Modification the ELISA Kit for diagnosis of "*Pseudomonas aeruginosa* and comparing it with ordinary ELISA kit"

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ABSTRACT

The first aim of the present study was to diagnosis *Pseudomonas aeruginosa* by many tests .This study consisted "200 patients " who suffered from burn wound and compare with 100 health individuals (male and female) as a control group, Vitek test was used to diagnose 118 (87 "local isolate ATCC 15692" with 31 other isolate of *Pseudomonas aeruginosa* ((ATCC 15690, ATCC 15688)) from 200 samples which were taken from burn patients. This result was similar to Analytical profile index (API) test (118 isolates of *P. aeruginosa* with 82 isolations of other bacteria. Then the detection *P. aeruginosa* isolate ATCC 15692 by new ELISA Technique and comparing its with modify the ordinary ELISA kit.

Keywords: coumarine, chromene, pyrmidine.

الخلاصة

الهدف الاول من الدراسة الحالية هو تشخيص بكتريا السيدوموناس ارجينوزا بعدة تقنيات فحص . تضمنت هده الدراسة 200 مريض مصابين بحروق الجروح وقورنت مع 100 شخص (رجال و نساء) كمجموعة سيطرة، استخدم فحص الفايتك للتشخيص النوعي، 118 شخص (87 حاله للأشخاص مصابون ببكتريا السيدوموناس ارجينوزا ذات العزلة اي تي سي 15692) مع 31 حاله مصابة ببكتريا السيدوموناس ذات سلالات اخرى من نوع (اي تي سي 15690، اي تي سي 15688) من اصل 200 عينة لمرضى مصابين بحروق. هده النتائج مشابهه لفحص الايبي (118 عينة مصابة ببكتريا السيدوموناس مع 82 عزلة لبكتريا اخرى). هو تحوير كت الاليزا الاعتيادي ثم تشخيص بكتريا السيدوموناس ذات السلاله اي تي سي 15692 عن طريق تقنيه الاليزا الجديدة ومقارنتها مع كت الاليزا الاعتيادي.

INTRODUCTION

The *Pseudomonas aeruginosa* includes bacteria that are straight or slightly curved rods *P. aeruginosa* ,almost a rod-shaped bacterium. All types of strains are motile by means of a polar flagellum [1].

The outer leaflet is composed primarily of lipopolysaccharide (LPS) projecting outside and the inner leaflet containing phospholipids and lipoproteins [2].

The LPS of Gram-negative bacterium consists of three different sectors:-Lipid, core polysaccharide comprising the inner and outer cores and O-specific polysaccharide chains projecting outward [3] .

The symptoms of such infections are generalized inflammation and sepsis. If such colonization's occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal [4] .Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is implicated in hot-tub rash. It is also able to decompose hydrocarbons and has been used to break down tar balls and oil from oil spills [5]. *P. aeruginosa* is not extremely virulent in comparison with other major pathogenic bacteria species – for example Staphylococcus aurous and Streptococcus progenies – and does not fare especially well under suboptimal atmospheric conditions nor aggregate into enduring biofilms [6].

Depending on the nature of infection, an appropriate specimen is collected and sent to a

bacteriology laboratory for identification. As with most bacteriological specimens, a Gram stain is performed, which may show gram-negative rods and/or white blood cells [7]. *P. aeruginosa* produces colonies with a characteristic "grape-like" or "fresh-tortilla" odor on bacteriological media. In mixed cultures, it can be isolated as clear colonies on Mac Conkey agar (as it does not ferment lactose) which will test positive for oxidase. Confirmatory tests include production of the blue-green pigment pyocyanin on cetrimide agar and growth at 42°C. A TSI slant is often used to distinguish non fermenting Pseudomonas species from enteric pathogens in faucal specimens [8].

Enzyme-linked Immuno sorbent Assays (ELISA) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme, ELISA can provide a useful measurement of antigen or antibody concentration [9].

SAMPLES AND METHODS

Subjects

All samples were collected from patients who attended Al-Kadhimiya Teaching Hospital and during the period from February 2014 to November 2014.

Laboratory diagnosis of Pseudomonas aeruginosa by many tests [Culture, Vitek ,API and ELISA KIT-Immunolab]and development of new ELISA Kit for Diagnosis of " *Pseudomonas aeruginosa* " IgG.

Methods steps:

1. Isolation and partial Purification of lipopolysaccharides of *Pseudomonas aeruginosa* by ordinary ELISA Kit

Extraction of LPS from *p. aeruginosa* was done using previous study (ATCC 15692).

