# Gel clot assay used for detection of *Candida spp.* infection in urinary tract

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## Abstract

The study including isolation and identification of *Candida spp.* causing UTIs from patients coming to Al- Yarmouk hospital. A local diagnostic kit was used for measuring canditoxin activity in the urine of patients and it's relation to *Candida spp.*

50 urine samples were collected from female patients of different ages with UTI. The percentage of *C. albicans* was (26%); *C. tropicalis* was (16%). The results showed a high significant difference between them (p=0.00, chi=21.0). The study comprised and attempts to prepare a local diagnostic kit from the lysate of *Homarus lobster*, used to indicate the presence of canditoxin and measure it's activity also in urine samples.

The study showed a significant increase in canditoxin activity in UTI patients, (69.2%) for *C. albicans*, (75%) for *C. tropicalis* while the percentage of the patients who have no fungal growth was 17.24%. The results showed that the percentage of positive LAL assay in-patients diagnosed with fungal infection was (40%) as compared with negative results (60%).

## Introduction

Gel clot LAL assay that has been obtained from aqueous extracts of the circulating ameobocyte of horseshoe crab (*Limulus polyphemus*) (1).
This assay used for estimation of bacterial endotoxins or fungal B-glucans in biological specimen (2).

*Candida albicans* was the most common single pathogen isolated from urine and made up just over half of the fungal isolates. *Candida* urinary tract infection usually associated with catheter use, pregnancy, diabetes, and broad-spectrum antibiotic used (3,4).

Approximately 80-90% of the cell wall of *C.albicans* is carbohydrate. Three basic constituents represent the major polysaccharides of the cell wall (B-glucans, chitin, Mannan), in addition to protein (6-25 %) and minor

Some strain of pathogenic *C.albicans* have the ability to produce endotoxins called canditoxin which cause physiological effect such as killing activity when give intravenous injection to lab. Animals' canditoxin had effectively on lysosome bodies that similar to endotoxin produce by gram-negative bacteria (6). Canditoxin activity could be measured by LAL assay (7).

Eleven species of both pathogenic and saprophytic yeast were tested for endotoxin activity by use of the Limulus assay. These included six species of the genes *Candida*. Such as *Candida albicans* (7). The principle of LAL assay depends on this figure (8).

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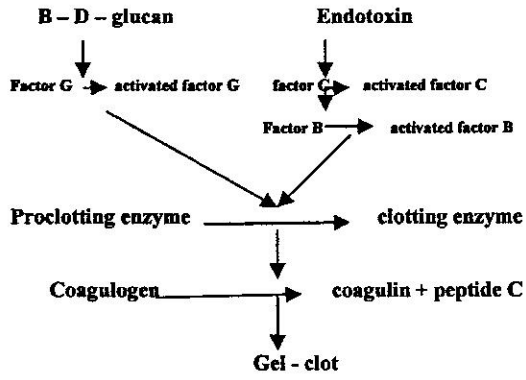


The aim of this study was to isolate and identify the *Candida* species in urine sample obtained from UTI patients and attempt to use new method for the detection of canditoxin activity on the same sample.

## Materials and Methods

- **Collection of Samples.**

50 urine samples were collected from UTI female patients of different ages during three months.

- **General urine examination (GUE).**

Microscopic examination of urine sediment is an essential part of all urinalysis in the presence of UTI Symptoms (9).

- **Cultivation of the samples.**

Samples were cultivated on Sabouraud agar then incubated at 37°C for 48-72-hrs (10).

- **Gram stain**

Gram stain was done to Candida colonies, which appearance like Spheroid – ovate shaped with blue – dark colour ($Gm^{+ve}$)(11).

- **Mycological Investigation.**

Identification of *Candida* species was based on germ tubes, chlamydospore production and biochemical characteristic (10).

- **Gel LAL assay**

## Preparation of hemolymph lysate

According to Nakamura *et al.* (1985) and Jorgensen & Smith, (1973)

a- Adult Shoecrabs were used in lysate preparation.

b- Equipment used for lysate preparation was rendered sterile & pyrogen – free by washing & rinsing with pyrogen – free distilled water, steam sterilization at 121 °C for 15 min. & dry – heat treatment at 170 °C for 2hr.

c- The crab dorsal joint area was cleansed with gauze moistened with 70% alcohol. Pyrogen – free syringe was inserted into the cardiac chamber by way of the dorsal junction of Cephalothorax of the Crab.

d- The hemolymph was allowed to flow directly into pyrogen – free Siliconized tubes.

e- The hemolymph was centrifuged at 1000 rpm for 10min at 4 °C, the blue supernatant fluid was discarded & the packed hemocytes (amoebocytes) were sediment, transferred to pyrogen – free centrifuge tubes and washed twice with pyrogen – free 3% NaCl with centrifugation at 1000 rpm for 10 min.

f- The cells were lysed by the addition of pyrogen – free distilled water at a 1:3 ratio of packed cells to water.

g- The cell suspension was thoroughly mixed with Vortex Mixer & allowed to stand at 4 °C for 18-24 hr.

h- The cellular debris was then removed by centrifugation at 8000 rpm for 30 min., and the lysate was decanted.

