**Assessment of some serological tests for the diagnosis of Acute human *Brucellosis*Saleem O.G.Al-Mawla (1), Rana H .H .Al-ubaid (2),Maisam Kh.Al-Anee (3), & Sahar  
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(4) Dept Of Basic Sciences /College Of Dentistry / Al- Anbar University. (2), (3)- Iraq****ABSTRACT***Brucellosis is a public health problem in many developing countries including Iraq. The most reliable  
diagnosis of an infectious disease is confirmed by isolation of its pathogen by culture ,but It is well  
known that culture for *Brucella* is relatively difficult, time consuming and very hazardous for  
laboratory workers therefore it is reasonable to use serological tests for routine diagnosis of this  
important disease in human patients . In order to choose and evaluate the most applicable test in term  
of speed of performance, and accuracy , three serological tests were assayed:  
Rose Bengal plate test (RBPT) , Modified Rose Bengal with 2ME ,and Standard tube agglutination test  
(STAT) for Brucellosis. The fourth test assayed was *anti brucella- IgM* by Enzyme-linked  
immunossorbant assay (ELISA) which was used as a referent test to compare the results of the above  
tests with it for its high specificity and sensitivity.  
Blood samples were obtained from 210 patients who were highly suspected of having Brucellosis .  
The above 4 serological tests were performed on all serum samples . Out of the total samples ,  
118(56.2%) gave positive results by Roes Bengal plate test (RBPT), 58(27.6%) by 2ME , 130  
(61.9%) by STAT ,and 70(33.3%) by Elisa. All negative cases by RBPT gave negative results by  
2ME. Twelve samples of negative results by RBPT and 2ME were found to be positive by STAT .  
The sensitivity of RBPT ,2ME ,and STAT were found to be 100%,82%,and 100% respectively ,while  
the specificity of these three serological tests were found to be 74%,82%,and 97% respectively as  
compared with the results of *anti Brucella –IgM* by ELISA .  
***Key words:*** Brucellosis , Serodiagnosis , Rose Bengal , ELISA  
**الملخص باللغة العربية**تعد حمى مالطا من المشاكل الصحية المهمة في الدول النامية بضمنها العراق. إن من أفضل الطرق لتشخيص المسببات المرضية  
البكتيرية بشكل عام هو الزرع البكتيري ولكن من المعروف ان استنبات الجرثومة المسببه لحمى مالطا يتميز بصعوبة نسبية إضافة  
إلى انه يستغرق وقتا طويلا كما أن فيه خطورة عالية على العاملين في المختبر وعليه فقد صممت الدراسة الحالية بهدف تقييم  
فاعلية ثلاث طرق مصلية لتشخيص المرض هي الفحص المباشر للمصل باستعمال طريقة Rose Bengalوالطريقة المحورة  
بإضافة 2MEوتخفيف الأنابيب القياسي STATوإجراء فحص الأجسام المضادة النوعية *anti Brucella –IgM*بطريقة الاليزا  
لغرض مقارنة النتائج به وتقييم مدى الاستفادة من هذه الفحوص لتشخيص المرض دون الرجوع إلى الزرع . تم جمع نماذج دم من  
210مريض يشتبه بإصابتهم بحمى مالطا وأجريت الفحوص المصلية الأربعة على كافة نماذج مصل الدم . بينت الدراسة الحالية  
أن من بين المجموع الكلي للعينات , 118عينة أعطت نتائج ايجابية باستخدام Rose Bengalو 58بطريقة 2MEو 62عينة  
بطريقة تقنية الاليزا،اما بالنسبة الى فحص STATفقد اعطت 12حالة نتيجة موجبة رغم انها كانت سالبة في الفحصين السابقين  
مما يعطي هذا الفحص أهميته . تبين ان حساسية الفحوص الثلاثة RBPTو 2MEو %100 STATو 82و% %100  
على التوالي ونوعيتها %74و %82و %97على التوالي بالمقارنة مع نتائج الاليزا .  
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***INTRODUCTION***Brucellosis is a chronic infection in animals  
mainly localized in reproductive organs (male  
and female),and are shed in in milk and urine.  
Transmission to humans usually occurs either  
through direct contact with infected animal  
tissues or ingestion of unpasteurized milk or  
milk products. Human to human transmission is  
rare (1,2).  
Human brucellosis is an infectious disease of  
worldwide importance .Due to the wide spectrum  
of manifestations of this disease, its diagnosis  
cannot be made solely on clinical grounds and it  
always essential to perform bacteriological and  
serological tests (3).  
This disease is characterized by acute ,chronic ,  
