consuming, (e.g. culture, germ tube, Vitek and API20C). So this study aims to produce a kit with more sensitivity, specificity, accuracy and safety than routine method to diagnose *C. albicans* to achieve this, the following approach were adopted:

1- Prepare a new kit for diagnosis of *Candida albicans* using (specific antibody conjugate with UV-stain).

2- Detect the immune complex by using harmless UV-light.

3- Design of protocol to diagnose other microorganisms depended on this principle study (more specific and sensitive than classical test).

Chapter two
LITERATURE REVIEW

2.1. Antigen –antibody Interaction

Antibody – Antigen interactions are much like other receptor-ligand interactions, physicochemical forces are involved in the interaction between an antibody and an antigen that are similar to those between an enzyme and its substrate (or competitive inhibitor) or between a receptor, such as an insulin receptor, and a ligand, such as insulin. These forces derive from four sources: (1) Ionic bond (2) hydrogen bonds (3) van der Waals forces, and (4) hydrophobic interactions. Many experimental approaches have been used to define the structure of the antibody – binding site for antigen (Zabriskie.,2009; Helbert., 2016).

The first correct description of the antigen-antibody reaction was given by Richard J. Goldberg at the University of Wisconsin in 1952 (Goldberg.,1952). It came to be known as "Goldberg's theory" (of antigen-antibody reaction) (Spiers., 1958).

Many serologic techniques are used to detect the interaction of antigens with antibodies. These methods are suitable for the detection and quantitation of antibodies to infectious agents, as well as microbial and non-microbial antigens. Antigen-Antibody reactions can be classified into two categories:

1- **Precipitation reactions**

Antibody and soluble antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate. Antibodies that aggregate soluble antigens are called precipitins. Although formation of the soluble Ag-Ab complex occurs within minutes, formation of the visible precipitate occurs more slowly and often takes a day or two to reach completion. Formation of an Ag-Ab lattice depends on the valency of both

the antibody and antigen. (Wild., 2001). In the clinical laboratory several applications of the precipitation reaction are used. These methods include:

1- Immunodiffusion: These are of two types: single and double immunodiffusion.
2- Electroimmunodiffusion (Stevens& Miller.,2016).

2- Agglutination reactions

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Agglutination reactions are similar in principle to precipitation reactions; they depend on the crosslinking of polyvalent antigens (Stites *et al*.,1997; Stevens& Miller.,2016 and Shoemark *et al*., 2017).

2.2. Factors affecting antigen antibody reactions

Many factors affect the interaction between antigen and antibody (Stevens & Miller., 2016); these include:

1- **Specificity:** The ability of a particular antibody to combine with one antigen instead of another is referred to as specificity.
2- **Temperature:** The optimum temperature needed to reach equilibrium in an antibody-antigen reaction differs for different antibodies. IgM antibodies are cold reacting with thermal range 4-22°C, and IgG antibodies are warm reacting, with an optimum temperature of reaction at 37°C.
3- **pH:** The optimum pH for all reactions has 7.0 is used for routine laboratory testing.
4- **Ionic strength:** The concentration of salt in the reaction medium has an effect on antibody uptake by the membrane bound erythrocyte antigens. Sodium and chloride ions in solution have inhibition effect.

5- **Concentration:** Under normal condition the concentration of antigen and antibody should be optimal but some time this thing fail to be happen in which excess antibody or antigen concentration will result in false reaction, some times known as zonal reaction. When the concentration of antigen is excess it is known as post zone reaction; excess antibody is referred as prozone reaction. This phenomenon can by overcome by serial dilution until optimum amount of antigen and antibody will present (Lisova *et al*.,2014).

6- **Cross reactivity:** When some of the determinants of an antigen are shared by similar antigenic determinants on the surface apparently unrelated molecules, a proportion of the antibodies directed against one kind of antigen will also react with the other kind of antigen. This is called cross reactivity (Wrammert *et al*.,2011).

2.3. Immunological diagnostic techniques

It's used usually to diagnosis human disease, these techniques have developed and used in the medical and biotechnology fields (Schultz., 2009), some type of immunological techniques:

2.3.1. Fluorescent immunoassay

Immunofluorescence use antibodies to which fluorescent compounds (fluorochromes) have been covalently attached. One of the fluorescent compound widely used by immunologists is fluorescein isothiocyanate (FITC), which couples to free amino groups on proteins. FITC emits a greenish light when exposed to ultraviolet(UV) light. Fluorescence microscopes equipped with UV sources are used to examine samples that have been exposed to fluorescent antibodies. This test is used widely to detect antigens in cells or tissue sections (Helbert.,2016; Shoemark *et al.,* 2017). **There are three types of Immunofluorescence:**

1- **Direct immunofluorescence:** This technique is used to detect antigen in clinical samples using specific fluorochrome labeled antibody.
2- **Indirect immunofluorescence:** Is uses two antibodies; the unlabeled first (primary) antibody specifically binds the target molecule, and the secondary antibody, which carries the fluorophore, recognizes the primary antibody and binds to it (Odell *et al*.,2013).
3- **Micro immunofluorescence:** This is a serological technique used to detect antibodies in patient serum. (Owen & Punt *et al*.,2013; Poot *et al*.,2016).

2.3.2. Flow cytometry

Flow cytometry is a technique used to enumerate cells that express an antigen. The cells are stained with antibody specific for the cell-surface antigen. The antibody is coupled to specific fluorescent reagents, such as FITC (several other different colored fluors are available), and is then passed through the flow cytometer. The number of stained cells can be counted, such as the number of $CD4^{+}$T cells (Helbert., 2016).

2.3.3. Radioimmunoassay

In radioimmunoassay, radioisotopes can be used to measure the concentration of antigen or antibody in serum sample. If antibody concentration is measured radioactive labeled antibody competes with patient unlabeled antibody for binding sites on a known amount of antigen. The main advantage of the radioimmunoassay method is the extreme sensitivity and ability to detect trace amounts of antigen or antibody. In addition, a large number of tests can be performed in a relatively short time

period. The disadvantage is the hazards and instability of isotopes (Turgeon., 2013).

However, with the proliferation of RIAs came increasing levels of worry about the amount of radioactivity generated by research and clinical laboratories and the associated risks to the technical staff and to the environment.

2.3.4. Enzyme linked immuno-sorbent assay, or ELISA

The Enzyme Linked Immuno-Sorbent Assay (ELISA) is a commonly used analytical biochemistry assay, first described by Engvall and Perlmann in 1972. This assay was used to measure antigen or antibody presence and concentration. ELISA is a very sensitive and simple test, it has been used as a diagnostic tool in medicine, plant pathology, and biotechnology, as well as a quality control check in various industries.

In the simplest form of an ELISA, antigens from the sample are attached to a well surface. Then, the antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change (Cheng., 2010; Helbert.,2016). There **are four types of enzyme linked immuno-sorbent assay, or ELISA:**

1- Direct ELISA

Antigens are detected by antibodies directly linked to the enzyme (Spence and Zachary.,2018).

2- Indirect ELISA

In indirect ELISA, both a primary antibody and a secondary antibody are used. In this case, the primary antibody is not labeled with an enzyme. Instead, the secondary antibody is labeled with an enzyme.

The primary antibody binds to the antigen Installer to the plate, and then the enzyme-labeled secondary antibody binds to the primary antibody. Finally, the enzyme linked to the secondary antibody reacts with its substrate to produce a visible signal that can be measured (Al-Lammi,2009; Schmidt *et al*.,2012).

3- Sandwich ELISA

In sandwich ELISA, however, it is the antibody that is immobilized to the plate, and this antibody is called capture antibody. In addition to capture antibody, sandwich ELISA also involves the use of detection antibodies, which generally include the unlabeled primary detection antibody and the enzyme-labeled secondary detection antibody. Antigens were added into the wells and antibodies are then added directly linked to the enzyme (Kragstrup *et al*.,2013).

4- Competitive ELISA

Also known as inhibition ELISA or competitive immunoassay, this assay measures the concentration of an antigen by detection of signal interference. The sample antigen competes with a reference antigen for binding to a specific amount of labeled antibody. The labeled antigen and the sample antigen (unlabeled) compete for binding to the primary antibody. The lower the amount of antigen in the sample, the stronger the signal due to more labeled antigen in the well (Charbonnet et al.,2014).

2.4. *Candida spp.*

The term *Candida* comes from the Latin word 'candid' a which means white, the spores of candida are harmless, polymorphic fungus that becomes invasive and pathogenic pseudohyphae when there is a defect in the balance of flora the host (Sharif.,2012 and Abood., 2014).

