DOI : 10.35124/bca.2019.19.2.4115

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 4115-4120, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

NEW IMMUNOLOGICAL TECHNIQUE FOR DIAGNOSIS OF *CANDIDA ALBICANS* IN DIFFERENT SITES

Russul Arkan Hassan*, Hazima Mossa Al-Abassi and Ali Abdul Al-Hussain Mahdi

Department of Biology, College of Education Ibn Al-Haitham, University of Baghdad, Iraq.
*e-mail: russularkan1995@gmail.com

(Received 29 April 2019, Revised 26 June 2019, Accepted 23 July 2019)

ABSTRACT : In this investigation, we attempted to produce a diagnostic Kit of *Candida albicains* infection (New Technique). This case-control study included 100 samples with various (fungi: *C. albicans*, *Aspergillus* spp., *Tricophactone* and bacterial *Staphylococcus aureus*) infection for immunocompromised women with average age (55-57) years and babies with average age (1-18) month in addition to 20 sample collected from apparently healthy individual (as control). Samples were collected from the skin scales(intertriginous)and skin swabs, mouth and vagina (swabs), the Samples were examined using the new technique compared to the routine methods of diagnosis, we found the new technique gave positive results for samples infected with *C. albicans*, while the rest of the samples non - *C. albicans* infection (fungal, Bacteria) gave a negative result. The results of the new technique were with (100%) sensitivity and (100%) specificity the same sensitivity and specificity (100%) to Vitek test were more sensitive (100%) and specialized (100%) for as compared to the results of other routine methods (Culture, Germ tube and API20C), according to the present result we can conclude that this new technique doesn't need a long time with low cost and high rate of accuracy and physician can use it through the attending visit of patient without having to be sent to laboratory.

Key words : Antigen-antibody reaction, *Candida albicans*, diagnosis.

INTRODUCTION

Immunological techniques are the wide varieties of methods and specialized experimental protocols devised by immunologists for inducing, measuring and characterizing immune responses. 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 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 speciûcity. The sensitivity of a test is deûned 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 binding with antibodies (eg. ELISA, Immune Fluorescent and Radioimmunoassay) (Carpenter, 1975; Schultz, 2009; Zabriskie, 2009 and Dunbbar, 2012).

Yeasts are opportunistic pathogens and cause disease in hosts, who are compromised by underlying local or systemic pathological processes. Yeasts can cause a number of diseases ranging from localized mild infections to deep-seated candidiasis (Noble and Johnson, 2007; Brown *et al*, 2012; Marttila *et al*, 2013).

Since the 1980s, there has been a significant increase in the number of *Candida* infections, especially in hospitalized patients which regarded to several factors. Predisposing factors include immunosuppression, prolonged administration of antimicrobial agents, surgery, burns, and indwelling catheters intravenous drug use. The increasing in number of important candida is not only observed from this year but also the incmrease in severity and resistance of all species (Raines *et al*, 2013 and Al-Jumaily *et al*, 2015).

C. albicans is a commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. *C. albicans* lives in 80% of the human population without causing harmful effects, although overgrowth of the fungus result in candidiasis [candidosis]. *C. albicans*is the most common and well-studied of the disease-causing Candida spp. that naturally colonizes the skin, genital and/or intestinal mucosa in up to 70% of healthy individuals. 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 (Vargas *et al*, 2005; Noble and Johnson, 2007; Ajah, 2016; Kühbacher, 2017).

IL-12 is a key immunoregulatory cytokine (Sinigaglia *et al*, 1999; Beadling and Slifka, 2006). IL-12 has multiple biological functions and importantly, it bridges the early nonspecific innate resistance and the subsequent antigenspecific adaptive immunity (Chehimi *et al*, 1994). The role of IL-12 in promoting endogenous protective immune responses to viral infections has been attracting more attention with time (Hussain *et al*, 2018).

C. albicans is also a predominant opportunistic fungal pathogen, leading to disease manifestations such as disseminated candidiasis and chronic mucocutaneous candidiasis (CMC). The differing host susceptibilities to the sites of *C. albicans* infection have revealed tissue compartmentalization with conditioning of immune responses based on site of infection (Kashem& Kaplan, 2016).The included ratio infection of candidiasis was for two years (2017 - 2018) in Iraq, respectively (15-14.8) / 10^5 aura of the population.

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, hence, immunodiagnostic and molecular techniques can be recommended for early and specific diagnosis (Deorukhkar and Saini, 2014).

In this study, we have attempted to prepare a new Kit for diagnosis of *C.albicans*using non-harmful UV-Light, and design of protocol to diagnosis of either microorganism depended in this principle study (More Specific and Sensitivity than classical Kit).