2. Preparation new ELISA kit to Diagnose *P. aeruginosa* IgG

I)- Coating

The antigen(LPS-Antigen) was diluted with Coating Buffer(sodium bicarbonate buffer (pH 9.6)(28.62 g/ l). and appropriate wells of ELISA plate were coated with the antigen by adding (100 ml)of the diluted antigen solution.

II)- Blocking

Add 200 ml of Blocking Buffer(1%) was added (Bovine serum Albumin)BSA to block the non-specific binding sites in the coated wells

III) Detection

•Reagents Provided

Category 1 ,Category 2 ,Category 3 ,Category 4, Category 5

Negative control: Protein solution (Bovine Serum Albumin)BSA diluted with phosphate buffer saline PBS(PH 7 ,0.1 N), containing no IgG antibodies against *P. aeruginosa*

•Enzyme Conjugate :anti-human-IgG-HRP (rabbit) (Hores radish peroxidase HRP), in protein-containing buffer solution.

•Substrate : (tetra methyl benzidine) TMB(32 mg/dl).

•Stop Solution : 15 mL, 0.5 M sulfuric acid.

Procedure

1- Added 100 µL each of the diluted (1:100) samples(1,2,3), standards(1,2) and negative control were pipetted.

2- The Plate was covered with the enclosed foil and incubate at room temperature for 60 minutes.

3-The wells of the plate were emptied (dump or aspirate) totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the micro titer plate on a tissue cloth.

4- Added 100 mL of each of ready-to-use conjugate was pipetted into the wells. One well was left empty for the substrate blank.

5- The plate was covered with the enclosed foil and incubated at room temperature for 30 minutes.

6- The wells of the plate were emptied (dump or aspirate) and add 300 ml of diluted washing solution. This procedure was repeated totally three times. Rests of the washing buffer were afterward removed by gentle tapping of the micro titer plate on a tissue cloth.

7-Added 100 ml of each of the ready-to-use substrate was pipetted into the wells. This time also the substrate blank is pipetted.

8- The plate was covered with the enclosed foil and incubated at room temperature for 20 minutes in the dark.
9- Added 100 ml of each of the ready-to-use stop solution was pipetted into the wells. The substrate blank was also pipetted.
10- After thorough mixing and wiping the bottom of the plate, the reading of the absorption at 450 nm was performed.

*Re-work same of samples, standers and negative control on ELISA kit (IgG) *P. aeruginosa*(ordinary ELISA kit) for comparing between results see Figure 1.

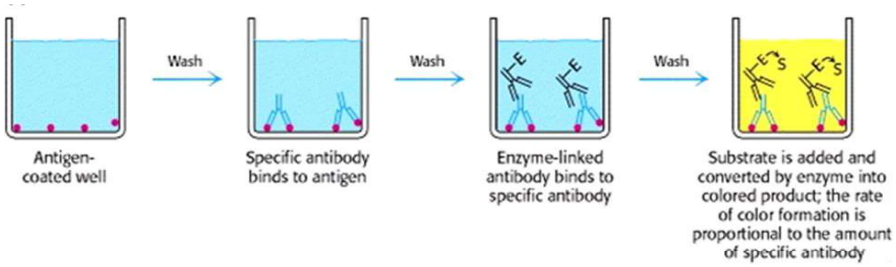

Figure 1: New ELISA kit (4)

RESULTS AND DISCUSSION

Table 1 and Figure 2 showed a data of the comparing new ELISA kit with ordinary ELISA Kit a by investigation of five samples to new and ordinary ELISA Kit these samples were:
Category 1: Serum of patient infected with *P. aeruginosa* isolate ATCC 15692.
Category 2 : Serum of patient infected with *P. aeruginosa* of other isolate .
Category 3: Serum of mice injected with by LPS from *P. aeruginosa* isolate ATCC 15692.
Category 4 : Monoclonal antibodies diluted with PBS, contains (10 mg/dl) concentration of IgG antibodies against *P. aeruginosa* (specific to isolate ATCC 15692).
Category 5 : monoclonal antibodies diluted with PBS, contains (10 mg/dl) concentration of IgG antibodies against *P. aeruginosa* (nonspecific to isolate ATCC 15692).

