Comparative study between Serological and Molecular Tests in Diagnosis of Human Brucellosis.

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Abstract:

Brucellosis is widespread zoonotic disease that afflicts both human and animals and the clinical signs and symptoms of human brucellosis are different and non –specific diagnosis of brucellosis depended on a positive assessment on laboratory based testing **,** the present study was designed to assess the validity of serological tests (Rose Bengal(RBT) and ELISA tests) and Polymerase Chain Reaction assay for diagnosis of brucellosis.

A clinical manifestation of fever cases included symptoms such as a temperature greater than 38C° on several occasion within three weeks. In patients suffering from Malta fever and according to recommendation of Ministry of Health to conduct this problem in Iraq. Blood samples were collected from the 50 patients during November 2017 to April 2018 after inform all patients about the aim of this study as a permission for ethics. Blood sera were prepared to conduct the serological tests (RBT and ELISA) then the *Brucella* was isolated on selective media such as *Brucella* agar media which confirmed molecularly by using Real-time PCR to amplify the primer of 16SrRNA *gene* and finally the method of *gene* sequencing was used to detect the species and biovars of *Brucella* by using the *rpoB gene*.

The results of screening (serological) test by using RBT showed that the occurrence percent of brucellosis in human was 45/50 (90%), while the results of ELISA was 42(84%). On the other hand ,the result of bacteria isolation on *Brucella* agar was 10(20%) , and the results of Real-time PCR was 38(76%) as

a confirmation test for *Brucella melitensis* isolates after extraction of DNA and amplification of primer belong to 16SrRNA gene .

The results of *rpoB gene* amplification for *Brucella melitensis* revealed the appearance of one distinct band after electrophoresis on agarose gel that had a molecular size 1091bp .

PCR product of *rpoB gene* was used for partial sequencing and blotting the phylogenetic tree of local isolates in comparison with some pathogenic standard world strains .Five local isolates were submitted to Gene Bank- NCBI for registration of sequences of *rpoB gene* .

The results of specificity ,sensitivity and accuracy values of the used methods in diagnosis revealed that the technique of PCR had a sensitivity 100%,specificity 30% and accuracy 44% in comparison with bacteria culture ,sensitivity 97.3%,specifity 33.3% and accuracy 82%when compared with RBT, at the last sensitivity 100%,specifity 66.6% and accuracy 92% in compared with ELISA test.

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Introduction:

Brucellosis is widespread zoonotic disease that afflicts both human and animals , it is prevalent in many regions of the world including Latin America ,the Mediterranean basin ,Middle east Asia and Africa(Young *et al*., 2014).According to WHO more than half a million new states of infection are reported in the different countries of the world (Wyatt, 2005).

Most species of *Brucella* can infect animals other than their preferred hosts ,when they get in close contact .*B.abortus* ,*Bmelitensis* ,*B.suis*, and *B.canis* are human pathogens, *B.melitensis* is the most virulent and causes the chronic and acute cases of brucellosis (Cutler *et al*., 2005).Since the clinical signs and symptoms of human brucellosis are different and non-specific diagnosis of brucellosis depended on appositive assessment in laboratory based testing(De Jong & Tsolis, 2012).

There are variety assays present for diagnosing *Brucella* in human and currently ,molecular ,serological and microbiological tests are popularly used to the goal (Hadush & Pal, 2013).Blood culture is a gold standard method for *Brucella* investigation but this method is time consuming ,elevate the risk of disease transmission to human and suffers from an acute case sensitivity of only 15 to70(Whatmore, 2009).Also it orders a high level of skill and safety parameters. Serological screening methods detection such as the Rose Bengal

commonly conducted in diagnostic laboratories (Padilla *et al*., 2010; Rock *et al*., 2016) It is well known that lipopolysaccharides display cross reaction with the Gram-negative bacteria (Fayaz et al., 2010; Goldman, 2016).ELISA was reported as the rapid and dependable diagnostic test for brucellosis(Memish *et al*., 2002).The ELISA test has the many advantage ,it can also detect the incomplete antibodies commonly observe in brucellosis

chronic phase(Araj, 2010).Molecular methods are also used for the determine of bacteria in culture, serum and blood samples. Real time- PCR has the ability to detect a very low level of bacteria in the sample and hence widely used as a tool for the diagnosis of infectious diseases(Goldman, 2016)).

The aim of present study was undertaken to evaluate the molecular tests classical PCR, Real -time PCR in comparative with the serological tests (Rose Bengal and enzyme linked immune- sorbent assay (ELISA) for diagnosis human brucellosis .

Methods:

-Samples collection and bacterial isolation:

To conduct the current work on antibody detection and molecular tests of *Brucella melitensis* detection in human , a total of 50 blood samples(5-7 ml.) were collected from human during November /2017 to April /2018 from different regions through Laboratory/ Samawah Gynecological and pediatrics Teaching hospital and private laboratories based on clinical symptoms for suspected cases which included body temperature ,headache ,sweating , arthritis ,bleeding and abdominal or back pain.Five ml of blood samples were withdrawn by disposable syringe under aseptic condition from each patient , placed in a sterile brain heart infusion broth bottle for incubation at temperature 37C for 4 weeks ,then the residues of blood sample(2 ml.) was separated by centrifugation at 3000 rpm for 5 minutes to make serological tests from apart of serum . Then stored by freezing (-20℃) until used. (Frangoulidis et al., 2003).

