**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 MENo(%)** | **Negative by 2 MENo(%)** |
| 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. ofpatients** | **GenderMale/female** | **ResidencyUrban/rural** |
| **1/320No(%)** | **1/160No(%)** | **1/80No(%)** |  |  |
| 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** |
| **1/20No(%)** | **1/40No(%)** | **1/80No(%)** | **1/160No(%)** | **1/320No(%)** | **1/640No(%)** | **1/1280No(%)** | **1/2650No(%)** | **1/5120No(%)** |
| 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) |
| Totalpositive | 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 ELISANo(%)** | **Negative by ELISANo(%)** |
| 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 ELISANo(%)** | **Negative by ELISANo(%)** |
| 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 .
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