Preparation of Nano-Antigen for Brucella Abortus by Ultrasound Technique and Evaluate their Sensitivity by ELISA Technique

Dania Dhafer Hameed[1], Siham Sabah Abdullah[2], Ali Abdullhussein Mahdi[3]

[1]Post Graduate, [2]Assist. Prof., Department of Physiology and Medical Physics, college of Medicine,Al-Nahrain University, Baghdad, Iraq, [3]Assist. Prof., Department of Pathological Analyzes, College of Health and Medical Technologies University, Baghdad, Iraq

Abstract

Background: The nanoparticle technology, or nanotechnology is the science that studies the treatment of matter on the atomic and molecular scale. Nanotechnology deals with measurements between 1 to 100 nanometers, i.e. deals with atomic clusters ranging from five atoms to a thousand atoms. They are much smaller than bacteria and a living cell palsy.

Method: In this study, it involved the production of a new technique for diagnosing infection with Brucella bacteria. This study included 45 samples of patients suffering from brucellosis (women and men), in addition to 36 samples from other Brucella. All were diagnosed Injuries by hospital medical advisory staff using various tests vitek, ELISA, API20C, Morphology, Brucella media test. The samples were examined using the new technique compared to the routine method of diagnosis. We found that the new technique gave a positive result to the samples infected with Brucella bacteria, while the rest of the samples without infection with Brucella bacteria gave a negative result.

Results: It was found that the tests of Elisa and API20C, Vitek were more sensitive 100% and specialized 100% for the diagnosis of Brucella bacteria than other method morpholgy with allergic 44%and specialized 86%, Brucella mediaTest, with allergy 88 % And specialty 86%. The results of the ELISA kit test were the nano-labs (the new technique) in this study to diagnose more successful bacteria compared to normal system and more allergic and specialized because of the antigen-containing.

Conclusion: The new kit use in nanotechnology is more Sensitivity and Specificity for diagnosis to the brucella, Roads routine to diagnose brucella is not effective compared with API, Vitek and ELISA .

Keywords: *Nanotechnology, Brucellosis, ELIZA Technology.*

Introduction

Nanotechnology is a term used to deal with the scaling matter at atomic or molecular level The word Nano means a one to one billion of the physical quantity. Considered one Nano unit length equivalent to one-billionth of a meter[(1)(2)]. When Nanomaterial's are manufactured from bulk material. Nanoparticles have emerged as powerful tools to initiate and modulate immune responses due to their inherent capacity to target antigen-presenting cells (APCs) and deliver coordinated signals that can elicit an antigen-specific immune response[(3)(4)]. Nanotechnology is now a fast and interdisciplinary scientific field, through combining distinctive sciences like biology, physics, medicine, and chemistry with engineering, also removing the conventional borders between them. The

Corresponding Author:
Dania Dhafer Hameed
Post Graduate, Department of Physiology and Medical Physics, college of Medicine,Al-Nahrain University, Baghdad, Iraq
e-mail: danyaphy24@gmail.com

nano-technology has the capability of controlling and recognizing molecules and atoms, also dealing with the structures with sizes in range of (1-100nm), 1nm has been equal to billionth of meter. The majority of current nano-materials might be provided into 4 types[5-8]: Carbon-based materials,Metal-based materials, Dendrimers Compounds Combine Nano-particles[9][10]. Brucellosis is a bacterial infection caused by Brucella, which is transmitted from animals to humans. In most cases, people are infected by consuming raw or unpasteurized milk products. Sometimes, the bacteria that cause Brucellosis may be transmitted through the air or in direct contact with infected animals[11].

Material and Method

The samples were collected from 45 patients. Blood samples and 36 other brucella individual (male and female) as control group. all samples were collected from Al-Kadhimiya Teaching Hospital & Baghdad Teaching Hospital during the period from October 2019–February 2020 and were investigation to diagnosis for Brucellaby many test [Culture, Brucella media Test, Vitek and API 20C].