About 14 *Candida* species have been implicated in human infections, with *C. albicans* being the most prevalent among the yeast isolates (Meurman, *et al*., 2007). The most frequently isolated species is *C. albicans*, but *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosisare* also emerging as important etiologic agents of Candida infection (Coleman *et al*.,2010 and Mikko *et al*.,2014).

Many fungal pathogens of humans such as *C. albicans* is capable of growing as unicellular budding yeast cells or as filamentous hyphae or Pseudohyphae it is called fungal polymorphic, which normally grow in filamentous forms outside the human body, but transform to yeast forms in human tissues (Vazquez and Sobel.,2003; Brown *et al*., 2012; Marttila *et al*., 2013).

2.5. *Candida albicans*

C. albicans lives in 80% of the human population without causing harmful effects, although the overgrowth of the fungus results in candidiasis [candidosis]. *C. albicans* is the most common and well-studied of the disease-causing *Candida spp*, that naturally colonizes in skin, genital and intestinal mucosa. Under normal circumstances, the fungus does not cause disease, but the absence of appropriate immune recognition and response mechanisms can lead to the inability to control *C. albicans* colonization and invasion. Candidiasis is often observed in immunocompromised individuals such as (HIV-infected patients (AIDS), Cancer Chemotherapy, Organ or Bone marrow transplantation]. In addition, hospital-acquired infections by *C. albicans* have become a cause of major health concerns (Vargas et al.,2005; David.,2010; Agha *et al*.,2011 and Ajah.,2016).

When grown in a laboratory, candida appears as a large, round, white or cream, which emit a yeasty odor on agar plates at room temperature. *C. albicans* ferments glucose and maltose to acid and gas, sucrose to acid, and does not ferment lactose, which helps to distinguish it from other Candida species (Vylkova *et al*., 2011). (Figure 2-1).

Figure (2-1). "Colony of *C. albicans* was growth on soubourod agar (Raines *et al*.,2013)

The human skin is commonly colonized by diverse fungal species. Some candida species, especially *C. albicans*, do not only reside on the skin surface as commensals, but also cause infections by growing into the colonized tissue. However, defense mechanisms at the skin barrier level are very efficient, involving residential non-immune and immune cells as well as immune cells specifically recruited to the site of infection. Therefore, the skin is an effective barrier against fungal infection. While most studies about commensal and pathogenic interaction of candida species with host epithelia focus on the interaction with mucosal surfaces. Such as the vaginal and gastrointestinal epithelia, less is known about the

mechanisms underlying Candida interaction with the skin. (Kashem & Kaplan.,2016and Kühbacher *e*t *al*., 2017).

2.6. Candidiasis

Candidiasis was one of the fungal infections caused by a type of yeasts that belong to the genus *Candida*. (Fenn *et al*.,1999; Noble&Johnson.,2007; Brown *et al*.,2012). candidiasis is also technically known as candidosis, moniliasis, and oidiomycosis. Candidiasis symptoms vary depending on the area of the body that is infected (Marttila *et al*.,2013). Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. Candida infections of the latter category are also referred to as candidemia and are usually confined to severely immunocompromised persons, such as cancer, transplant, and AIDS patients, as well as nontrauma emergency surgery patients (Noble&Johnson.,2007; Marttila et al.,2013).

2.7. Classification of Candidiasis infection

Candidiasis may be divided into the following types: (Marttila et al.,2013)

2.7.1. Superficial Infection

2.7.1.1. Oral Candidiasis

The mucous membranes lining the mouth are infected by oral candidiasis also known as oral thrush (Melo *et al*.,2004). The mouth is the main pathway through which most pathogenic bacteria enter Therefore, the study of oral health contributes to the reduction of multiple bacterial infections within the body (Todar.,2002). Of the most resulting infections

common of *candida albicans* yeast, they appear as a simple tumor or ulceration in any part of the oral cavity and then develop into white spots that may combine to form a membrane containing large numbers of Pseudo hyphae and playstyle spores as well as endothelial cells (Noltte.,1982). This type of injury appears in newborns mainly because they acquire yeast when passing through the mother-birth canal (Emmons et al.,1977; Kwon-Chung and Bennett.,1992), as the bacteria passes from the mother to the child through saliva directly. During kissing, talking or sneezing on the face of the child or using contaminated substances such as spoons and toothbrushes (Steven.,1996). The infection in adults is chronic and characterized by the formation of the membrane thicker with the presence of heartburn and dehydration in the affected part and more frequent in people with pulmonary tuberculosis and leukemia or HIV infection (AIDS) (Meurman *et al*.,2007; Mahdi.,2015). (Figure 2-2).

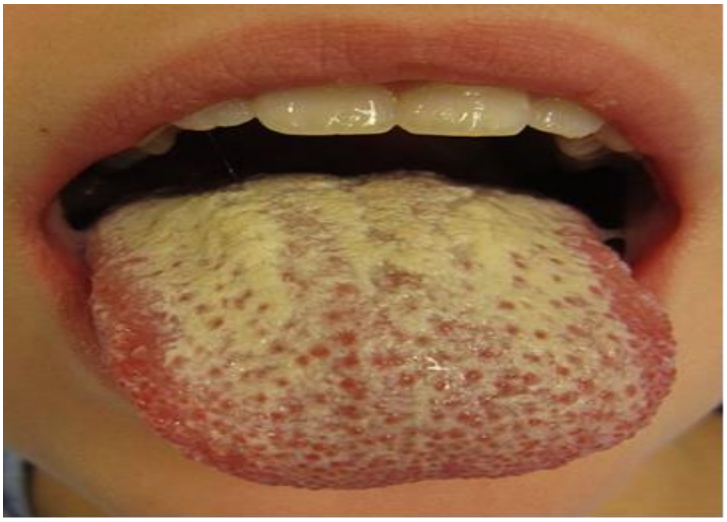

Figure (2-2) Oral candidiasis [thrush] (Coleman *et al*.,2010)

2.7.1.2. Cutaneous candidiasis

The infection in adults is chronic and characterized by the fact that the membrane, which is formed thicker with the presence of heartburn and dryness in the affected part and the incidence of infection in people suffering from tuberculosis, Acute leukemia or HIV / AIDS. The infection occurs in the outer part of the body (skin, hair, and nails) by the *Candida albicans* because of its possession of the two types of enzymes are proteinase and keratinize. The injury includes an area of Infra-axillaries, Infra-mummeries and Inter-triginous, where these places are characterized by warmth and humidity (Figure 2-3). The Included ratio infection of Cutaneous Candidiasis was 85% in Iraq (Minster of health). The infection begins in the form of vesicles, and then turns into specific spots that are dark red and wet accompanied by itching and movement and tend the outer layer to be scaly and fast division. Also, may be accompanied by a secondary infection with bacteria *Staphylococcus aureus* (Notle.,1982; Negi *et al*.,1984).

The infection of the nail known as chronic Paronychia which occurs as a result of the infection of candidiasis of the subcutaneous plate causing inflammation of the pterygium with redness and painful swelling and the collection of inflammatory secretions. In case of people who use water for a long time and people with diabetes (Vazquez and Soble.,1995; Hay.,2018), infected nails and become a brown color.

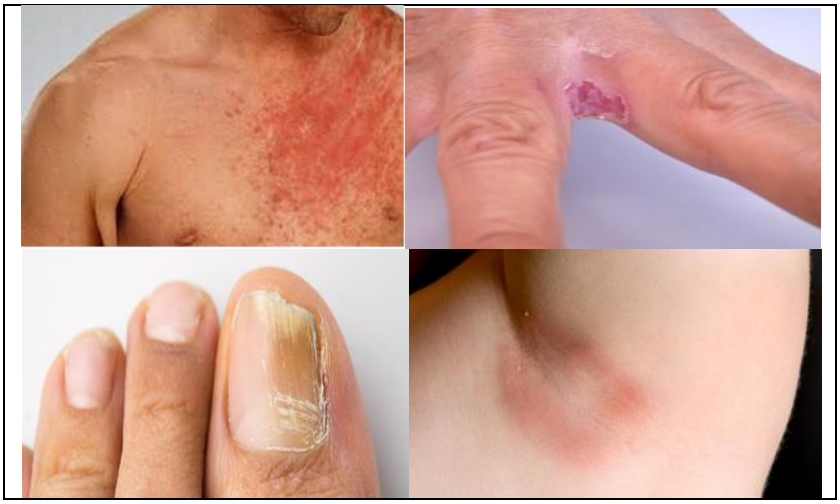

Figure)2-3(Cutaneous Candidasis

2.7.1.3. Vaginal candidiasis

C. albicans yeast is a natural flora in vaginal secretions in the case of a balance between the bacteria and this yeast, but when there is an imbalance, this yeast grows and becomes more increasing and this indicates the occurrence infected with this yeast (Murray *et al.*,2000). Vaginal candidiasis is a common fungal infection that occurs under predisposing conditions such as, diabetes, antibiotic therapy, and contraceptive. This disease is a common disorder among women. (Watson and Calabretto,2007). It is caused by the overgrowth of Candida species in the vagina and is characterized by vaginal secretion white or yellow thick textures accompanied by heartburn at the site of injury, the appearance of secretions varies from one case to another depending on the severity of the infection (Fidel,2004; Sobel, 2007; (Achkar and Fries, 2010 and Abood., 2014).