MATERIALS AND METHODS

Subjects

This case-control study included 100 samples with various (fungi and bacterial) infection for immunocompromised women with average age (55-57) years and babies with average age (1-18) month in addition to 20 sample collected from apparently healthy individual (as control). A patients were attended to AL-Dowaly Private Hospital in Baghdad during the period January 2019 to February 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: (1) 30- samples for patients with *C. albicans* infection (skin sacles). (2) 15-Samples for patients with *Aspergillus* spp. infection (skin scales). (3) 15-Samples for patients with *Tricophactone* (infection (skin scales). {Skin scales and skin swabs were isolated from fingers (intertriginous)} (4) 10-Samples for patients with *C. albicans* infection (skin swabs). (5) 10-Samples for patient's infected with *Staphylococcus aureus* (skin swabs). (6) 10-Samples for babies (1-18) month infected with *C. albicans* (mouth swabs). (7)10-Samples for patients with *C. albicans* infection (vagina swabs).

Preparation of the specialized kit for the diagnosis of *C. albicans*

The kit was prepared using the following materials:(1) Kit for specific antibodies for *C. albicans*. (2) Ultraviolet -Stain. (3) Distilled water.

Principle of the test : Specific (monoclonal antibody) for *C. albicans* conjugate with UV stain, react with antigenic determinant of *C. albicans*. The immune-complex exposed to UV-Light source and the positive sample with glow while the negative sample doesn't glow.

Preparation of kit

1. 1ml kit of specific antibody for *C. albicans* was diluted by 1ml for distilled water, the mixture was mixed and 1 ml of UV-Stain was added.
2. The mixture incubated at 37°C for 24 hours.

Assay procedure

1. The samples (Skin Scales, Swabs) was collected from patients with fungal and bacterial infections (*C. albicans*, *Aspergillus* spp, *Trichophacton* and *Staphylococcus aureus*) and from non-infected individuals.
2. Skin scales was placed on slide then fixed by the burner.
3. About 0.01ml of prepared kit was placed on fixed samples by pipette.
4. Incubated at room temperature for 1 minute.
5. Samples washed twice by D.W.
6. Samples were exposed to UV-Light (harmless) to detect the samples with their glow and non-glow, when the sample was glow this mean a positive result and the sample that does not glow this mean a negative result.

Detection of *C. albicans* infection by new Kit

Experiments were done on the samples to check the validity and specialty of the kit (specific to diagnosis of *C. albicans*).

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 as mentioned above.

2. The second experiment was conducted on infected skin scales from women with *C. albicans* and *Aspergillus*. These samples have been treated as mentioned above .

3. The third experiment was conducted on infected skin scales from women with *C.albicans* and *Tricophacton*. These samples have been treated as mentioned above.

4. The fourth experiment was carried out on skin-swabs from the infected skin with bacteria *Staphlococcusauries* (swabs) from women. These samples have been treated as mentioned above.

5. The fifth experiment was carried out on the isolation of the mouth infected *C. albicans* (swabs) from babies. These samples have been treated as mentioned above.

6. The sixth experiment was carried out on the isolation of the vagina infected *C. albicans*. These samples have been treated as mentioned above.

RESULTS AND DISCUSSION

Isolation and diagnosis of *C. albicans* infected samples

Table 1 showed the comparison among some types of tests used to diagnose *C. albicans* and other fungi and bacteria infections. It had been shown that the Vitek test was used to diagnose (+60) samples of *C. albicans* (skin scales, skin swabs, mouth and vaginal swabs) versus zero isolations from non *C. albicans* infection, while API20C test was used to diagnose (+60) samples of *C. albicans* opposite (+1) isolations from non *C. albicans* infection, as well as to culture test (to diagnosed (+58) isolations of *C. albicans* opposite (+3) isolations from non *C. albicans* infection) and Germ tube test had (to diagnosed (+59) isolations of *C. albicans* opposite (+2) isolations from non *C. albicans* infection). As for the new technical technique for diagnosis of *C. albicans* which diagnos 60 isolates infected with *C. albicans* and gave a positive result.

Sensitivity and Specificity for the New Kit

Results in Table 2 showed True Positive, False Negative, True Negative and False Postive to 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.

With regard to experiments carried out on the samples using the new technique and give results as follows: in pictures (2) give a positive result glow of the skin scale infected with *C. albicans* and give a negative result non-glow of the skin scale (Control), as well as for two pictures (3) (4) give a positive result glow of the skin scale infected with *C. albicans* and give a negative result non-glow of the skin scales infected with *Aspergillus* spp. and *Tricophactone*, while results in three Figs. 5, 6, 7 give a positive result glow of the skin swabs and (mouth, vagina) swabs infected with *C. albicans* and give a

Table 1 : Diagnosis *C. albicans* by Routine methods and New Technique.