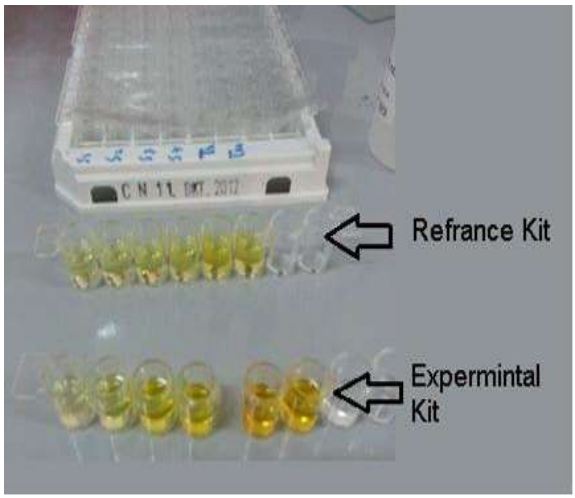

Figure 2: Comparing between new ELISA Kit and ordinary ELISA Kit (4)

Table 1: Comparing between antibody reading of new and ordinary ELISA kit

Sample	Type Antibody	Read of absorption of 450 nm New ELISA Kit	Read of absorption of 450 nm ordinary ELISA Kit
Category **1**	specific to isolate ATCC 15692 **[**human serum]	**2.2**	**1.2**
Category **2**	Non-specific to isolate ATCC 15692 **[**human serum]	**0.9**	**1.9**
Category **3**	specific to isolate TCC 15692 [mice serum]	**1.8**	**0.6**
Category 4	specific to isolate ATCC 15692 [Artificial]	**2.1**	**1.6**

Category **5**	Non-specific to isolate ATCC 15692 [Artificial]	**0.5**	**1.90**
Negative control	Without Ab	**0.2**	**0.1**

Negative control: protein solution diluted with PBS, contains no IgG antibodies against *P. aeruginosa.* Table 1 and Figure 3 showed that Category 4 > 3 (monoclonal antibodies specific to isolate ATCC 15692) was to ELISA kit (absorption 2.1 at 450 nm), than ordinary ELISA kit(absorption 1.6 at 450 nm), per contra, the result of Category 5 (monoclonal antibodies nonspecific to isolate ATCC 15692) was less sensitive to new ELISA kit (absorption 0.5 at 450 nm) than ordinary ELISA kit(absorption 1.90 at 450 nm).

This result was similar to comparison of results inCategory 1 (serum of patient was infected with *P. aeruginosa* isolate ATCC 15692) and with Category 1 > 2 (serum of patient was infected with *P. aeruginosa* other isolate ATCC 15692), which showed that Category 1 (absorption 2.2 at 450 nm) was 1than Category 2(absorption 0.9 at 450 nm) in new ELISA Kit ,while in ordinary kit, opposite result was obtained since Category 1 (absorption 1.2 at450nm) was less sensitive than Category 2 (absorption 1.9 at 450 nm) in ordinary ELISA Kit than new ELISA. In Category 5 (monoclonal antibodies nonspecific to ATCC 15692], new ELISA Kit had LPS, but it was specific to ATCC 15692 only ,therefore, Category 5 was more sensitive to antigen in ordinary ELISA kit than new ELISA kit These results agree with previous study [5]. Category (1) was also taken from patients and infected with *P. aeruginosa* with the isolate ATCC 15692 ,it was shown that the new ELISA kit was more sensitive than ordinary ELISA Kit because it had specific antibody to *P. aeruginosa* [ATCC 15692 isolate], whereas in Category 2 which was taken from patients who were infected with *P. aeruginosa* of other isolate, new ELISA kit was more sensitive than the ordinary ELISA kit ,while Category 3 which was taken from infected mice with *P. aeruginosa* isolate ATCC 15692, was similar to Category 1 result "relatively". All above results in Table 1 showed that the new ELISA kit was successful for diagnosis of *P. aeruginosa* isolate ATCC 15692 compared with ordinary ELISA kit, and it was more sensitive and specific due to having pure LPS, and manufacturing of new ELISA kit. These results agree other study.

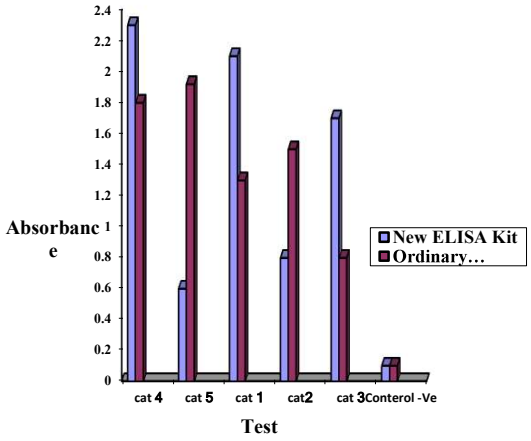

Figure 3: Comparing between the absorbency new ELISA kit with ordinary ELISA kit**.**

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