2.7.2 Systemic Infections

This infection included different system such as: -

1- **Urinary tract candidiasis**
2- **Respiratory tract candidiasis**
3- **Alimentary tract infection**
4- **Candida of central nervous system**
5- **Candidal meningitis**
6- **Endocarditis**
7- **Disseminated candidiasis** [e.g. "hepatosplenic candidiasis", which sometimes follows neutropenia; or "candidemia", a form of septicemia] (Vylkova *et al*.,2011 and Antinori *et al*.,2016).

2.8. Pathogenicity and virulence of *C. albicans*

Pathogenicity is a qualitative characteristic of the micro-organism by which it can induce disease (pathogen) or not (non-pathogen). Virulence is a quantitative trait that indicates the amount of damage induced by the pathogen on the host. The most virulent pathogen causes the most serious damage (disease) to the host. Both pathogenicity and virulence are controlled by pathogenicity and virulence genes. The dimorphism, germ tube formation and hemolysis are indicators of pathogenicity of *C. albicans*. *C. albicans* possess a group of virulence factors that include surface molecules aids in adhesion to cell surface of the host, acidic protease enzymes and ability to transform to the filamentous form inside the body. The later factor enables the fungus to escape from the bloodstream and penetration of epithelial tissues then the growth in the internal tissues. It aids also in the resistance of phagocytes where it ruptures the cell and continue growing (Hidalgo and Vazquez,2005; Shirtliff *et al*.,2009).

The ability of *C. albicans* to switch morphology between yeast and hyphal form is crucial to its ability to adhere surfaces and colonize tissue (Saville *et al*.,2003). The ability of the pathogenic strains to transform from white cells to opaque cells is associated with changes in the size, shape, adhesion, hyphae formation, sensitivity to drugs and neutrophils and pathogenicity. The white cells are more virulent in systemic infections while the opaque is more successful in skin infections. (Williams, *et al*.,2000; Munro *et al*.,2005; Forche *et al*.,2008 and Hassan *et al*.,2014).

2.9. Immune responses to *Candida spp.*

The emergence of novel pathogenic fungi and the lack of fungal vaccines have focused on an acute interest in illustrating immune defense mechanisms against fungi. And these mechanisms of immune defense occur by two pathway innate and adaptive immune responses (Devine and Marsh, 2009; LeibundGut-Landmann *et al*., 2012).

Innate immunity was the first line of unspecific host defense against pathogens. First step for the immune responses interacts with epithelial cells that play a critical role in protecting the body against invasion, the *C. albicans* can recognized by many receptors including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), mannose receptor (MR) that can induce pro-inflammatory cytokine, chemokine (Kiyoura and Tamai, 2015). Beyond the critical role of phagocytosis in host defense, phagocytosis was one of the first processes of innate immunity, carried out by macrophages, neutrophils, and dendritic cells to engulf *C. albicans* (Underhill, 2005; Wellington et al., 2009). The *C. albicans* cell wall components (mannan, glucan and chitin) considered as immunostimulatory for activate phagocytosis (Kiyoura and Tamai, 2015). Mannan, which known as the serotypes of distinguish *Candida spp.* located on the outer surface of the *C. albicans* cell wall. The structure of *C. albicans* mannan involves in its

recognition through macrophages (McKenzie *et al*., 2010). Heat-killed *C. albicans* raised level of surface expression to 1, 3-glucan, that commonly available within mannan in the pathogen cell wall (Jouault *et al*., 2006; Wellington *et al*., 2009). Phagocytosis of glucans by macrophages can improve killing pathogen through production of reactive oxygen species (ROS), which considered as crucial chemical reactive to get rid of pathogen, while a live *C. albicans* inhibition ROS production in process of phagocytes (Wellington *et al*., 2009; Kankkunen *et al*., 2010).

Subsequently, producing many antimicrobial peptides that secreted and synthesized by different cells involving epithelial cells, neutrophils and immune cells such as LL-37, β Defensin and Histatins. LL-37 was chemotactic produce by neutrophils and monocytes. Which have advantage in suppress *C. albicans* adhesion on plastic surfaces (LópezGarcía *et al*., 2005; Tsai *et al.*, 2011;). The β Defensin family, produced by epithelial cells have antimicrobial activity against gram negative and gram positive bacteria, enveloped viruses and fungi in vitro. Histatins were proteins that secreted by human parotid and submandibular, sublingual saliva in humans, that have fungicidal activity against *C. albicans* and also may have role in causing small membrane defects (Kiyoura and Tamai, 2015).

Adaptive immune cells, such as T cells and B cells, express genetically rearranged surface receptors that are exquisitely specific for a particular antigen. Innate and adaptive immune responses are inextricably intertwined, and a successful adaptive immune response is a coordinated effort requiring activation of tissue-resident cells, antigen-presenting cells, and antigen-specific T and B cells. recovery of tissue homeostasis involves a balance of TH1- and TH17-type responses and Treg cells. For example, activation of CLRs in vivo leads to the development of TH1- and TH17-

type CD4+ T cells. The TH1 response provides protective immunity against fungi through enhancing the functions of phagocytic cells through the production of IFN-γ and promotion of B-cell production of opsonizing antifungal antibodies. TH17 responses are also critical because they activate tissue cells, such as epithelial cells and fibroblasts, resulting in the production of chemokines that recruit phagocytes to the site of the immune response, through the production of (IL-17 family of cytokines particularly IL-17A and, IL-22) (Verma *et al*.,2015; Jolink *et al*.,2017 and Bartemes & Kita.,2018).

Th1 and Th17 cells are the principal T helper subsets that contribute to protective immunity to several pathogenic fungi. (Kagami *et al*,2010; Saijo *et al*,2010). Apart from conventional Th1/Th2 responses, Th17 cells have recently been described as an important Th cell subtype conferring protection against extracellular bacterial and fungal infections (Curtis and Way, 2009). IL-17A, the major cytokine secreted by Th17 cells, possesses multiple proinflammatory functions, such as recruiting neutrophils (Brown ,2011), activating neutrophil/macrophage phagocytosis activity, and inducing b-defensin release (Kao *et al*,2008). Therefore, IL-17 is regarded as an important component in host defense against *C. albicans* infection. (Acosta-Rodriguez *et al*,2007; Tomalka *et al*,2011).

For a long time, it was assumed that cell-mediated immunity (CMI) was remarkable, but humoral immunity had no or little role in immunity. Whatever, it was admitted now that CMI importance mechanism in defence, but that specific kinds of antibody response were protective. Usually, Th 1-type CMI wanted for riddance of a fungal infection, while Th2 immunity commonly leads to susceptibility to infection (Blanco and Garcia, 2008).

2.10. Methods Laboratory diagnosis of *C. albicans*

2.10.1. Direct examination

Direct microscopic examination is a rapid method for diagnosis of candidiasis. It requires less expertise (Deorukhkar & Saini.,2014). The swab (sample) with KOH (10%) solution is examined and fixed on the slide. The solution analyzes the epithelial cells accompanying the sample, leaving the yeast cells, allowing to see 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 result, but is inaccurate (Kerawala and Newlands.,2010).

2.10.2 Culture

The sample 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 (Sabroad Dextrose Acar, Blood acar medium, Candida Chrome acar medium). And 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 sample can be contaminated making it useless in diagnosis (Purkait., 2011).

2.10.3 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 and is important as a diagnostic character to distinguish it from other types of *Candida* (Milne., 1996), as Campbell *et al*.,1998 mentioned that *C. dubliniensis* and has the ability to produce the germ tube.

In this test the observer must be able to differentiate between the germ tube and the pseudohyphae. The elongated daughter cells from the mother cell without constriction at their origin are referred to as germ tubes whereas constriction at the origin of the mother cells is called the pseudohyphae (Kim *et al*., 2002). A criterion for germ tube positivity is observation of minimum five germ tube in the entire wet mount preparation. Negative results are confirmed by examining at least 10 high power fields for the presence of germ tubes (Deorukhkar *et al*., 2012 c). (Figure 2-4).