Sub culture of bacterial growth were grown on brain heart infusion broth on blood base agar, chocolate agar and *Brucella* agar, the rate of two dishes for

each sample from each these media then incubated the dishes at a temperature of 37 and left dishes for 2 to 3 days(MacFaddin, 1985).

-Identification of bacterial isolates

-Colonies characteristics:

For the purpose of identifying the isolated bacteria, colonies that grown in culture media were transferred to two glass slides, then stained with gram stain and examined under the oil lens to investigate the shape and arrangement of the bacteria. showing the rod bacilli of the negative to gram stain(Sam *et al*., 2012).

- Biochemical tests(API 20E system):-

This test was done according to the manufacturer's instructions (Biomerieux). The results was obtained with numerical profile and matched with analytical profile index (Elsaghir & James, 2003)

- Serological tests:-

1-Rose Bengal Test

Test Serum (0.03 ml) was mixed with an equal volume of antigen on a white tile or enamel plate to create a zone approximately 2 cm in diameter. The mixture was agitated mildly for four minutes at ambient temperature, and then observed for agglutination.Any visible reaction was reflected to be positive (Naureen *et al.*, 2007).

2- Enzyme Linked Immuno-Sorbent Assay(ELISA):

The Diagnostic Automation Inc. *Brucella* IgM Antibody ELISA Test Kit has been used for the the detection and the quantitative determination of

specific IgM antibodies against *Brucella* in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Diagnostic Automation by using Elisys Uno Human(Bricker, 2002) .

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Molecular test:-

-Genomic DNA Extraction

Genomic DNA was extracted from blood ,serum, broth culture samples by using Genomic DNA Mini Kit (Geneaid. USA) and done according to company instruction .

The extracted DNA was checked by using Nanodrop spectrophotometer for measurement the purity of DNA through reading the absorbance in at 260/280 mm.

-Amplification of*16S RNA gene* in *Brucella melitensis* using Real-Time PCR:-

Real Time PCR was performed for detection of *Brucella melitensis* from blood, serum and bacteria broth samples by using the specific primers and TaqMan probe specific for 16S ribosomal RNA gene specific for *Brucella melitensis* this technique was carried out according to method described by Sun et al.(2016) .

qPCR master mix was prepared by using **NEXpro™ qPCR Master Mix (Probe)**and this master mix done according to company instructions (table 1)

Table (1)q PCR Master Mix:

PCR Master mix	Volume

DNA template	5µL
16S ribosomal RNA gene (10pmol)	1µL
16S ribosomal RNA gene (10pmol)	1µL
16S ribosomal RNA gene probe (20pmol)	1 µL
qPCR master mix	10µL
PCR water	2 µL
Total volume	20µL

After that, these PCR master mix component that mentioned in table above transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in Real-time PCR Thermocycler (BioRad . USA).

Real-Time PCRthermocycler conditions was set according to primer annealing temperature and RT-PCR TaqMan kit instructions by Biorad Real-Time PCR thermocycler system (table 2).

Table (2): RT PCR thermocycler conditions

Step	Condition	Cycle
Pre-Denaturation	95 °C 5 min	1
Denaturation	95 °C 20 sec	45
Annealing/Extension	55 °C 30 sec	
Detection (Scan)		

qPCR data analysis was performed by calculation the threshold cycle number (CT value) that presented the positive amplification of *Brucella melitensis* in Real-Time PCR cycle number.

-Statistical Analysis:-

Sensitivity: This was measured by dividing true-positive test results over all patients with the disease. {=a/ (a+c)}.

Specificity: This was measured by dividing true- negative test results over all patients without the disease. {=d/ (b+d)}.

Negative predictive value (NPV):. This was measured by dividing true-negative test results over all negative test results. {=d/(c+d)}.

Positive predictive value (PPV): is measured by dividing true- positive test results over all positive test results. {=a/ (a+b)}.

Overall accuracy was measured by dividing true-positive + true negative test results over all tests. {(a+d)/ (a+b+c+d)}.

Where by:

a= true positive. c= false negative.

b= false positive d= true negative.

Results and Discussion:-

-Serological tests:

Rose –Bengal test was carried out on 50 samples of patients suspected of brucellosis, 45 (90%) of which were positive. This test was used as a screening and diagnostic test because it is an easy, fast and efficient test.(Abbas *et al*., 2012) The effectiveness of this test is specifically limited to the detection of immunoglobulin IgG1 and depend in the sensitivity to the immunoglobulin IgM and IgG also this test detects the infection in the early stages(Al-Ouqaili, 2006).