Preparing the blood agar: The medium has been prepared based on the specifications that have been provided by the manufacturing company. 37.50g of the powder has been dissolved in 1 liter of the Distal water (DW), auto-calved for 15 minutes at 121C° (15psi), cooled to 50 -55C° and aseptically 5-7% v/v sterile human blood has been added.

Enterotoxin B Ab Preparation

Based on the manufacturing leaflet, 1ml of the DW has been added into 0.20mg of the enterotoxin B Ab. 500µl has been obtained from the enterotoxin B Ab. and diluted with 9500µl DW for obtaining the 10µg/ml concentration.

Brucella Diagnosis using the vitek system:

1. 3 colonies have been utilized for preparing the Brucella suspension in saline tubes (0.50ml). The suspension has been adjusted to McFarland standard (0.50) utilizing Vitek colorimeter (450nm filter, transmittance ranging between 45% and 55%).
2. After the labeling of the Brucella cards with marker, placed card at a transfer tube with filling stand which has been in Brucella suspension.
3. The card has been inoculated by the module of the filling.
4. The cards have been closed by the sealer module, and incubated afterwards, for 25hrs or 56hrs at 36C, according to type of the readings which have been provided by the instrument types.

The destruction of the cell wall and the isolation of Ag

- The *Brucella* has been cultured on the blood agar plates and incubated overnight at a temperature of 36˚C.
- The growth has been harvested with the use of the microscopic slide via curettage.
- DW has been added into the growth of the bacterial for making 5 ml suspension.
- Brucella suspension has been treated by the lysis buffer (50ml of 50ml of 1% Acetate and 2% SDS). The resultant suspension has been incubated for 20min in the boiling water and vortexed vigorously afterwards.
- One bacterial suspension volume has been mixed with two chloroform volumes. Such suspension has been vigorously vortexed and exposed to the ultra-sounds at 50C° for 30min.
- The suspension has been centrifuged 5min at 4,400rpm. Ten volumes of supernatant has been precipitated with the use of 1 saturated ammonium sulfate (NH4)2SO4 volume after that, it has been mixed at 40˚C on hot plate magnetic stirrer.
- The mix has been dialyzed with the use of the dialysis bag (14,000) against the sucrose for obtaining stock (i.e. the concentrated protein)

The dialysis is a commonly utilized approach, it is simple, yet, time consuming due to the fact that separation is dependent on the diffusion. The sample has been placed within a dialysis bag which has been prepared from a tube which is made from semi-permeable membranes. Relying on the dialysis tube which is available commercially, merely small molecules with sizes <10kDa has been eliminated from sample to the medium around. Which is why, this approach is typically utilized for removing the salts from the protein solution. Dialysis can be utilized to concentrate protein solutions as well. The molecules of the water are eliminated from the inside of the dialysis bag with the use of a hydrophilic polymer like the poly-ethylene glycol.

Purifying enterotoxin B using the gel filtration chromatography: 50ml of the sephacryl S300 has been packeted in the column (2.50cm diameter×78cm length, volume of the column: 379.90ml). 5 ml of the specimen which has been concentrated through the ammonium sulfate has been applied to the sephacryl S300 column.

The elution has been accomplished at a 5ml fraction flow rate with the use of the DW as buffer, each fraction's absorbance has been measured at 280ηm. The peaks have been obtained and concentrated afterwards via the dialysis against the sucrose.

Detecting Ag using the ELISA kit:

Principles: The concept has been accomplished based on manufacturing leaflet. This immunoassay of the enzyme has been based on the immuno-capture concept for quantitatively detecting of the enterotoxin B Ag. The micro-well plate has been coated by anti-enterotoxin B Ab. Throughout the test, the sample has been added into the antibody coated micro-well plate and incubated after that. In the case where the sample includes the enterotoxin B Ag, it will be binding to antibody which is coated on micro-well plate for forming immobilized complexes of Ag-Ab. Following the first incubation, the micro-well plate has been washed for removing the unbound materials. Anti-enterotoxin B Ab has been added into micro-well plate and incubated afterwards. This antibody will be binding to complexes of Ag-Ab. The enzyme-conjugated have been added into micro-well plate and incubated afterwards. The enzyme-conjugated will be binding to the present immune complexes. The solution of the sulfuric acid has been added into the micro-well plate for stopping the reaction which produces a change of the colour from the blue to the yellow. The intensity of the color corresponding to the enterotoxin B Ag amount which is present in the sample has been measured by a micro-plate reader at 450/630-700ηm or 450ηm.