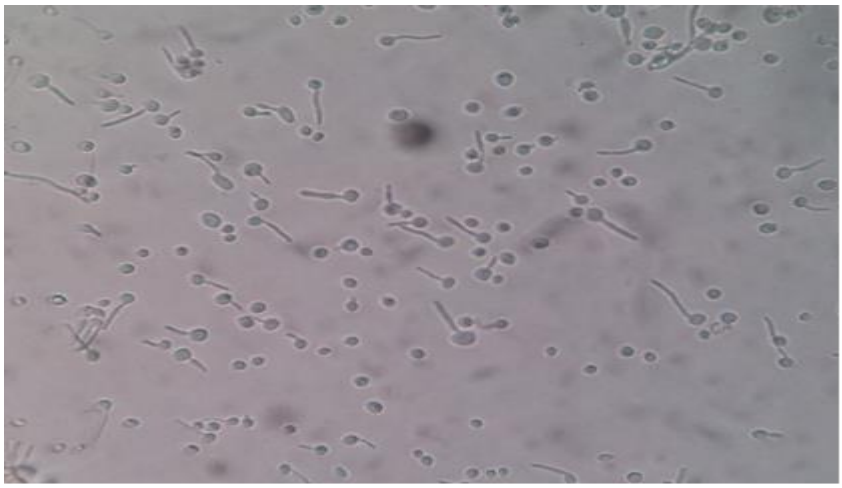

Figure (2-4). Germ tube formation of *C. albicans* (Bedini *et al.*,2006)

2.10.4. 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 b-maltosidase, a-amylase, b-xylosidase, b-glucuronidase, urea hydrolysis, N-acetylb-glucosaminidase, and b-galactosidase). Inoculation of the wells was performed by adding a yeast suspension to the dehydrated substrates. The results were read after incubation for 18 to 24 hat 35°C.A four-digi tnumerical 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. (Colin *et al*.,1999; Hazen and Howell., 2003).

2.10.6. Vitek Yeast identification system [specific biochemical reaction]

2.10.5. Polymerase chain reaction (PCR)

One of the methods used for accurate diagnosis is a DNA-based method known as Polymerase Chain Reaction (PCR) (White *et al*., 2006). They are widely used if an expansion by a small fraction of DNA that is estimated by micrograms and by decoupling the DNA bar and then re-link with new tapes and this process is done by controlling the temperature (Mothershed and Whitney.,2006) and it passes by three stages:

1- **Denaturation.**
2- **Annealing.**
3- **Elongation.**

The basis of PCR work is to rely on a thermal cycle that uses thermodynamics from DNA reactions by rotating the heating and cooling of PCR samples after the temperature is determined (Sharkey *et al*.,1994).

This technique was depended in the clinical diagnosis of Candida, in addition to other pathogenic fungi(Kato.,2001). And it is one of the most effective techniques in the diagnosis of yeast and mold, which is better than the traditional methods as well as accompanying many of the obstacles in the diagnosis of isolates within the same type and in addition to that, it takes a long time (weeks or more) for the emergence of the result (Thomas ., 2003) in addition to the similarity of the behavior of yeast in many of the parietal characteristics, and on the agricultural circles and the difficulty of distinguishing them even using the differential chromar Acar (Mancini and Ossi ., 2005 and Al-khafaji.,2017).

2.11. Diagnosis of disseminated candidiasis

As compared to superficial and mucocutaneous candidiasis, the clinical presentations of Disseminated Candidiasis (DC) are non-specific and complicated. Therefore, the rapid and precise laboratory diagnosis of DC is crucial, not only for species identification, but also for timely institution of appropriate antifungal treatment (Ellepola and Morrison, 2005; Pappas et al., 2009). The laboratory diagnosis of DC involves the use of immunodiagnostic and other non-cultural methods in addition to culture. The varied nature of clinical forms the specimen collection difficultin DC. In most cases, invasive procedures are necessary for the specimen collection (Segal & Elad., 2005 and Mikko et al., 2014).

Chapter three

MATERIALS AND METHODS

3. Materials and Methods

3.1. Subjects

The case study included 100 samples of the patient (immunocompromised women with average age (55-57) year and babies with average age (1-18 month), patients infected with various (fungi, bacteria) infection, in addition to 20 samples for an apparently healthy individual (a control). Patients were attended to AL- Dowaly Private Hospital in Baghdad during the period January 2019 to June 2019. All infections were diagnosed by consultant medical staff at the hospital by using several tests including (germ tube, culture, Vitek and API20C). Cases enrolled in this investigation were as follow:

- Thirty - Samples from patients with *C. albicans* infection (skin scales).
- Fifteen -Samples from patients with *Aspergillus* spp. infection (skin scales).
- Fifteen -Samples from patients with *Tricophactone* (infection (skin scales).

{Skin scales were isolated from fingers (intertriginous)}

- Ten -Samples from patients with *C. albicans* infection (skin swabs).
- Ten -Samples from patient's infected with *Staphylococcus aureus* (skin swabs).
- Ten -Samples from babies (1-18) month infected with *C. albicans* (mouth swabs).
- Ten -Samples from patients with *C. albicans* infection (vagina swabs).

The Samples were examined using the new technique and compared with the routine methods of diagnosis.

3.2. Equipments

Instruments and equipment used in this study are listed in the tables below:

Table (3-1) general Equipments utilized in this study.

Equipment	Company	Country
Cover slip	Surgiphath	**China**
Filter paper	Whatman	**U.S.A**
Forceps	Surgipath	**China**
Glass microscope slide	Surgipath	**China**
Gas burner	boiBact	**China**
Loop	Surgipath	**China**
Micro- pipette	Bioche-max	**China**
Petri dishes	Surgipath	**China**
Scalped blade		**China**
Swab culture		**China**
Test tube	corporation	**USA**
Tips-Pipette 1ml ،and,200ml 1ml	Medeco	**China**
Wooden applicator stick	Cardinal Health	**UK**

Table (3-2) Materials utilized in this study

Materials	Company	Country
Normal saline	Baxter	**USA**
Potassium hydroxide solution	Biomax	**UK**
Sabouraud agar	Biomax	**UK**
SDS (sodium dodecyl sulfate)	Bio-Rad	**Germany**

Table (3-3) devices which used in the study

Devises	Company	Country
Hood Lab	Fine Tech	**Korea**
Incubator	MEMERT	**Germany**
Microscope	Optika	**Italy**
Refrigerator	Beko	**Turkey**
UV-Light System	Major Ccience	**Taiwan**

Table (3-4) Kit and material

Material Kit and	Company	Country
Antibody Specific *C. albicain*	Elabscience	**China**
UV-Stain	LibreTexts	**California**

Protocol

100 Samples(infection) / 20 Samples (control)

Type of Samples: Skin scales, Skin swabs, Mouth and vaginal swabs.

Samples were collected from AL- Dowaly Private Hospital in Baghdad during the period January 2019 to May 2019.

Diagnosis of samples by consultant medical staff using routine methods

- **Germ tube**
- **Culture**
- **Vitek**
- **API20C**

Diagnosis of samples by a new technique

Kit for specific antibodies for *C. albicans*+ UV-stain

The samples were diagnosed by UV –light (harmless)

3.3. Methods

3.3.1. Laboratory diagnosis of *C. albicans*

3.3.1.1. Culture:

Sabouraud's dextrose agar (65 g) was suspended in 1L distilled water. And well until a uniform suspension was obtained. After heated with frequent agitation and boiled then sterilized at 121°C for 15 min, then after 48, and 72 hours of incubation.

3.3.1.2. Germ tube test

Principle assay:

The Germ Tube Test is a screening procedure used to differentiate *C. albicans* from other yeast.

Procedure assay:

1. 0.5 ml [11 drops] of sheep serum was add in a 10 x 75 mm tube.

2. A light suspension of the suspect yeast colonies was made by touching 1-2 large colonies or 3-4 smaller colonies with a sterile wooden applicator stick and then the sheep serum was inoculated with the applicator stick.

Note: Too large inoculum will inhibit germ tube formation.

3. The tube was incubated for 2-3 hours in a 35 - 37°C incubator.

Warning: Do not over-incubate the tube. Candida tropicalis may produce pseudo-germ tubes after 3 hours of incubation.