sub acute , localized and relapsing arthralagia  
and the main clinical features are undulating  
fever ,headache ,night sweats ,fatigue and  
anorexia (4).  
The diagnosis of brucellosis is based on clinical  
features and the results of laboratory tests  
(5).The common serological test used for the  
diagnosis to brucellosis is Rose Bengal Plate test  
(RBPT) based on agglutination of colored  
particulate antigen (Killed Brucella organisms )  
by the antibodies present in the patient’s serum  
.Although it is a simple ,cheap and effective test  
, the RBPT is generally considered to be less  
sensitive than other tests like standard tube  
agglutination test (STAT),complement fixation  
test (CFT)and enzyme linked immunosorbant  
assay ( Elisa).Elisa has been claimed to be a  
good screening test whether used alone or in  
combination with the (RBPT) (6). the standard  
tube *Brucella* agglutination test (STAT) except  
for the addition of 2ME to a final concentration  
of (0.05 M) in each agglutination tube .The 2ME  
disrupt disulfide bonds ,making immunoglobulin  
(IgM) inactive and permitting only *Brucella*agglutination by immunoglobulin IgM  
agglutinating antibodies that are resistant to 2ME  
(7).  
The aim of the present study was to assess the  
validity of 3 serological methods (RBPT ,2ME ,  
and STAT ) in the diagnosis of acute human  
brucellosis in comparison to the results of *antiBrucella – IgM* by ELISA .  
***MATERIALS AND METHODS***Blood samples were obtained from 210 patients  
who were highly suspected of having Brucellosis  
.Both genders were included .Ages of patients  
ranged from 10 to 65 years .All blood samples  
were centrifuged to get serum , and all serum  
samples were subjected to 4 serological  
diagnostic methods including Rose Bengal Plate  
Test ( RBPT),2-Mercaptoethanol test (2ME)  
,Standard Tube agglutination Test (STAT),and  
*Anti-Brucella-IgM* by Elisa . Tests were done as  
described by (8-10).  
Standard Tube Agglutination was performed as  
described by (11) as fellows :  
A series of 10 test tubes were placed in a rack,  
and then 0.9ml saline was delivered in the first  
test tube and 0.5ml in each of the remaining test  
tube. After that, 0.1ml of the tested serum was  
added to the first test tube .After mixing ,0.5ml  
of the diluted serum was transferred to the  
second test tube . Then 0.5ml diluted serum was  
transferred from the second test tube to the third  
test tube, and so on until the contents of tube 10  
were mixed, from which 0.5ml diluted serum was  
discarded. The resulting dilutions in the 10 test  
tubes ranged from 1:10 in tube no. 1 to 1:5120 in  
tube no 10 .As an antigen control another tube  
was added to the series containing 0.5ml saline  
.Then 0.5ml *B.abortus* antigen diluted 1:50 in  
saline , was added to each tube to make a final  
dilution varying from 1:20 to 1:10240 . After  
shaking the rack well , it was placed in a 37˚C  
water bath for 48 hours .The same procedure  
was repeated at the same time for positive and  
negative controls. Sensitivity , specificity , and  
accuracy of each test were calculated as follows :  
sensitivity = no. of True positive/(no. of true  
positive + no. of false negative )  
specificity = no. of true negative /(no. of false  
positive +no. of true negative ).  
***RESULTS***The total number of patients enrolled in the study  
was 210 . The ratio of males to females was  
58/60. Ages of patient ranged from 10 – 65 years  
.The ratio of patients from urban to rural area  
was 44/74.Of the total serum samples ,118  
samples gave positive results by RBPT as shown  
in Table 1.  
Out of 118 positive cases with different titers of  
RBPT , only 58(49.1%) were found to be  
positive by 2Me and the remaining 50.9% gave  
negative results ( Table 2).  
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**Table (1) :Distribution of positive results by RBPT according to gender , residency and age groups  
Table (2) : Results of 2 ME in comparison to RBPT titers**