The lysate was stored in sterile pyrogen – free vials at - 20 °C.

## Gel clot lysat assay.

LAL assay was conducted by adding a 0.1-ml urine sample of UTIs patients to 0.1ml of lysate in disposable glass test tubes. Negative controls were performed by including a tube containing 0.2 ml of the saline lysate only and tube with 0.1 ml of the saline diluent used for particular test material added to 0.1 ml of lysate. The reaction mixtures were incubated for 60 min. at 37°C (1). The resultant reaction was observed and grading for degree and quality of gelation (Table 1).

**Table 1: Grading of lysate gelation.**

| REACTION | DESCRIPTION |
|---|---|
| Negative | No visible increase in viscosity or opacity. |
| 1+ | Very weak gel with slight opacity & with some starch-like floccules adhering to sides of tube. |
| 2+ | Weak gel with slight to moderate opacity & adhesion of starch like floccules to sides of tube when tube is slanted. |
| 3+ | Soft gel with moderate to considerable opacity. |
| 4+ | Firm gel with considerable opacity. |

## Result and Discussion

Data from 50 UTI patients were reviewed. The identification and differentiation fungal isolates from urine samples for study groups were done depending on Bergey' s manual of determinative bacteriology (13). Which included, GUE, cultivation on suitable media, Gram stain & mycological investigation such as (germ tube & chlamydospore production) and biochemical test by using API system. Table (2) shows that the *C.albicans* isolates presented (26%) from patients with present UTI, while the *C.tropicalis* isolates presented (16%) there is high significant difference between them (p= 0.00, chi = 21.0).

The comparison of fungal culture results with respective results of gel clot LAL assay as shown in table (4). A positive LAL assay was identified in (69.2%) for *C.albicans*, (75 %) for *C.tropicalis* and (17.24%) for No. Growth culture. This result show significant difference between them (p= 0.01, chi = 25.45).

This result shows the high specificity of the qualitative gel clot test in the diagnosis of Candida infections in UTIs patients. About (69.2%) of all patients with *Candida albicans* have a positive LAL test and (75%) patients with *C. tropicalis* where as (17.24%) of no growth culture were associated with LAL-Positive assay, this result may be due to drug treatment during LAL-assay.

The data presented in table (3) demonstrated the positive result of gel clot LAL assay was (40%). There's highly significance between positive and negative result of LAL assay (p= 0.00, chi 50), this results range between (Grad+1of gelation ⟶ Grad +4), this indicate that LAL assay detect small molecular mass of endotoxins . This results is in agreement with previous studies which found that LAL assay could not detect fungal infection and it 's specificity to gram – negative endotoxin bacteria (7,14).

In conclusion, the gel clot LAL assay used in this study is practical tool, in that the time required for incubation is 1 hr and it can used for detecting fungal infection in urine.

**Table (2): Fungus isolates from urine sample**

| FUNGUS ISOLATES | NO. | PERCENT % |
|---|---|---|
| *C.albicans* | 13 | 26% |
| *C.tropicalis* | 8 | 16% |
| Total | 21 | 42% |

**Table (3): Grading results of lysate gelation**

| LYSATE RESULTS | NO. | PERCENT% |
|---|---|---|
| Negative | 30 | 60% |
| Positive | 20 | 40% |
| Total | 50 | 100% |

**Table (4): Relationship between qualitative gel clot assay and fungal culture results.**

| LYSATE FUNGUS TEST ISOLATES | NEGATIVE | PERCENT % | POSITIVE | PERCENT % |
|---|---|---|---|---|
| *C.albicans* | 4 | 30.8 % | 9 | 69.2% |
| *C.tropicalis* | 2 | 25% | 6 | 75% |
| No growth | 24 | 24% | 5 | 17.24 % |

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# فحص جل الخثرة المستخدمة للتحري عن الإصابة بـ *Candida spp.* في القناة البولية

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**الخلاصة:-**

شملت الدراسة عزل و تشخيص أنواع الـ*Candida* المسببة لخمج المجاري البولية من المرضى الوافدين إلى مستشفى اليرموك، و استعمال عترة تشخيصيه محليه لقياس فعالية canditoxin في إدرار المرضى و علاقته بأنواع الـ*Candida* .جمعت (50) عينة إدرار من الإناث المصابات باخماج المجاري البولية بأعمار مختلفة و كانت نسبة الإصابة بـ*C.albicans* (26%) و *C. tropicalis* (16%) و أظهرت النتائج بوجود فرق معنوي بينهما(p=0.00, chi=21). شملت الدراسة محاولة تحضير اختبار تشخيصي محلي من حلالة الخلوية للدم *Homarus lobster* للتحري عن تواجد canditoxin و قياس فعاليته في عينات الإدرار. أظهرت النتائج وجود فرق معنوي في فعالية canditoxin للمرضى المصابين بـ *C. albicans* (69.2%) ولـ *C. tropicalis* (75%) في حين كانت النسبة (17.24%) للمرضى الخالين من النمو الفطري وكذلك أظهرت النتائج نسبة التفاعل الإيجابي لفحص LAL في المرضى المشخصة إصابتهم بالفطريات كانت40% مقارنة بالفحص السالب 60%.