4. A drop of the suspension was placed on a slide using a Pasteur pipette and coverslip.

5. The wet mount was examined microscopically for production of germ tubes (long tube-like projections extending out from the yeast cells). (Mahdi.,2015).

3.3.1.3. Vitek yeast identification system (automatic system).

3.3.1.4. API 20C Yeast identification system {Done according to company biomerieux}

Procedure assay:

1- The basal medium was melted in the ampoules by placing them in an autoclave for 2 minutes or in a boiling water

2- The ampoules were placed in a water bath at 48 to 50 C, and were allowed to cool.

3- An incubation tray was preped. A squeeze bottle was used to dispense 20 ml of water into the tray, and then the strip was placed into the incubation tray.

4- The ampoules according were opened to the manufacturer's instructions, and The molten medium were inoculated with an applicator stick that has touched one or two colonies (>2 mm diameter), adjusted to a density just below 1+ on a Wickerham card.

5- The strip was inoculated (20 cupules; approximately 0.2 ml each) by using a Pateur pipette and following the manufacturer's directions, and then the lid was placed on the tray.

6- The trays were incubated at 28 to 30 C for 72 hours. record the results were read and recorded after 48 hours (David.,2010).

3.3.2. Laboratory diagnosis of the other samples such as (*Aspergillus spp* , *Tricophacton* and *Staphylococcus aureus* bacteria) was diagnosed by consultant medical staff at the Dowaly Private Hospital in Baghdad ,and using the methods for special diagnosing these samples such as (Culture ,and other methods).

3.4. Preparation new kit to diagnose Ab-*C. albicans*

3.4.1. Procedure

I) Preparation of stain (Manufactured stain)

1. 200ml of distilled water added to 100ml from concentrated liquid stain (100%).

2. The mixture has been shaken for one min.

3. Figure (3-1) shows the composition of the UV-stain. Tables (3-5), (3-6) showing basic information and chemical properties of stain (López-García.,2007).

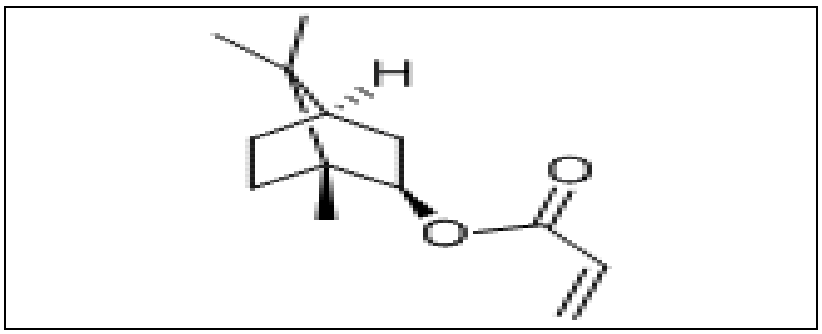

Figure (3-1) Isobornyl acrylate structure

II) Preparation of conjugated reagent

1. 100ml of stain solution added to 50 ml from (Antibody-*C. albicans*).

Note: The concentration of the antibody is (10%).

2. Shake the mixture for one min.

3. The mixture incubated 37°C for (24 hours**)** was the optimization time to incubate the mixture and happened association between the stain and antibody- *C. albicans*. Because (5,10,15,20) hours are not appropriate for a link to occur (Mahdi.,2015).

4. Then kept at 4°C until to use.

III) preparation procedures to diagnosis the samples

1. Infected samples (skin scales, skin swab, mouth and vagina swabs) were taken by a doctor.

2. Samples were placed on the slide and fixed by exposure to heat (benzene burner).

3. Add 2 drops (0.02ml) from conjugated reagent then left it for (1 minute) was the best time to incubate the sample and give the result. Because more than 1 minute gives false results due to the strength of the dye concentration that makes all the components of the sample to be pigmented.

4. Finally washed by Distilled water.

5. Then the samples are examined by UV-light (+ve result was glowing, -ve result was non-glowing.

Table (3-5) Isobornyl acrylate basic information

Product Name:	Isobornyl acrylate
Chemical Abstract Service (CAS)	**5888-33-5**
Molecular formula	C13H20O2
Molecular weight	208.3
EINECS:	227-561-6
Hazard	Moderately toxic by ingestion. Low toxicity by ski n contact and mild eye irritant.

Table (3-6) Isobornyl acrylate chemical propertie

Melting Point	<-35°C
Boiling Point	119-121 °C15 mm Hg(lit.)
Density	0.986 g/mL at 25 °C(lit.)
Refractive Index	*n*20/D 1.476(lit.)
Fahrenheit Point	207 °F
Water Solubility	Difficult to mix in water.
Sensitive	Light Sensitive
CAS DataBase Reference	5888-33-5(CAS DataBase Reference)

3.5. Diagnosis of *C. albicans* infection by New technique

Experiments were performed on the samples to check the sensitivity and specificity of the new technique

1- The first experiment was carried out on infected skin scales from women with *C. albicans* and non-infected skin scales (Control). These samples have been treated in mentioned steps previously in the procedure.

2- The second experiment was conducted on infected skin scales from women with *C. albicans* and *Aspergillus spp.* These samples have been treated in mentioned steps previously in the procedure.

3- The third experiment was conducted on infected skin scales from women with *C. albicans* and *Tricophacton spp*. These samples have been treated in mentioned steps previously in the procedure.

4- The fourth experiment was carried out on infected skin-swabs with *C. albicans* and bacteria *Staphlococcus auries* (swabs) from women. These samples have been treated in mentioned steps previously in the procedure.

5- The fifth experiment was carried out on the infected mouth-swabs with *C. albicans* from babies. These samples have been treated in Mentioned steps previously in the procedure.

6-The sixth experiment was carried out on the infected vagina-swabs with *C. albicans*. These samples have been treated in mentioned steps previously in the procedure.

Chapter four
RESULT

4.1. Isolation and diagnosis of *C. albicans*

Table (4-1) showed the comparison among some types of tests used to diagnose *C. albicans* and other fungi, bacterial infections. It have been shown that the Vitek test was used to diagnose (+ve 60) samples of *C. albicans* (skin scales, skin swabs, mouth and vaginal swabs) versus zero isolations for a non *C. albicans* infection, while API20C test was used to diagnose (+ve 60) samples of *C. albicans* versus (+ve 1) isolations for a non *C. albicans* infection ,as well as to culture test (diagnosed (+ve 58) isolations of *C. albicans* versus (+ve 3) isolations for a non *C. albicans* infection) and Germ tube test (diagnosed (+ve 59) isolations of *C. albicans* versus (+ve 2) isolations for a non *C. albicans* infection). As for the new technique which diagnose (60)+ve isolate of *C. albicans* versus to zero for a non- *C. albicans*.

Table (4-1) diagnosis *C. albicans* and other (fungi, bacteria) Infection by routine methods and new technique

Types of samples(fungi, bacteria)	No. samples	New technique	Routine techniques			
C. albicans infection	60	+ve 60	Culture +ve 58	Germ Tube Test +ve 59	Vitek +ve 60	API20C +ve 60
		-ve 0	-ve 2	-ve 1	-ve 0	-ve 0
Other (Fungi & Bacteria) infection	40	-ve 40	-ve 37	-ve 38	-ve 40	-ve 39
		+ve 0	+ve 3	+ve 2	+ve 0	+ve 1

(+ve) Number of samples infected with *C. albicans*.

(-ve) Number of non - infected samples with *C. albicans*.

4.2. Sensitivity and specificity for diagnosis of *C. albicans*

Results in table (4-2) showed (True Positive, False Negative, True Negative and False Postive) of samples per all tests to diagnosis of *C. albicans*.

- (TP) The number of samples infected with *C. albicans* and have a positive result.
- (FN) The number of samples non-infected by *C. albicans* and have a negative result.
- (TN) The number of samples non-infected by *C. albicans* and have a negative result (other fungi and bacteria).
- (FP) The number of samples non- infected with *C. albicans* and have a positive result.

Table (4-2)The reliable of the test by using sensitivity and specificity

Tests for Diagnostic of *C. albicans*	(TP)	(FN)	(TN)	(FP)
Colonial morpholy (Culture)	60	2	40	3
Germ tube test	60	1	40	2
Vitek	60	0	40	0
API20C	60	0	40	1
New technique	60	0	40	0

Table (4-3) and Figure (4-1) showed the comparing between some types of tests used in *C. albicans* diagnosis. It was shown that the Vitek test was more sensitivity (100%) and specificity (100%) for diagnosis of *C. albicans* than other methods (API20C sensitivity (100%) and specificity

(97%), Colonial morphology "culture" sensitivity (96%) and specificity (93%) and germ tube test sensitivity (98%) and specificity (95%).