Process:

A-Ab. Coating:

- To capture Ab. in the wells' bottoms, 100µl of diluted enterotoxin B Ab. (10µg/ml) has been added into the 96 wells of micro-titre plate.
- The plate has been covered by sealer of the plate and then incubated overnight at room temperature.
- Every one of the wells has been washed three times by 100µl of the working wash buffer for each one of the wells, after that, the liquid has been washed away.

The micro-well plate has been turned upside down for few seconds on an absorbent tissue. Each well was entirely cleaned and dried.

B-Blocking

- To block the area in the wells' bottoms, 100µl of blocking buffer has been added into the wells.
- The plate has been covered by sealer of the plate, then, incubated for 30min ± 2min at room temperature.
- Every one of the wells has been washed three times by 100µl of the working wash buffer for each one of the wells, the liquid has been washed away. The micro-well plate has been turned upside down for a few seconds on an absorbent tissue. Each well was entirely washed then dried.

C- Assay process:

- The serial dilutions for Ag have been produced: 0.50ml of Ag has been added into the first one of the eight tubes, every one of which contains 0.50ml DW, 0.50ml has been obtained from the first tube and added into second one, etc.
- Al has been left as a blank well.
- 50 micro-liter of every one of the dilutions has been added into assigned wells and carefully mixed through swirling micro-well plate for 30sec. on flat bench.

The micro-well plate has been covered by a plate sealer and incubated for 30min ± 2min at room temperature.

- Those assigned wells have been washed three times by 100µl of a working wash buffer for each one of the wells.
- 50 micro-liter of the diluted enterotoxin B Ab has been added into specified wells.
- The plate has been covered by the sealer of the plate and incubated for 30min ± 2min at room temperature.
- Those assigned wells have been washed 3 times by 100µl working wash buffer for each one of the wells, the liquid has been washed away.

The micro-well plate has been turned upside down on an absorbent tissue for seconds. Each well has been entirely washed and dried.

- 50 micro-liter of the conjugate has been added into specified wells with the exception of the blank one.
- The plate has been covered by sealer of the plate and incubated for 30min. ∓ 2min at a room temperature.
- Those assigned wells have been washed three times by 100µl of the working wash buffer for each one of the wells.
- 50 micro-liter of the TMB has been added into the specified wells.
- Two-tree minutes later, 50µl of the stop solution has been added into those specified wells.
- Absorbance has been read at 450ŋm. The enterotoxin B Ag concentration has been specified through the comparison of absorbance to the Brucella curve (IgG Ab.) ELISA Kit.

Method

1. Isolatione of bacteria by culture of blood sample on Brucella media .
2. Add 0.5 ml from pure colony bacteria to 0.5 ml DW (Mix-bacteria)
3. Exposure Bacteria (Mix-bacteria) of Brucella antigen to ultrasound by sonication bath for (1 min, 5 min and 10 min 30 min). …..(R1)(this step to Cracking proteins into smaller particles due to collision of high frequency sound waves)
4. Add 100 Ml (10 M/dl concentration) from R1 to plastic wells ELISA (to coating of Ag on basic of plastic wells)
5. Add 100 Ml (10 M/dl concentration) from Bacteria of Brucella antigen without exposure of ultrasound (R2) plastic wells ELISA as control
6. Waiting for 24 h.
7. Add 100 Ml Conjugated reagent to R1 & R2 (Routine work for preparation of ELISA Kit)
8. Wash 3 time all wells by washing reagent
9. Add 100 Ml from TMB and waiting 10 min
10. Add 50 Ml from stop reagent (to Stop Interaction)
11. Read on 450 nm
12. Comparing between R1 & R2 of absorbance

Results

Isolation and Diagnosis of Brucellaisolation: Table [1] and Figure (1) showed the comparison of some test types that are utilized in the Brucella diagnoses. It has been shown that Vitek and API 20-C of a higher sensitivity 100% and specificity 100% for diagnosing Brucella compared to other approaches (Colonial morphology "culture" 44 %and Brucella media test 88.0%), the Vitek test has been used to diagnose 32 isolates.