There are several studies indicated the approaching the percentage of brucellosis like(Abbas *et al*., 2012) who recorded cases for the brucellosis suspected samples were 58 Cases (58%) that positive for Rose Bengal test which in the acute phases from100 samples .(Agasthya *et al*., 2012) showed that found (15.69%) serum samples were positive out of 652 suspected case from veterinary personnel.(Al-Ouqaili, 2006) who examined 84 of tested samples he found 78(92.9%)patients with acute brucellosis and 80(82.5%)out of 97 patients with chronic brucellosis gave positive results for Rose Bengal test.(Al-Bayatti & Al-Thwani, 2009)found that 40 tested samples (80%)were positive out of 50 suspected cases in Baghdad city .

The above studies reveal differences in their results which can be clarified considering the next trails such as site of studies ,number of samples , study duration and RBT test is still the main stay of serological diagnosis of acute Brucellosis. So the Prozone phenomenon sometimes occur in this test. The immunoglobulin M(IgM) is major agglutinating antibody formed especially in first week , followed by IgG and IgA antibodies in chronic infection .All these antibodies are active in RBT test. Prozone phenomenon due to IgG and IgA can give false–negative. .

Also false–positive test due to immunological cross-reactivity have been associated with *Brucella* skin testing , *Cholera* vaccination , or infection with *Vibrio cholera, Francisella tularensis , Yersinia enterocolitica* duetomsimilar O-antigen side chain of lipopoly saccharide of *Brucella* with these microbes (Al-Bayatti & Al-Thwani, 2009; Al-Ouqaili, 2006).

An enzyme-linked immunosorbent assay (ELISA) designed at detecting anti *Brucella* IgM antibody was also used to test the 50 patient samples that had previously been examined by the Rose Bengal Test. Of the 45(90%) samples that had positive the Rose Bengal results, 42(84%) were also positive in the ELISA test. 42 serum samples were found to contain had IgM antibodies against *Brucella* antigen. The 5 serum samples that were negative with the Rose Bengal test were also negative in ELISA test. Results of this study showed that ELISA test was the best technique in diagnosis of acute infection of *Brucella* microorganism.

These results elucidate that the test was more sensitive than Rose Bengal test. Because the ELISA test was very sensitive and could as simply be made specific for antibodies(Agasthya *et al*., 2012). Investigation from other studies of patients with acute brucellosis showed that the ELISA was the most sensitive diagnosis test such (AL-Kha,2012), showed that found 150(100%) patients of acute infection with *Brucella* microorganism were positive. (Ahmed et al., 2010)showed serum samples a high seropositivity of 95 (43%) of the 221 positive samples positive for IgM.

(Alim *et al*., 2015) showed in *Brucella* ELISA test results, 96 (44.2%) IgM antibodies out of 217 cases. I- ELISA. This high seropositivity exclusively to I-ELISA could only be best ascribed in its nature being a primary binding assay which can detect 1/100 of the antibodies to those detected by secondary binding assay (Sharma, 2016).I-ELISA present study make it a highly sensitive test in brucellosis serology. Further, the above finding was supported by the work of (Sharma *et al*., 2017) who revealed that some culture Were negative while positive by I-ELISA.

-Isolation and identification of *Brucella* sp. :-

The results showed that of the total of 50 samples blood of the patients suspected of brucellosis, 10 (20%) samples were isolated a positive result of *Brucella* from the patient while 40(80%) samples were given negative result, all these samples were cultured on brain heart infusion broth through (48-72) hours and also perform sub culture in the week on blood agar ,chocolate agar, *Brucella* agar.

The results of culture on the blood agar, chocolate agar, *Brucella* agar showed after culturing during period (24-72) hour in 37C growth colonies of *Brucella* In the form of colonies yellow and yellow ovaries and rough with a convex appearance and is not shiny and dry and large size about 3 mm and irregular edges as in the following

forms. On the other hand, the results of the microscopic examination of the colonies after the staining of the Gram-stain showed the existence of a bacilli-rod bacteria that result in groups of parallel, negative, to, Gram-stain. They may appear in coccobacilli form, as well as single and chains, short chains or small clusters, and their axis is straight and the ends are rounded and the sides are parallel or convex

The results of isolation of *Brucella* bacteria indicated that the percentage of isolation in patients examined was different compared to other studies such as study(Yong *et al*., 2015) found A total of 41(100%) *Brucella* spp. isolates from blood culture of 41 patients were obtained from various parts of Malaysia .(Tabibnejad *et al*., 2016) showed a total of 100 patients with suspected brucellosis 39 cases (39%) had positive results when tested by the BACTEC system, and 61 cases (61%) became negative 23 culture. (Abbas *et al*., 2012) presented the rate isolation of brucellosis that (20 %).

These above differences may be related to the patients had been taken antibiotics before made the culture , to the different percentage of brucellosis habited according to the geographic region ,also to the technique used to the isolation bacteria(Bryan, 1989).