The results of New Technique assay for *C. albicans* was similar to the results of Vitek test in sensitivity and specificity (100%), but it was different regarding all these tests due to its dependent on antibodies (against *C. albicans*), in preparation the specialized kit to diagnose of *C. albicans* Consists of (Antibody conjugated with UV-Stain) ready for interaction with *C. albicans* antigens. The sensitivity and specificity of the new technique was recognized by the two laws:

- Sensitivity= TP/(TP+FN)

- Specificity= TN/(TN+FP)

Table (4-3) sensitivity and specificity for diagnosis of *C. albicans* by Routine methods and new technique.

Tests for Diagnostic of *C. albicans*	Sensitivity **TP/(TP+FN)**	Specificity **TN/(TN+FP)**
Colonial Morpholy (Culture)	96%	93%
Germ Tube Test	98%	95%
Vitek	100%	100%
API20C	100%	97%
New Technique	100%	100%

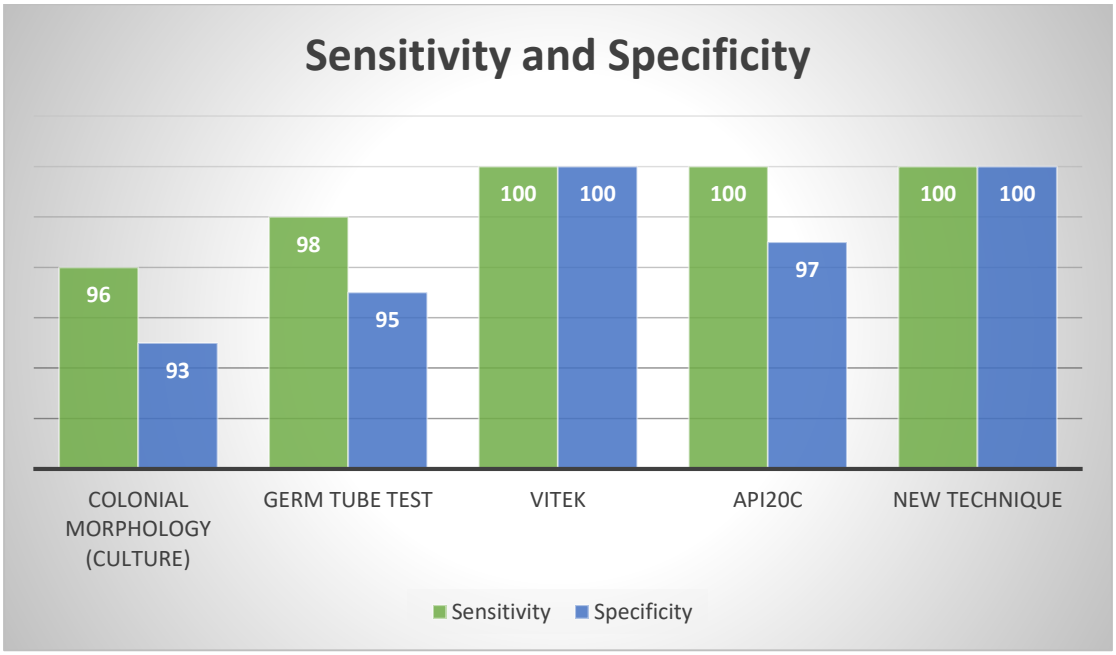

Figure (4-1) sensitivity (%) and specificity (%)for diagnosis of *C. albicans* by routine methods and new technique

4.3. Experiments results in which a new technique was used to diagnose samples

1- The first experiment results

As shown in figure (4-2), the result was (+ve) for a glowing sample of skin scale infected with *C. albicans* as compared with the (-ve) result for a non-glowing sample (non-infected) of skin scale (as control/negative).

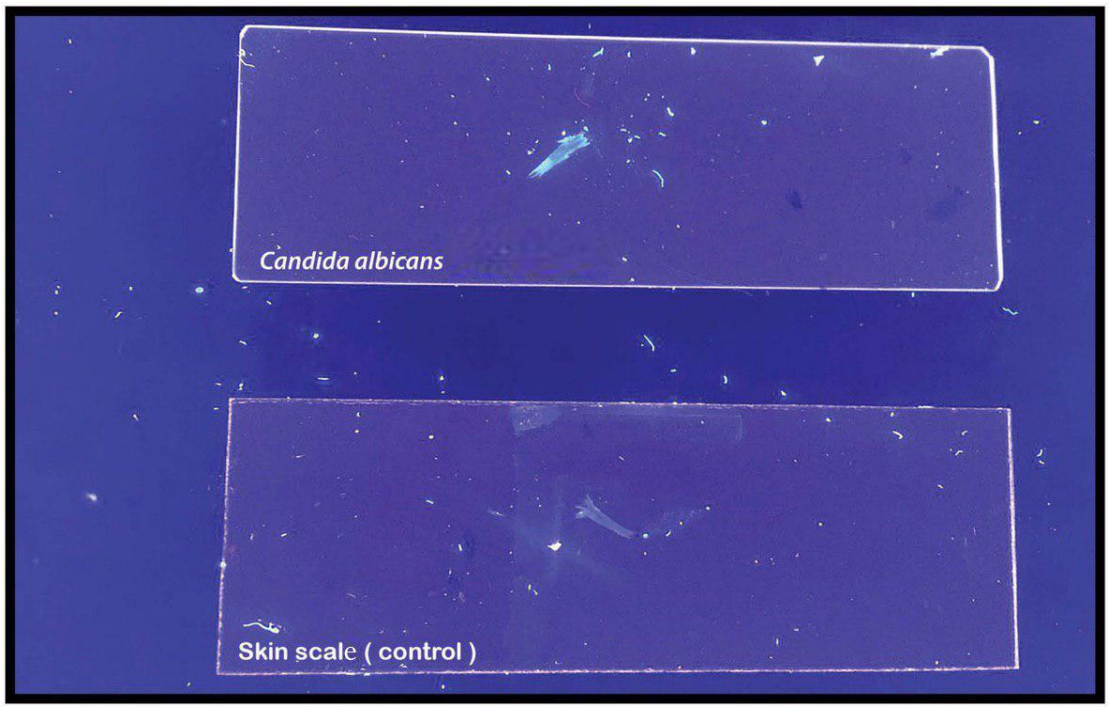

Figure (4-2) shows the difference between sample of skin scale infected with *C. albicans* and a non-infected sample of skin scales (as control/negative) under UV. Light.

2- The second experiment results

As shown in figure (4-3), the result was (+ve) for a glowing sample of skin scale infected with *C. albicans* as compared with the (-ve) result for a non-glowing sample of skin scale infected with *Aspergillus spp*.

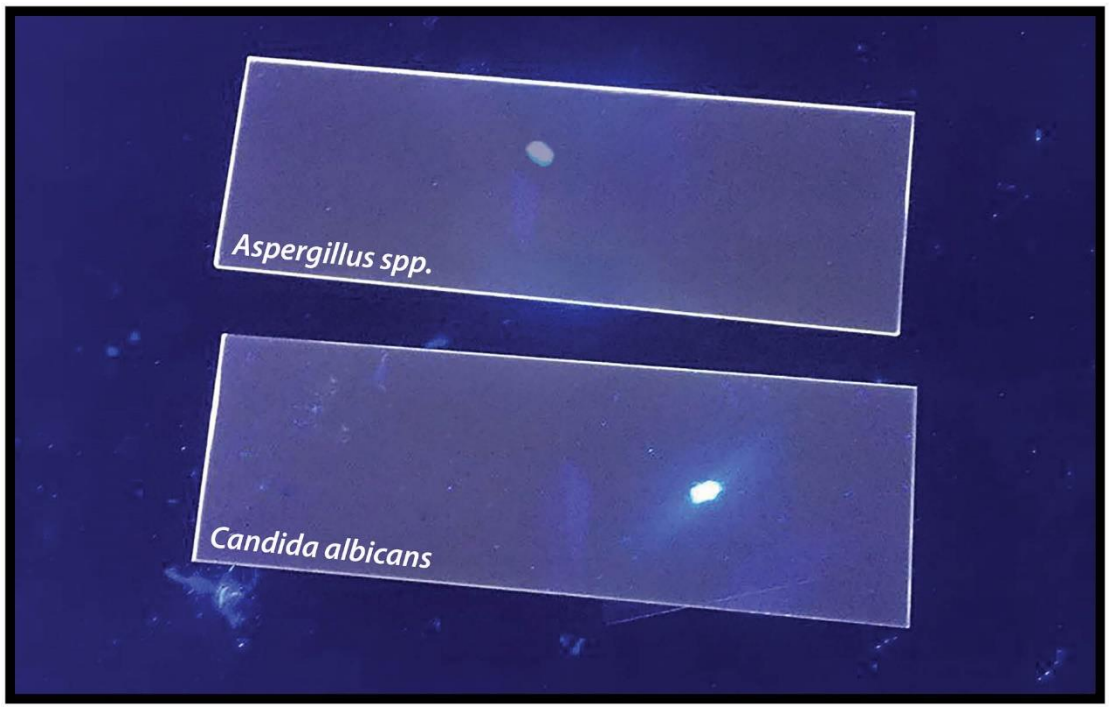

Figure (4-3) shows the difference between sample of skin scale infected with *C. albicans* and sample of skin scale infected with *Aspergillus spp*. under UV. Light.