Table [1] Diagnostic test for Brucella

Test	Sensitivity* TP/ (TP + FN)	Specificity** TN/(TN + FP)	Brucella	False Positive (FP)	False Negative (FN)	Other Brucella	Total
Colonial morphology	44 %	86%	20	2	12	36	45
Brucella media Test	88 %	86 %	28	2	4	39	45
Vitek	100 %	100 %	32	0	0	47	45
API 20C	100 %	100 %	32	0	0	47	45
ELISA[IgG]	100 %	100 %	32	0	0	47[-]	45

*The sensitivity of a test is the ability of the test to identify correctly the affected individuals (111)
**The specificity of a test is the ability of the test to identify correctly non-affected individuals (111)

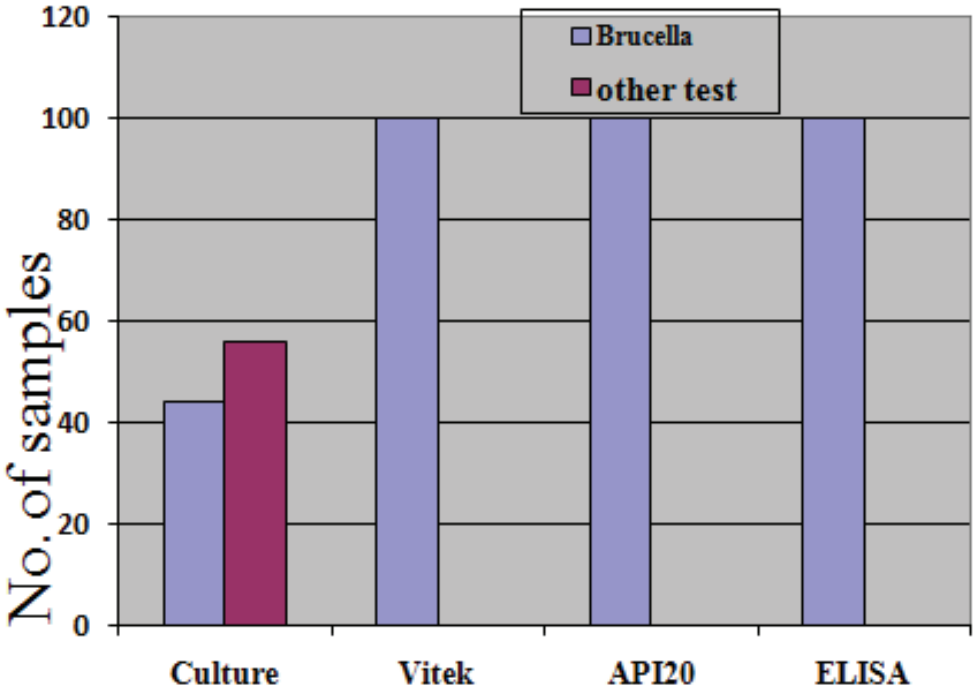

Figure (1): Sensitivity of tests for diagnosis

The results showed in Fig (2) and the figure a high absorbency for the samples that were prepared with nanotechnology and whose absorbance was measured as being more high than the samples prepared by traditional method .