The biochemical tests were conducted on the bacteria that grew on the selective medium, since the isolated *Brucella* was positive for catalase and oxides and produced a H2S gas. These results are compatible with what was indicated(Scholz *et al*., 2008).

-Molecular tests :-

-Real-Time PCR:-

Real-time PCR assay targeting the 16S rRNA gene detected infection in 38 (76%) from 50 patients blood samples. Serological tests showed different results RBT90%, I-ELISA 84%and only 10% of blood samples were positive with all three tests, which makes interpretation of the serological results very complicated(Fig.1).

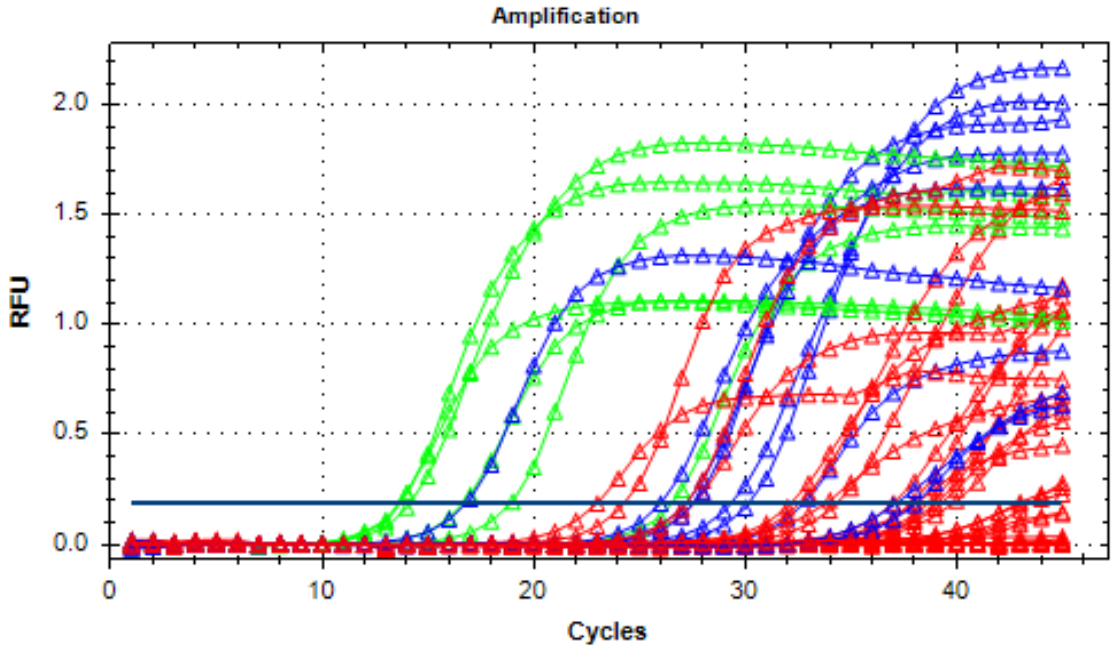

Figure (1) : Real-Time PCR Amplification plot of 16SrRNA gene that used in detection *Brucella melletansis*. Where, Red plot from blood samples, Blue plot from serum samples, and Green plot from culture isolates samples.

Mustafa *et al*.(2017) found the results of real- time PCR assay with *16SrRNA* genes of isolates from patients belonged to genus *Brucella* and species *B. melitensis*. (Pelerito *et al*., 2017)examined 259 samples were tested by real –time PCR assay43 (16.6%) were positive for *Brucella* spp., being *B. melitensis* the only species detected in the analyzed cases As described, the real-time PCR assay will allow the confirmation of bacterial isolates as *Brucella* spp., or *B. melitensis* within 2 to 3 h. The inclusion of a genus specific primers-probe set assists in the recognition of infrequently isolated *Brucella* species and the identification of atypical *Brucella* strains.

Conventional methods for *Brucella* isolation and detection may take days to weeks to perform and often need the preparation of heavy suspensions of these highly infectious pathogens. Our laboratory performs Gram stain, oxidase, and catalase testing as a primary screening test for suspected *Brucella* isolates. If a slow-growing, gram-negative coccobacillus is observed, the isolate is then tested by real-time PCR method described here uses heat to inactivate the micro-organisms and greatly reduces the hazard of laboratory-acquired infection with *Brucella*. Finally, the multiplex format of the technique will reduce reagent cost and staff time required to perform testing for brucellosis.

Results obtained were compared the diagnosis of brucellosis in human in four different tests bacteria isolation, serological tests RBT and ELISA ,and real-time PCR ,the seven blood samples for which the result was obtained with RBT and four samples in ELISA , inhibited in the real-time PCR ,while twenty three samples wasn't detection in culture table (3)

Table(3) Occurrence of brucellosis in human by RBT,ELISA, culture and PCR tests.