|  |  |  |  |
| --- | --- | --- | --- |
| **RBPT titer** | **Total** | **Positive by 2 ME No(%)** | **Negative by 2 ME No(%)** |
| 1/320 | 37 | 27(73) | 10(27) |
| 1/160 | 24 | 18(75) | 6(25) |
| 1/80 | 57 | 13(22.8) | 44(77.2) |
| Total positive | 118 | 58(49.1) | 60(50.9) |
| Negative | 92 | 0(0) | 92(100) |

Tube agglutination tests were standardized into 9  
dilutions starting in 1/20 to 1/5120 as shown in  
Table (3) .All positive cases by RBPT gave  
positive results by STAT in different titers in  
addition to 12 cases that were negative by RBPT  
were found to be positive by STAT.  
Among the total positive cases by RBPT  
62(52.5%) gave positive results by Elisa while  
all negative cases by RBPT were found to be  
also negative by Elisa( Table 4 ).  
All positive cases by 2ME gave positive results  
by ELISA while 3 of negative cases gave  
positive results by ELISA . Among 130 positive  
cases by STAT , only 70(53.8%) were found to  
be positive by ELISA while all negative cases by  
STAT were found to be also negative by ELISA  
(Table 5 ).  
The validity of the above three serological tests  
in comparison with Anti Brucella – IgM was  
calculated as %sensitivity, %specificity , and  
%accuracy as shown in table 6 .  
***DISCUSSION***Brucellosis has a worldwide distribution and  
remains a major problem in humans and animals  
in Middle Eastern and Mediterranean countries ,  
where the prevalence is high (12).  
Isolation and identification of the causative  
agents remains the gold standard in the  
diagnosis of infectious diseases , however , the  
isolation of *Brucella* bacteria from blood is  
difficult and time consuming and the rate of  
success range from 47- 94% depending on the  
method used for cultivation and period of  
incubation (13,14). Furthermore , the clinical  
diagnosis of Brucellosis is difficult because the  
disease affect many organs and the symptoms  
may be non- specific (15).Particular problems  
for the final diagnosis are inadequate data on  
case history, course of the disease, chronic  
period, infections caused by microorganisms  
which are alike in terms of antigens, and also  
eventual treatment with antibiotics. Due to a  
number of subjective and objective problems  
which are the result of pathogen isolation, which  
is not even possible in the most of the cases, it  
takes a lot of time to isolate the pathogens even  
when some of the modern microbiologic methods  
are applied. If all of this is taken into  
consideration, immunological methods in  
brucellosis diagnosis are obligatory with a good  
reason (16).  
IgM antibodies are present in acute brucellosis  
and it potentially aid in the diagnosis of the  
disease (17) .In the present study , we evaluated  
the diagnostic value of three serological tests to  
be compared to the results obtained by *antiBrucella – IgM* by ELISA for its high sensitivity  
, specificity , and accuracy in the diagnosis of  
acute Brucellosis. Many investigators proved that  
ELISA is the most sensitive and specific test  
serological test in the diagnosis of acute  
brucellosis and it can be used as a referent test  
(18-20).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Age groups  in years** | **RBPT titer** | **No. of patients** | **Gender Male/female** | **Residency Urban/rural** |
| **1/320 No(%)** | **1/160 No(%)** | **1/80 No(%)** |  |  |
| 10-20 | 27 | 12/15 | 8/19 | 12(44.5) | 5(18.5) | 10(37) |
| 21-30 | 26 | 13/13 | 10/16 | 8(30.8) | 6(23) | 12(46.2) |
| 31-40 | 33 | 17/16 | 14/19 | 11(33.4) | 8(24.2) | 14(42.4) |
| 41-50 | 19 | 7/12 | 6/13 | 3(15.8) | 4(21) | 12(63.2) |
| >50 | 13 | 9/4 | 6/7 | 3(23) | 1(7.7) | 9(69.2) |
| Total | 118 | 58/60 | 44/74 | 37(31.4) | 24(20.3) | 57(48.3) |

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**Table (3) : Results of STAT titers in comparison to RBPT titers**

|  |  |  |
| --- | --- | --- |
| **RBPT** | **Total** | **STST titer** |
| **1/20 No(%)** | **1/40 No(%)** | **1/80 No(%)** | **1/160 No(%)** | **1/320 No(%)** | **1/640 No(%)** | **1/1280 No(%)** | **1/2650 No(%)** | **1/5120 No(%)** |
| 1/320 | 37 | 21(56.4) | 13(35.1) | 1(2.7) | 2(5.4) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| 1/160 | 24 | 8(33.3) | 7(29.2) | 5(20.8) | 2(8.3) | 0(0) | 2(8.3) | 0(0) | 0(0) | 0(0) |
| 1/80 | 57 | 5(8.8) | 13(22.8) | 10(17.5) | 11(19.3) | 10(17.5) | 8(14) | 0(0) | 0(0) | 0(0) |
| Total positive | 118 | 34(28.8) | 33(27.9) | 16(13.5) | 15(12.7) | 10(8.5) | 10(8.5) | 0(0) | 0(0) | 0(0) |
| negative | 92 | 0(0) | 1(1) | 2(2.2) | 2(2.2) | 1(1) | 2(2.2) | 4(4.3) | 0(0) | 0(0) |