Type of fungi and bacteria	NO. Samples	New Technique	Routine techniques			
C.albicans (Skin scales & swabs)	60	+ 60	Colonial Morphology (Culture)	Germ Tube Test	Vitek	API20C
			+58	+ 59	+60	+60
		-0	-2	-1	0	0
Non *C. albicans* infection (Other Fungi & Bacteria)	40	- 40	-37	-38	-40	-40
		+ 0	+ 3	+ 2	0 +	1

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

Table 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

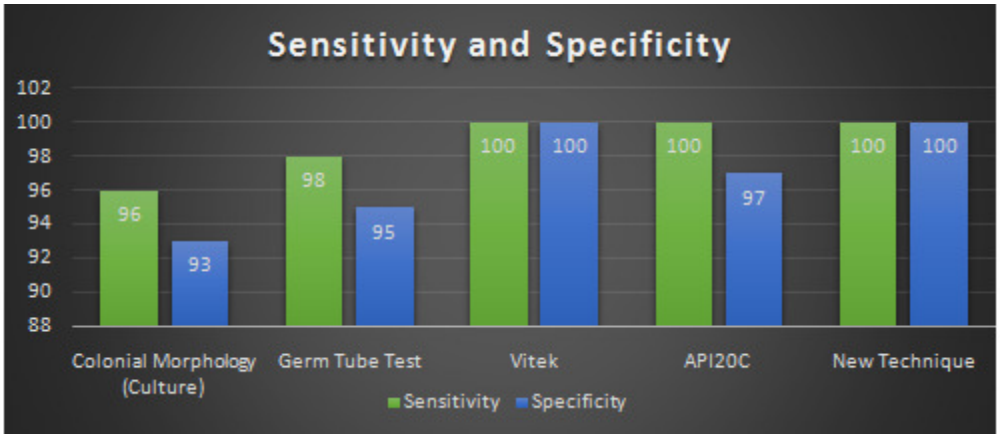

Fig. 1: Sensitivity (%) and Specificity (%) for diagnosis of *C. albicans* by Routine methods and New Technicque.

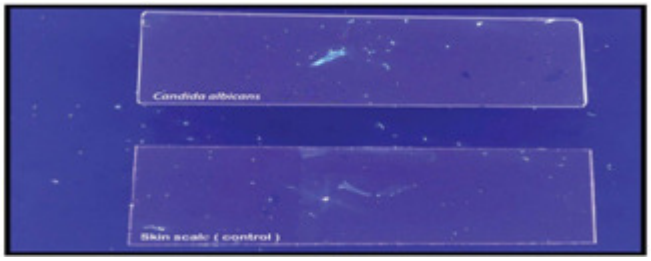

Fig. 2 : The difference between a skin scales infected with *C. albicans* and a non-infected skin scales (control) under UV. Light.

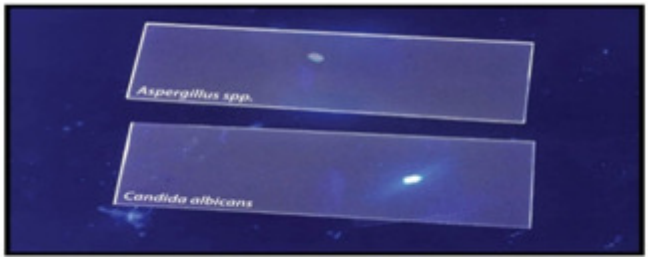

Fig. 3 : The difference between a skin scales infected with *C. albicans* and an infected skin scales *Aspergillus* spp. under UV. Light.

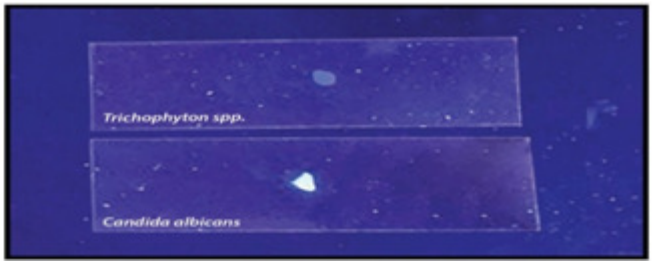

Fig. 4 : The difference between a skin scales infected with *C. albicans* and non-infected skin scales *Tricophacton spp*. under UV. Light.