Technique	No. tested	Patients			
		Sample- positive		Sample-negative	
		No.	%	No.	%
RBT	50	45	90	5	10
ELISA	50	42	84	8	16
Culturing	50	10	20	40	80
PCR	50	38	76	12	24

The table(4) shows the sensitivity, specificity and accuracy of the methods used (culture and RBT) in the diagnosis of brucellosis in humans. Of the 50 samples examined by the test of the Rose Bengal test and culturing of bacteria were 10 positive samples of the RBT and the culture and 35 samples of the positive in the RBT and the negative in the culture, the sensitivity , specificity and accuracy (100%) (12.5)%, (0.3%) respectively

Table (4): Sensitivity and specificity of techniques that used (Culture and RBT) in diagnosis of *Brucella melitensis*

Test *RBT*	*Culture*		Total	Sensitivity %	Specificity %	Predictive value %		Accuracy rate %
	Positive	Negative				Positive	Negative	
Positive	10 T.P	35 F.P	45	100%	12.5%	22.2%	100%	0.3%
Negative	0 F.N	5 T.N	5					
Total	10	40	5o					

* T.P= True positive ** F.P = False

*** T.N= True negative **** F.N = False negative

Table(5) shows the sensitivity, specificity and accuracy of the methods used (culture and ELISA) in the diagnosis of brucellosis in humans. Of the 50 samples examined by the test of the Enzyme Linked Immuno Sorbent Assay and culturing of bacteria were 10 positive samples of the ELISA and the culture and 32 samples of the positive in the ELISA and the negative in the culture, the sensitivity , specificity and accuracy (100%) (20)%, (36%) respectively

Table (5): Sensitivity and specificity of techniques that used (Culture and ELISA) in diagnosis of *Brucella melitensis*

Test	*Culture*		Total	Sensitivity %	Specificity %	Predictive value %		Accuracy rate %
ELISA	Positive	Negative				Positive	Negative	
Positive	10 T.P	32 F.P	42	100%	20%	23.8%	100%	36%
Negative	0 F.N	8 T.N	8					
Total	10	40	5o					

* T.P= True positive ** F.P = False positive

*** T.N= True negative **** F.N = False negative

The table(6) shows the sensitivity, specificity and accuracy of the methods used (culture and PCR) in the diagnosis of brucellosis in humans. Of the 50 samples examined by the test of the Polymerase Chain Reaction and culturing of bacteria were 10 positive samples of the PCR and the culture and 28 samples of the positive in the PCR and the negative in the culture, the sensitivity , specificity and accuracy (100%) (30)%, (44%) respectively.

Table(6): Sensitivity and specificity of techniques that used (Culture and PCR) in diagnosis of *Brucella melitensis*

Test / *PCR*	*Culture*		Total	Sensitivity %	Specificity %	Predictive value %		Accuracy rate %
	Positive	Negative				Positive	Negative	
Positive	10 T.P	28 F.P	38	100%	30%	26.31%	100%	44%
Negative	0 F.N	12 T.N	12					
Total	10	40	5o					

* T.P= True positive ** F.P = False positive

*** T.N= True negative **** F.N = False negative

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Table(7) shows the sensitivity, specificity and accuracy of the methods used (RBT and ELISA) in the diagnosis of brucellosis in humans. Of the 50 samples examined by these tests were 42 positive samples of the ELISA and the RBT and 3 samples of the positive in the RBT and the negative in the ELISA, the sensitivity , specificity and accuracy (100%) (62.5)%, (94%) respectively.

Table(7): Sensitivity and specificity of techniques that used (RBT and ELISA) in diagnosis of *Brucella melitensis*

Test	*ELISA*		Total	Sensitivity %	Specificity %	Predictive value %		Accuracy rate %
RBT	Positive	Negative				Positive	Negative	
Positive	42 T.P	3 F.P	45	100%	62.5%	93.3%	100%	94%
Negative	0 F.N	5 T.N	5					
Total	42	8	5o					

* T.P= True positive ** F.P = False positive

*** T.N= True negative **** F.N = False negative

Table(8) shows the sensitivity, specificity and accuracy of the methods used (RBT and PCR) in the diagnosis of brucellosis in humans. Of the 50 samples examined by these tests were 37 positive samples of the PCR and the RBT and 8 samples of the positive in the RBT and the negative in the PCR, while, one sample was positive in the PCR but negative in the RBT, the sensitivity , specificity and accuracy (97.3%) (33.3)%, (82%) respectively

Table(8): Sensitivity and specificity of techniques that used (RBT and PCR) in diagnosis of *Brucella melitensis*

Test RBT	*PCR* Positive	*PCR* Negative	Total	Sensitivity %	Specificity %	Predictive value % Positive	Predictive value % Negative	Accuracy rate %
Positive	37 T.P	8 F.P	45	97.3%	33.3%	82.2%	80%	82%
Negative	1 F.N	4 T.N	5					
Total	38	12	5o					

* T.P= True positive ** F.P = False positive

*** T.N= True negative **** F.N = False negative

Table (9) shows the sensitivity, specificity and accuracy of the methods used (ELISA and PCR) in the diagnosis of brucellosis in humans. Of the 50 samples examined by these tests were 38 positive samples of the PCR and the ELISA and 4 samples of the positive in the ELISA and the negative in the PCR, the sensitivity , specificity and accuracy (100%) (66.6)%, (92%) respectively