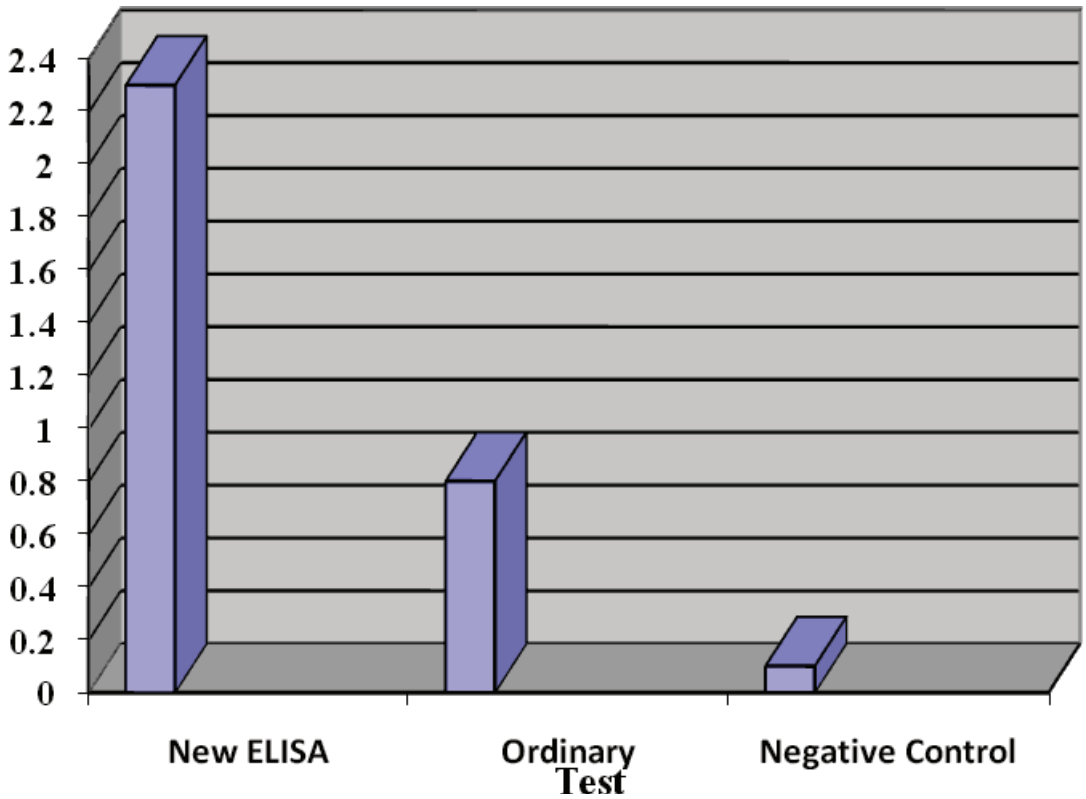

Fig. (2): Comparing new ELISA kit with ordinary ELISA Kit

Discussion

Samples have been gathered from 45patients. Blood sample and 36 others brucella individuals (males and females) as the control group.Every sample has been taken from Al-Kadhimiyya as well as Baghdad Teaching Hospitals during the period from october 2019 – February 2020 andhave been investigated by diagnosing for the Brucella by several tests [Culture, Brucella mediaTest, Vitek and API 20-C].

In table (1) Fig(1) The results have shown that the percentage of sensitivity in the exams API,Vitek and ELISA were (100%), while the examinations of Morphology (44%) and (88) Brucella media test, respectively, while the Specificity rate (100%) for API,Vitek and ELISA were (100%), while its percentage was lower (86) for the Brucella media test and Morphology test, This is because the tests were dependent on the external appearance, color difference, and morphology of the microorganisms,The results proved that the best way to diagnose bacteria is by means of API 20C,Vitek and ELISAThis is consistent with the findings of Mahdi[12].

The results proved that the extracted and purified antigen by Nano-technique method was more effective than the purified antigen in the routine way because in the event that the large molecule of the antigen is broken, which contains multiple epitopes on the surface of the large molecule where the precise cracking of the large molecule allows to distribute the space of the epitope more widely and publish it accurately and broadly ., When using standard antibodies manufactured specifically for specialized isolates of Brucella by the Nano scale method, the results showed a high correlation with the Nano antigen and was detected by absorbance measurements on wavelength 450 nm,Whereas, there was no reaction interaction between antibodies and normally made antigens, this is consistent with the findings of Kishimoto,Maldonado[13].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

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