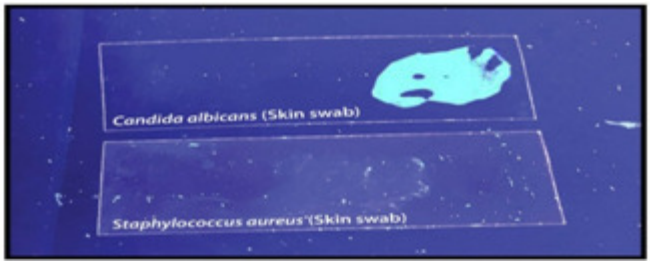

Fig. 5 : The difference between a skin swab infected with *C. albicans* and non-infected skin swab *Staphylococcus aureus* under UV. Light.

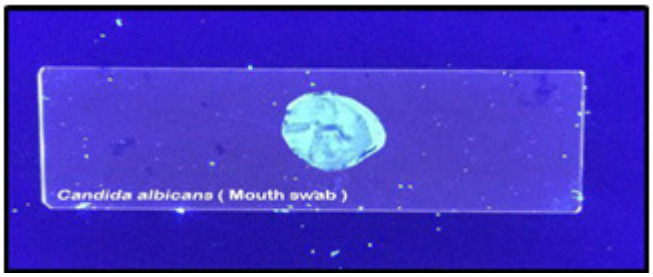

Fig. 6 : The Mouth-swab of *C. albicans*.

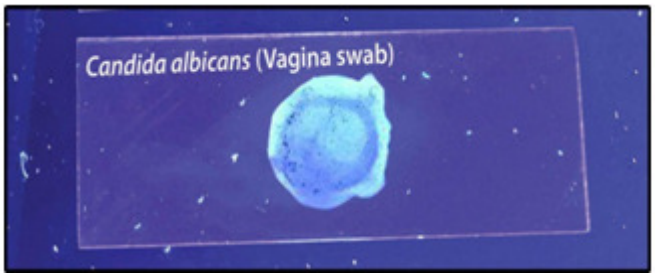

Fig. 7 : The Vagina-swab of *C. albicans.*

negative result non-glow of the skin swab infected with *Staphlococcus aures*.

Age : The patients age from new born babies with average (1-18 month) and women (immunocompromised) with average (55-57) years, this result is disagreeing with previous research, which recorded age average (10-70) years (Al-Rayahi *et al*, 2019).

The comparison between the new diagnostic kit with the current routine was performed to demonstrate the feature of the new kit, and the comparison are as follows:

Table 3, Fig. 1 shows 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). The culture medium can be incubated at 28^{0}C or/and at 37^{0}C. Candida 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 technique does not take a long time it need one minute to get result. These results agree with studies (Segal and Elad, 2005 and Purkait, 2011).

Table 3, Fig. 1 shows that the new technique is more sensitive and specialized (100%) compared with the germ tube test which has a sensitivity (98%) and specificity (95%). It is also known as Reynolds-Braude Phenomenonas relative to the name of the two scientists Which reported by Reynolds and Braude, 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^{0}C for 2 hours. Due to the time required to prepare human serum and inherent 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, trypticase soya broth and various peptone media. Trypticase soya broth is found to be more stable, effective and safe than other media for production of germ tube. 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 of Milne (1996), Kim *et al* (2002) and Deorukhkar *et al* (2012).

Table 3, Fig. 1 shows 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 commercial systems are costly, they have several advantages like rapid identification, require no or less supplemental tests.These results agree with Deorukhkar and Saini (2014).

Table 3, Fig. 1 shows that the new technique of diagnosing *C.albicans* is similar to the Vitek test with (100%) sensitivity and specificity (100%). This is because the Vitek system widely used for rapid identification and susceptibility testing of yeast and yeast like organisms, and that the principle consists of chemical compounds which generate charged molecules and measure their mass to charge ratio. such molecules (signatures) can be used for rapid yeast or bacteria identification from isolate colonies. As well as by new technique it is one of the methods that give guaranteed results depending on the source of the UV-light system, where the result is determined by the glow of the sample or not glow. The sample that is glowing gives a positive result and the non-glowing sample gives a negative result. These results agree with Graf *et al* (2000) and David (2010).

Table 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%

CONCLUSION

The New Technique is a "Kit and source for the UV-light device" Where the kite is a mixture of UV-stain conjugated with the antibody (specialist of the *C. albicans*), if the samples (infected with *C. albicans* and non-Candida infected) were treated with Kit and exposed to UV-light. The sample that is glowing give a positive result, the sample infected with *C. albicans*, due to the formation of the immune complex, which is the conjugation with antibody with its antigens, and the sample that gave a negative result doesn't to glow as a result of the absence. The present diagnostic kit is considering a good tool to detect *C. albicans* infection in all infected area (skin, mouth and vagina).

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