Table (9): Sensitivity and specificity of techniques that used (ELISA and PCR) in diagnosis of *Brucella melitensis*

Test	*PCR*		Total	Sensitivity %	Specificity %	Predictive value %		Accuracy rate %
ELISA	Positive	Negative				Positive	Negative	
Positive	38 T.P	4 F.P	42	100%	66.6%	90.4%	100%	92%
Negative	0 F.N	8 T.N	8					
Total	38	12	5o					

* T.P= True positive ** F.P = False positive

*** T.N= True negative **** F.N = False negative

The serological tests are believed a good laboratory test which it is used as screening test because it is high sensitivity and accuracy . The sensitivity and specificity percent of the PCR compared with RBT and ELISA techniques in human is significant (Authority, 2009) found higher sensitivity and specificity of the PCR than the serological methods to the diagnosis of brucellosis Among the 50 patients whose nested PCR assays were initially positive, 43 (86%) were negative 6 months after completing treatment. Relapse founded in five (10%) patients within 6 months after treatment and all were PCR positive. PCR and indirect-ELISA give a significant advantage over conventional serological techniques in the diagnosis of brucellosis in endemic geographical area. The PCR test results can be particularly significant in human with clinical symptoms

and signs, and negative serological tests, permitting the rapid confirmation of the brucellosis(Corbel, 2006).

Brucella have slow growth time, and the culture result are not obtainable for several days or weeks. The number of bacteria in clinical samples may vary broadly, with the isolation of *Brucella* being highly dependent on the phase of disease (acute vs. chronic), antibiotic pretreatment, the existence of an suitable clinical specimen and the culturing methods used(Corbel & Banai, 2005). Recently, lysis centrifugation method and automated blood culture systems enhanced the speed of finding but are still too slow to create a rapid diagnosis, (Millar *et al*., 2007) polymerase chain reaction (PCR) is fast and can be performed on any clinical sample(Dreier *et al*., 2007).

Although PCR is very hopeful, standardization of extraction methods, infrastructure, equipment and expertise are absent, and a better understanding of the clinical significance of the results is still wanted , PCR-based laboratory tests have been projected, they cannot be considered a routine diagnostic technique yet. These limitations make serology for antibody recognition the most useful instrument for the laboratory diagnosis of brucellosis(Constance, 2010).

Serological tests are used for the first diagnosis of human brucellosis as well as throughout treatment follow-up. In our study, RBPT had a sensitivity and specificity of compared to ELISA. The RBPT can be used as a screening test in endemic region especially in rural population. But one should remember its presentation is poor in patients formerly and/or repeatedly showing to the agent(Authority, 2009). In high-risk populations, testing of diluted sera using the RBT might be a sensible

alternative, as this would reduce the need for a significant number of confirmatory tests(Al Dahouk *et al*.,2013).

Laboratories can use ELISA as it is the most sensitive test. Though RBT is cheaper and easier test turnaround time is longer. In chronic and acute cases, ELISA is more domino effect in acute cases. The detection of the IgG antibody class by ELISA is more sensitive than IgM detection(Al Dahouk & Nöckler, 2011).

ELISA techniques are low cost, need less time to complete and less preparation for interpretation compared with RBT(Nielsen, 1998). These advantages give explanation their widespread use in recent years. However the sensitivity and specificity of ELISA for recognition of antibodies against *Brucella* spp. differ among studies. (Gomez *et al*., 2013) give a sensitivity of 60% for IgM and 84% for IgG, while the combined specificity for IgG and IgM was 100% .However (Mantur *et al*., 2010) found a combined IgG and IgM ELISA sensitivity of 100% but joint specificity of 71.3% . (Welch & Litwin, 2010)reported a 92.3% combined sensitivity and a combined specificity of 55%.

Mitka *et al*.(2007) described that PCR is a very useful apparatus not only for the diagnosis of acute brucellosis, but also as a predictive indicator for the course of the disease and the post treatment follow-up, which is valuable for the early determining of relapses. In the present study, there was statistically insignificant difference as regards results of PCR in relation to ELISA and this agrees with (O'Leary *et al*., 2006)who recommended that there was advantage in using PCR methods over standard serological and bacteriological methods in the detection of *B. melitensis* ,samples may take from whole blood or lymph nodes.

However, molecular techniques have been shown to be more suitable, accurate, highly sensitive, rapid, and simple to need small sample volumes to reduce the risks of handling tissues and more specific for the diagnosis of brucellosis from whole blood samples or from sera (Bounaadja *et al*., 2009).