1- The third experiment results

As shown in figure (4-4), the result was (+ve) for a glowing sample of skin scale infected with *C. albicans* as compared with the (-ve) result for a non-glowing sample of skin scale infected with *Tricophacton spp*.

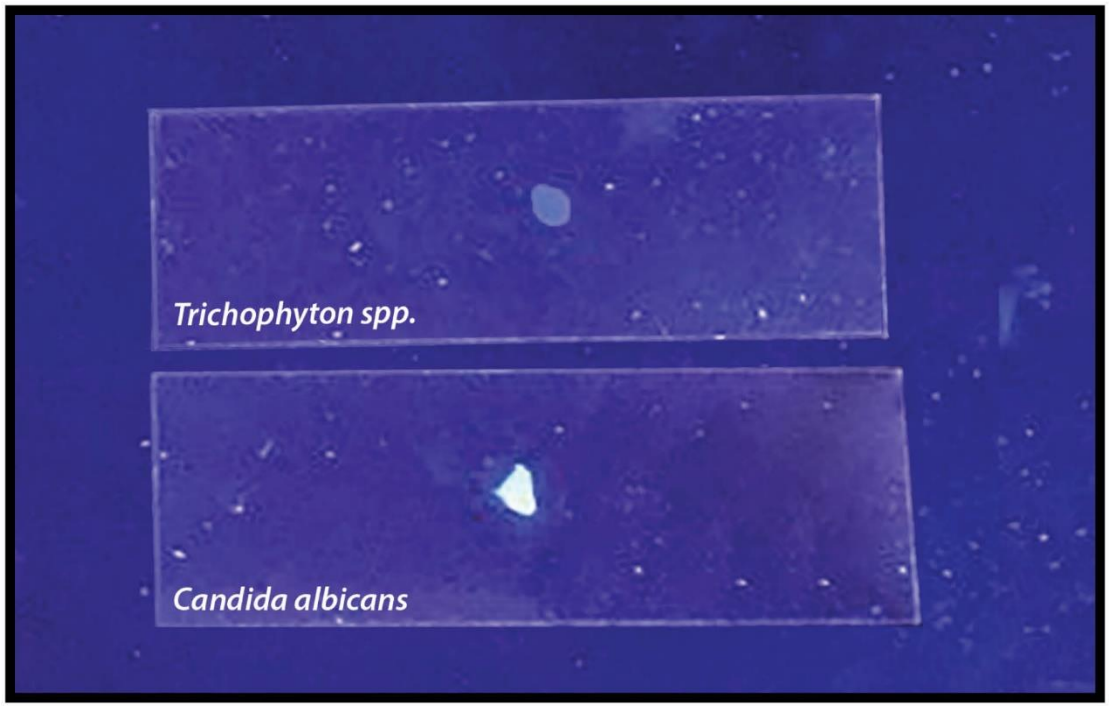

Figure (4-4) shows the difference between sample of skin scale infected with *C. albicans* and sample of skin scale infected with *Tricophacton spp*. under UV. Light.

4- The Fourth experiment results

As shown in figure (4-5), the result was (+ve) for a glowing sample of skin swab infected with *C. albicans* as compared with the (-ve) result for a non-glowing sample of skin swab infected with *Staphylococcus aureus*.

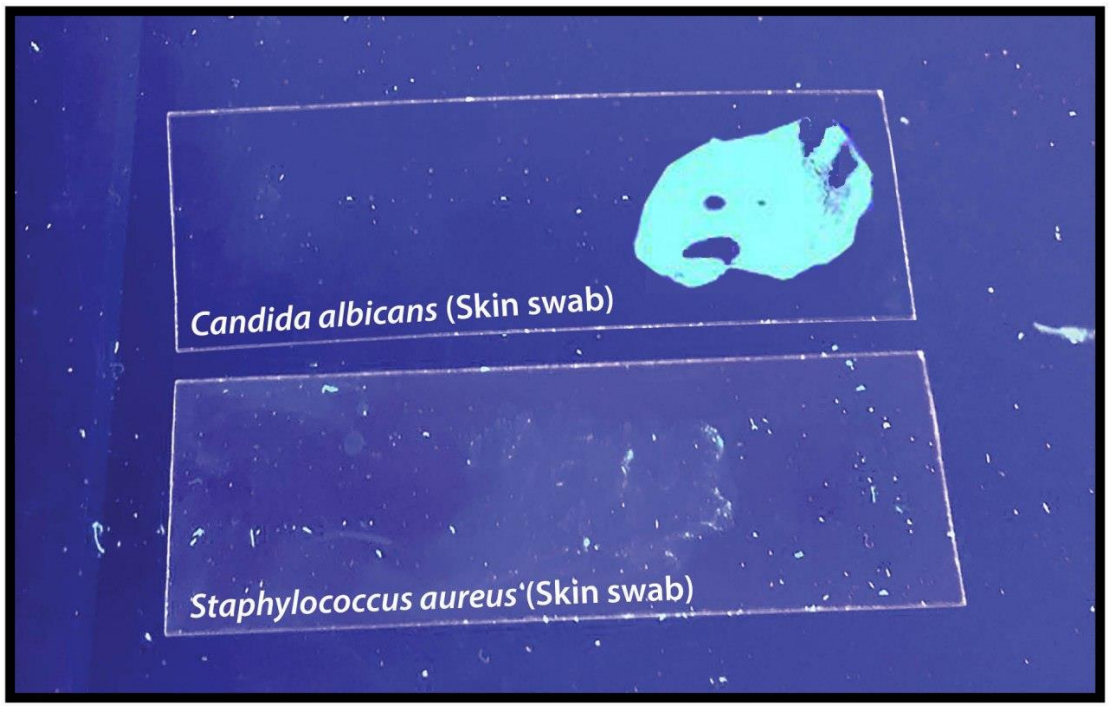

Figure (4-5) shows the difference between sample of skin swab infected with *C. albicans* and sample of skin swab infected with *Staphylococcus aureus* under UV. Light.

1- The fifth experiment results

As shown in figure (4-6), the result was (+ve) for a glowing sample of mouth swab infected with *C. albicans*.

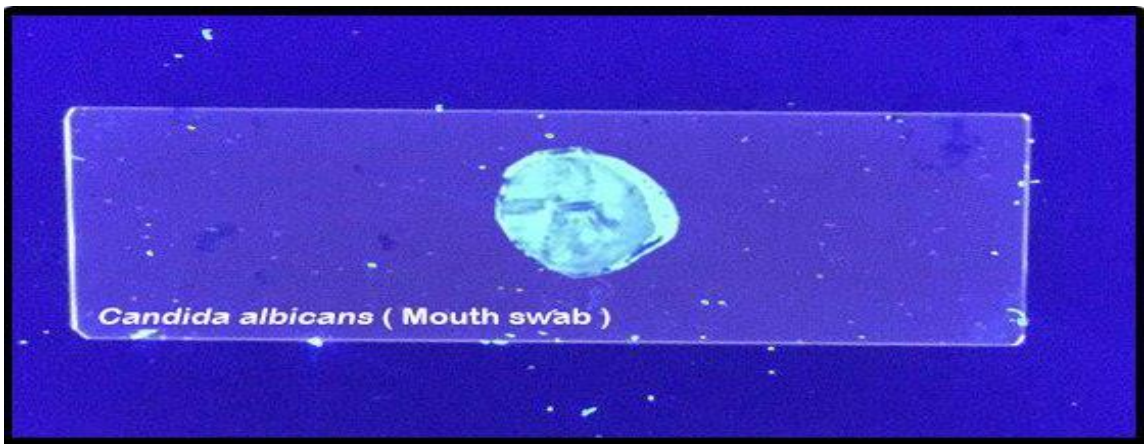

Figure (4-6) shows the mouth-swab infected with *C. albicans*

6- The sixth experiment results

As shown in figure (4-7), the result was (+ve) for a glowing sample of vagina-swab infected with *C. albicans.*

Figure (4-7) shows the vagina-swab infected with *C. albicans*

Chapter five
DISCUSSION

1.5 Discussion

The case study included 100 samples of the patient (immunocompromised women with average age (55-57) year and babies with average age (1-18 month), patients infected with various (fungi, bacteria) infection, in addition to 20 samples for an apparently healthy individual (a control). Patients were attended to AL- Dowaly Private Hospital in Baghdad during the period January 2019 to June 2019. All infections were diagnosed by consultant medical staff at the hospital by using several tests including (germ tube, culture, Vitek and API20C).