**Table (4) : Results of Anti-Brucella-IgM by ELISA according to RBPT titers**

|  |  |  |  |
| --- | --- | --- | --- |
| **RBPT titer** | **Total** | **Positive by ELISA No(%)** | **Negative by ELISA No(%)** |
| 1/320 | 37 | 31(87.8) | 6(12.2) |
| 1/160 | 24 | 19(79.2) | 5(20.8) |
| 1/80 | 57 | 12(21) | 45(79) |
| Total positive | 118 | 62(52.5) | 56(47.5) |
| Negative | 92 | 0(0) | 92(100) |

**Table (5) : Results of 2ME and STAT as compared to Anti-Brucella-IgM by ELISA**

|  |  |  |  |
| --- | --- | --- | --- |
| **Test** | **Total** | **Positive by ELISA No(%)** | **Negative by ELISA No(%)** |
| Positive by 2ME | 58 | 58(100) | 0(0) |
| Negative by 2ME | 152 | 3(2) | 149(98) |
| Positive by STST | 130 | 70(53.8) | 60(46.2) |
| Negative by STAT | 80 | 0(0) | 80(100) |

**Table (6) : validity of RBPT , 2ME , and STAT as compared to Anti-Brucella – IgM by ELISA results**

|  |  |  |
| --- | --- | --- |
| **Test** | **% sensitivity** | **%specificity** |
| RBPT | 100 | 74 |
| 2ME | 82 | 82 |
| STAT | 100 | 97 |