References:

- Abbas, B. A., Mohammed, H. Q., & Tofah, J. A. (2012). Diagnostic Study on Brucella melitensis isolated from Human in Wassit province. *Al-Mustansiriyah Journal for Pharmaceutical Sciences, 12*(2), 8-24.
- Agasthya, A. S., Isloor, S., & Krishnamsetty, P. (2012). Seroprevalence study of human brucellosis by conventional tests and indigenous indirect enzyme-linked immunosorbent assay. *The Scientific World Journal, 2012*.
- Al-Bayatti, S., & Al-Thwani, A. (2009). DNA polymorphism investigation among local Brucella isolates. *Iraqi Journal of Veterinary Sciences, 23*.
- Al-Ouqaili, M. (2006). *Molecular, bacteriological and immunological aspects in the diagnosis of human brucellosis.* Ph. D. thesis in medical microbiology. College of Med. Univ. of Baghdad,
- Al Dahouk, S., & Nöckler, K. (2011). Implications of laboratory diagnosis on brucellosis therapy. *Expert review of anti-infective therapy, 9*(7), 833-845.
- Al Dahouk, S., Sprague, L., & Neubauer, H. (2013). New developments in the diagnostic procedures for zoonotic brucellosis in humans. *Rev Sci Tech, 32*(1), 177-188.

- AL-Kha,J.K. (2012). Efficacy of immunological tests in diagnosis of acute human brucellosis . thesis in medical microbiology. College of Med. Univ. of Babylon,
- Alim, A., Oguzkaya-Artan, M., & Artan, C. (2015). The seroprevalence of brucellosis among undiagnosed family members of brucellosis positive patients. *Nigerian journal of clinical practice*, *18*(5), 620-625.
- Araj, G. F. (2010). Update on laboratory diagnosis of human brucellosis. *International journal of antimicrobial agents*, *36*, S12-S17.
- Authority, E. F. S. (2009). Porcine brucellosis (Brucella suis). *EFSA Journal*, *7*(6), 1144.
- Bounaadja, L., Albert, D., Chénais, B., Hénault, S., Zygmunt, M. S., Poliak, S., & Garin-Bastuji, B. (2009). Real-time PCR for identification of Brucella spp.: a comparative study of IS711, bcsp31 and per target genes. *Veterinary microbiology*, *137*(1-2), 156-164.
- Bricker, B. J. (2002). Diagnostic strategies used for the identification of Brucella. *Veterinary microbiology*, *90*(1-4), 433-434.
- Bryan, C. S. (1989). Clinical implications of positive blood cultures. *Clinical microbiology reviews*, *2*(4), 329-353.
- Constance, J. A. (2010). The future of molecular diagnostics. *Innovative technologies driving market opportunities in personalized medicine*.
- Corbel, M., & Banai, M. (2005). Brucella Meyer and Shaw 1920, 173 AL. *Bergey's Manual® of Systematic Bacteriology*, 370-386.
- Corbel, M. J. (2006). *Brucellosis in humans and animals*: World Health Organization.
- De Jong, M. F., & Tsolis, R. M. (2012). Brucellosis and type IV secretion. *Future microbiology*, *7*(1), 47-58.
- Dreier, J., Störmer, M., & Kleesiek, K. (2007). Real-time polymerase chain reaction in transfusion medicine: applications for detection of

bacterial contamination in blood products. *Transfusion medicine reviews*, *21*(3), 237-254.

- Elsaghir, A. A., & James, E. A. (2003). Misidentification of Brucella melitensis as Ochrobactrum anthropi by API 20NE. *Journal of medical microbiology*, *52*(5), 441-442.
- Fayaz, A. M., Balaji, K., Girilal, M., Yadav, R., Kalaichelvan, P. T., & Venketesan, R. (2010). Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. *Nanomedicine: Nanotechnology, Biology and Medicine*, *6*(1), 103-109.
- Foulongne, V., Bourg, G., Cazevieille, C., Michaux-Charachon, S., & O'Callaghan, D. (2000). Identification of Brucella suis genes affecting intracellular survival in an in vitro human macrophage infection model by signature-tagged transposon mutagenesis. *Infection and immunity*, *68*(3), 1297-1303.
- Goldman, L. (2016). Schafer A. Goldman-Cecil Medicine. In: Philadelphia: Elsevier.
- Gomez, G., Pei, J., Mwangi, W., Adams, L. G., Rice-Ficht, A., & Ficht, T. A. (2013). Immunogenic and invasive properties of Brucella melitensis 16M outer membrane protein vaccine candidates identified via a reverse vaccinology approach. *PLoS One*, *8*(3), e59751.
- Hadush, A., & Pal, M. (2013). Brucellosis-An infectious re-emerging bacterial zoonosis of global importance. *Int J Livest Res*, *3*(1), 28-34.
- MacFaddin, J. (1985). Mannitol salt agar (MSA). *Media for isolation-cultivation-identification-maintenance of medical bacteria. Baltimore: Williams & Wilkins*, 483-486.
- Mantur, B., Parande, A., Amarnath, S., Patil, G., Walvekar, R., Desai, A., Patil, S. (2010). ELISA versus conventional methods of diagnosing

endemic brucellosis. *The American journal of tropical medicine and hygiene*, *83*(2), 314-318.