Results of new technique was as follow: in figure (4-2) the result was (+ve) for a glowing sample of skin scale infected with *C. albicans* as compared with the (-ve) result for a non-glowing sample (non-infected) of skin scale (as control). As shown figures (4-3)and (4-4) the result was (+ve) for a glowing sample of skin scale infected with *C. albicans* as compared with the (-ve) result for a non-glowing samples of skin scales infected with *Aspergillus spp* and *Tricophactone spp.*, while shown in figures (4-5),(4-6)and (4-7) the result was (+ve) for a glowing sample of skin swab and (mouth, vagina) swabs infected with *C. albicans* as compared with the (-ve) result for a non-glowing sample of skin swab infected with Staphylococcus aureus.

The comparison between the new technique with the current routine tests was performed to demonstrate the feature of the new technique, and the comparison are as follow:

Table (4-3) figure (4-1) shown that the new technique 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, these results agree with studies (Segal and Elad.,2005 and Purkait., 2011), while the new technique does not take a long time it need one minute to get a result and there is no contamination.

Table (4-3) figure (4-1) shown that the new technique is 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. It's 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. These results agree with studies (Milne., 1996; Kim et al., 2002 and Deorukhkar et al., 2012), while the new technique dissent need any factors of the above.

Table (4-3) figure (4-1) show that the new technique 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. These results agree with (Deorukhkar and Saini., 2014), while the new technique is low cost commercially.

Table (4-3) figure (4-1) shows that the new technique of *diagnosing C. albicans* is similar to the Vitek test with sensitivity and specificity (100%) This is because the Vitek system is widely used for rapid identification and susceptibility testing and sensitivity testing for many microorganisms (e.g. Yeasts, bacteria, viruses, and parasites) these studies agree with (Mondelli et al., 2012), it's a fast and accurate diagnostic technique in all routine laboratories. The study which perform by (Ligozziet.,2002) that proved This technology has evolved so that it can also, diagnose the specific antibodies for each pathogenic microorganism by diagnostic card. These results agree with (Graf et al., 2000; David., 2010 and Deorukhkar &Saini,.2014; Q. Badr and Abaas., 2017). The new technique is used to measure the antigens' presence of *C. albicans* and is done by adding conjugated similar to the conjugated of ELIZA, but the difference is the addition of a stain and its binding to the Fc-antibody part instead of the enzyme, this association is caused by the hydrophobic effect Because nonpolar molecules are clustered together, away from water molecules, Large molecules can contain non-polar regions, they tend to be near to each other and cause a change in the shape of the molecule so that it becomes near to each other and away from water molecules (Jordan and

Gibb.,2015). This is why Strong physical bonds were formed between the stain, which is a non-polar molecule with a hydrophobic surface with the antibody security acid (hydrophobic). The immune-complex exposed to UV-Light as a source. The positive sample glowed while the negative sample doesn't glow. The present diagnostic kit is considering a good tool for a diagnosis of *C. albicans* infection in all infected areas (skin, mouth, and vagina).

Conclusions and recommendation

Conclusions

1- The new technique was more sensitivity (100%) and specificity (100%)compared to the routine method.

2- The present diagnostic kit is considering a good tool to diagnosis of *C. albicans* infection in all infected area (skin, mouth and vagina).

3- It does not take a long time Where the duration of its work to give the result to be one minute, and their cost is low.

4- Local Kit for diagnosis of *C. albicans* was successfully prepared and produce in this study.

Recommendation

1- Development of the new technique to diagnose other samples, not skin only, for example blood, sputum and other samples infected with *C. albicans.*

2- Preparation of a kit to diagnose the rest of fungi and bacteria.

3- The new technique can be used by the physicians without having to be sent to the laboratory.

4- To determine the precise accuracy, we recommend to apply this diagnostic kit on infected animals by inoculation infection in mouth and skin or blood then observe the animals to get more safety and accuracy.

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الخلاصة

في هذه الدراسة ، تضمنت إنتاج تقنية جديدة لتشخيص الاصابة بفطر *Candida albicans* (الجلد والفم والمهبل). اشتملت هذه الدراسة 100 عينة من المرضى يعانون من نقص المناعة (نساء تتراوح اعمارهم بين (55–57 سنة) واطفال تتراوح اعمارهم بين (1–18 شهر) ، والمرضى المصابين بااصابات مختلفة (فطرية وبكترية) ، بالإضافة إلى 20 عينة من الاشخاص الاصحاء (control), تم جمع عينات من مستشفى الدولي الاهلي في بغداد خلال الفترة من يناير 2019 إلى يونيو 2019. تم تشخيص جميع الإصابات من قبل الطاقم الطبي الاستشاري في المستشفى باستخدام العديد من الاختبارات تظمنت الحالات المسجلة في هذا الدراسة كانت على النحو التالي:

- ثلاثون عينة للمرضى المصابين بفطر *C. albicans*(قشور الجلدية)
- خمسة عشر عينة لمرضى المصابين بفطر *Aspergillus spp.* (قشور الجلد)
- خمسة عشر عينة للمرضى المصابين بفطر *Tricophactone*(قشور الجلد)

{تم عزل قشور الجلد من بين الأصابع القدم}

- عشرة عينات للمرضى المصابين بفطر *C. albicans*(مسحات الجلد)
- عشرة عينات من المرضى المصابين ببكتيريا المكورات العنقودية الذهبية (مسحات الجلد).
- عشرة عينات لمرضى مصابين بفطر *C. albicans*(مسحات الفم)،
- عشرة عينات للمرضى مصابين بفطر *C. albicans* (مسحات المهبل).

فحصت العينات باستخدام التقنية الجديدة مقارنة بالطرق الروتينية للتشخيص ، وجدنا أن التقنية الجديدة أعطت نتيجة إيجابية للعينات المصابة بـ *C. albicans* ، في حين أن بقية العينات غير المصابة بـ *C. albicans* (لفطرية ، البكتيريا) أعطت نتيجة سلبية.

وقد تبين أن اختبار Vitek كان أكثر تحسسية بنسبة (100 ٪) ونخصصية (100 ٪) لتشخيص *C. albicans* من الطرق الأخرى (API20C ذات تحسسية (100%) وتخصصية (97%)، الزرع الاحيائي كمظهر المستعمرات (Culture) ذات تحسسية (96 ٪) وتخصصية (93 ٪) واختبار تكوين الانبوب الجرثومي (Germ tube) ذات تحسسية (98 ٪) وتخصصية (95 ٪). كانت نتائج اختبار التقنية الجديدة في هذه الدراسة لتشخيص *C. albicans* مماثلة لنتائج اختبار Vitek ذات تحسسية (100 ٪) وتخصصية (100 ٪) ، فوائد هذه التقنية الجديدة هي توفير معلومات عالية الدقة ، اقتصادية (منخفضة التكلفة) وتقليل استهلاك الوقت. على سبيل المثال ، يمكن للأطباء استخدامها من خلال الزيارة التي يقوم بها المريض دون الحاجة إلى إرسالها إلى المختبر.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد
كلية التربية للعلوم الصرفه/ ابن الهيثم
قسم علوم الحياة

تقنية مناعية جديدة لتشخيص الاصابة بفطر المبيضات البيضاء

رسالة مقدمة

الى مجلس كلية التربية للعلوم الصرفة (ابن الهيثم) – جامعة بغداد وهي جزء من متطلبات نيل درجة ماجستير علوم في علوم الحياة / علم المناعة

من قبل

رسل اركان حسن

بكالوريوس علوم الحياة/ كلية التربية للعلوم الصرفه (ابن الهيثم) / جامعة بغداد (2016)

باشراف

الاستاذ المساعد الدكتورة

حازمة موسى خليل العباسي

ذو الحجة/1440هـ أب /2019م