Comparing these results with classical methods  
of serological diagnosis, (21), who described the  
competitive immunoenzyme test (cELISA) as a  
more selective test for detection of and  
differentiation between infected and uninfected  
animals in comparison to all other serological  
tests, including iELISA. On the basis of the  
analysis of the results on sensitivity and  
specificity of the tests applied, it was concluded  
by the above researchers that the most sensitive  
were iELISA (100 %) and it can be used as a  
referent test in the diagnosis of acute cases of  
brucellosis . Out of the total number of cases  
assayed ,118 cases gave positive result by RBPT  
, only 62 cases were found to be positive by  
ELISA while the remaining 56 cases were found  
to be negative. Both genders were affected and  
patient rural area were affected more than those  
from urban , these findings are supported by  
many investigators (22,23).  
Comparing the results of RBPT and 2ME ,  
27(69%) of high titer RBPT results gave positive  
results by 2ME while 10 (27%) were found to  
be negative , and in low titer RBPT (1⁄160 - 1⁄80  
), only 18(70 %) and 1(20%) gave positive  
results by 2ME respectably But despite the  
differences in percentage positivity , negative  
results were found to be almost equal in RBPT  
and 2ME. 2-Mercaptans (2ME) cause the  
cleavage of disulphide bonds of IgM and loss of  
agglutinin activity , Thus , comparisons of  
results obtained in the absence or presence of  
theses agent is often used to distinguish IgM  
from IgG activity and differentiate between  
early and persistent infection (24, 25), which  
may explain the differences between the  
percentage positivity in these two methods .  
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The present study has shown that there are  
differences in the ability of the three serological  
methods in detection of the specific anti  
*Brucella* antibodies in patient′s sera .The  
majority of positive cases by RBPT gave  
positive result by 2ME and all negative cases  
by RBPT gave negative results by 2ME(Table  
2),however , it was reported that 2ME can detect  
the disease at 18 months after the onset of the  
disease because it detects IgG only (26). In the  
present study as shown in table 3 and 4, out of  
92 negative cases by RBPT were 12 cases were  
found to be positive by STAT however in Elisa  
All negative cases by RBPT were found to be  
also negative by Elisa . Similar results obtained  
by (27), while (22) stated that diagnosis of  
Brucellosis cannot be achieved sometimes by  
STAT because of the low titer antibodies , and  
the presence of blocking antibodies . Kostoula  
*et. al* reported that Elisa is more sensitive than  
STAT because Elisa detects the specific IgM or  
IgG (23). On the other hand, (24) reported that  
in patients with brucellosis the sensitivity of  
either Elisa –IgM or IgG is higher than of STAT  
.  
It is considered that a sensitive test will  
determine the most true – positive patients and a  
specific test will determine the most true –  
negative patients(28).In the present study , the  
three serological tests were found to be sensitive  
but with different specificity ,however the whole  
3 serological tests are simple , inexpensive , and  
rapid when compared by culture , ELISA ,PCR  
,and other diagnostic methods (23).  
Based on the analysis of results obtained in the  
present study , STAT was recommended as the  
best serological test in the diagnosis of  
Brucellosis because it detect false negative cases  
and its sensitivity and specificity was the highest  
.Each of serological tests has its advantages and  
limitations and requires careful interpretation and  
advanced techniques e.g. PCR and other  
molecular methods are still required to confirm  
diagnosis because of the complicity of the  
disease .  
***REFERENCES***1. Richard AH, Champe PC, and Bruce  
DF.(2007).Microbiology 2nd edition.:334.  
2. Schellig E,Diguimbaye C,Daoud S,Nicolet  
J,Boerlin P,Tanner M, and Zinsstag J. (2003).  
Brucellosis and Q-fever seroprevalances of  
nomadic pastoralists and their livestock in  
chad.prev.vet.med.61:279-293.  
3.Godfroid J,Cloeckaert A, Liautard  
JP,KohlerS,Fretin D,Walravens K,Garin B,  
Letesson JJ.(2005).From the discovery of the  
Malta fevers agent to the discovery of a marine  
mammal reservoir .brucellosis has continuously  
been a reemerging zoonsis.Vet.Res.36:313-326.  
**International Journal for Sciences and Technology Vol. 8, No. 1, March 2013** 41  
4. Dhand NK,Gumber S,Singh BB, Aradhana  
BMS, Kumar H, Sharma DR,Singh J, and  
Sandhu KS.(2005). A Study on the epidemiology  
of brucellosis in Punjab (India) using survey  
Toolbox.Rev.Sci.Tech.24:879-885.  
5. Mainar- Jaime RC,MunozPM,Miguel  
MJ,GrilloMJ,Marin CM, Moriyon I, and Blasco  
JM. (2005). Specificity dependence between  
serological tests for diagnosing bovine  
brucellosis in Brucella-free farms showing false  
positive serological reactions due Yersinia  
enterocolitica O:9.Can.Vet.J.46:913-916.  
6. Jacques I,Olivier- Bernardin V.,and Dubray  
G.(1998).Efficacy of Elisa compared to  
conventional tests (RBPT and CFT) for the  
diagnosis of Brucella melitensis infection sheep.  
Vet.Microbiol.64(1):61-73.  
7. Buchanan TM., Sulzer CR, Frix MK,and  
Feldman RA.(1974).Brucellosis in the united  
states ,1960-1972:an abattoir.associated  
disease.2.Diagnostic aspects .Med .53:415-426.  
8. Araj GF, Lulu AR.,Mustafa MY.,and Khateeb  
MI.(1986). Evaluation of ELISA in the diagnosis  
of acute and chronic brucellosis in human  
beinge. J.Hyg.(Lond) 97:457.  
9. Bettelheim KA.,Maskill WJ.,and Pearce  
J.(1983). Comparison of standard tube and  
microagglutimation techniques for determining  
Brucella antibodies. J.Hyg.Camb. 90:33.  
10. Marmonier A.,Stahl JP., Metz A, and  
Micaud M. (1979).Ineret et Valeur dela  
technique immunoenzymatique ELISA  
appliqute on diagnostic serologique des  
brucellosis humanies . Medicine et Maladies  
infectieuses .99:664.  
11. Faydi Y, and AL-Khlil S.(1992). Laboratory  
diagnosis of Brucellosis using the slids and  
standard tube agglutination methods. An.Najah  
.J.Res. 2(35):2417-2418.  
12. Teoman Z, Apan M, Yildrim M, and  
Istanbulluoglu E. (2007) .Seroprevalence of  
Brucellosis in Human ,Sheep ,and Cattle  
Populations in Kirikkale  
(Turkey).Turk.J.Vet.Anim.Sci. 31(1):75-78  
13. Ruiz J,Lorente I, and Perez J. (2008).  
Diagnosis of brucellosis by using blood. Oxford  
Printers.  
14. Ozturk R,Mert A, and Kocak F.(2002). The  
diagnosis of brucellosis by use of BACTEC  
9240 blood culture System .Diagn Microbiol.  
Infect. Dis. 44:133-135  
15. Young EJ. (2000). Brucella species  
.Principles and Practice of Infectious Diseases  
5th end. (Eds.Mandell GL, Bennett JE,Dolin R)  
Philadelphia ,Churchill Livingstone 2386-2393.  
16. Emmerzaal A., De Wit JJ, Dijkstra T, Bakker  
D, and Van Zijderveld FG. (2002). The Dutch  
Brucella abortus monitoring programme for  
cattle: the impact of false-positive serological  
reactions and comparasion of serological. J. Vet