- Millar, B. C., Xu, J., & Moore, J. E. (2007). Molecular diagnostics of medically important bacterial infections. *Molecular Diagnostics: Current Technology and Applications*, *9*(1), 21-39.
- Mirabella, A., Terwagne, M Mirabella, A., Terwagne, M., Zygmunt, M., Cloeckaert, A., De Bolle, X., & Letesson, J. (2012). Brucella melitensis MucR, an orthologue of Sinorhizobium meliloti MucR, is involved in resistance to oxidative, detergent and saline stresses and cell envelope modifications. *Journal of bacteriology*, JB. 01336-01312.
- Mitka, S., Anetakis, C., Souliou, E., Diza, E., & Kansouzidou, A. (2007). Evaluation of different PCR assays for early detection of acute and relapsing brucellosis in humans in comparison with conventional methods. *Journal of clinical microbiology, 45*(4), 1211-1218.
- Mustafa, A. S., Habibi, N., Osman, A., Shaheed, F., & Khan, M. W. (2017). Species identification and molecular typing of human Brucella isolates from Kuwait. *PLoS One*, *12*(8), e0182111.
- Naureen, A., Saqib, M., Muhammad, G., Hussain, M. H., & Asi, M. N. (2007). Comparative evaluation of Rose Bengal plate agglutination test, mallein test, and some conventional serological tests for diagnosis of equine glanders. *Journal of veterinary diagnostic investigation, 19*(4), 362-367.
- Nielsen, K. (1998). Brucellosis: Development and success using ELISAs for diagnosis. *Toward livestock diseases diagnosis and control in the 21st century*, 35-45.
- O'Leary, S., Sheahan, M., & Sweeney, T. (2006). Brucella abortus detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. *Research in veterinary science, 81*(2), 170-176.

- Pelerito, A., Cordeiro, R., Matos, R., Santos, M. A., Soeiro, S., Santos, J., . . . Paixão, E. (2017). Human brucellosis in Portugal—Retrospective analysis of suspected clinical cases of infection from 2009 to 2016. *PLoS One, 12*(7), e0179667.
- Rock, K. T., Mugizi, D. R., Ståhl, K., Magnusson, U., & Boqvist, S. (2016). The milk delivery chain and presence of Brucella spp. antibodies in bulk milk in Uganda. *Tropical animal health and production, 48*(5), 985-994.
- Scholz, H. C., Hubalek, Z., Sedláček, I., Vergnaud, G., Tomaso, H., Al Dahouk, S., . . . Cloeckaert, A. (2008). Brucella microti sp. nov., isolated from the common vole Microtus arvalis. *International Journal of Systematic and Evolutionary Microbiology, 58*(2), 375-382.
- Sharma, V. (2016). *SERO-PREVALENCE STUDIES OF BRUCELLOSIS AMONG GOATS AND HUMANS USING DIFFERENT SEROLOGICAL TESTS.* Division of Veterinary Public Health and Epidemiology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu,
- Sharma, V., Sharma, H., Ganguly, S., Berian, S., & Malik, M. (2017). Seroprevalence Studies of Brucellosis among Human using Different Serological Tests. *Int. J. Curr. Microbiol. App. Sci, 6*(5), 1793-1800.
- Sun, M.-J., Di, D.-D., Li, Y., Zhang, Z.-C., Yan, H., Tian, L.-L., . . . Fan, W.-X. (2016). Genotyping of Brucella melitensis and Brucella abortus strains currently circulating in Xinjiang, China. *Infection, Genetics and Evolution, 44*, 522-529.
- Tabibnejad, M., Alikhani, M. Y., Arjomandzadegan, M., Hashemi, S. H., & Naseri, Z. (2016). The optimization of molecular detection of clinical isolates of brucella in blood cultures by eryD transcriptase gene

for confirmation of culture-negative samples. *Iranian Red Crescent Medical Journal*, *18*(4).

- Whatmore, A. M. (2009). Current understanding of the genetic diversity of Brucella, an expanding genus of zoonotic pathogens. *Infection, Genetics and Evolution*, *9*(6), 1168-1184.
- Wyatt, H. (2005). How Themistocles Zammit found Malta Fever (brucellosis) to be transmitted by the milk of goats. *Journal of the Royal Society of Medicine*, *98*(10), 451-454.
- Yong, T. B., Hashim, R., Noor, A. M., Hamzah, S. H., & Ahmad, N. (2015). Identification of Brucella spp. isolated from human brucellosis in Malaysia using high-resolution melt (HRM) analysis. *Diagnostic microbiology and infectious disease*, *81*(4), 227-233.
- Young, E. J., Roushan, M. R. H., Shafae, S., Genta, R. M., & Taylor, S. L. (2014). Liver histology of acute brucellosis caused by Brucella melitensis. *Human pathology*, *45*(10), 2023-202.
- Zeder, M. A., & Hesse, B. (2000). The initial domestication of goats (Capra hircus) in the Zagros Mountains 10,000 years ago. *Science*, *287*(5461), 2254-2257.