Evaluation of Rapid Chromatographic Immunoassay with Latex Agglutination Test and (ELISA) for Diagnosis of Human Toxoplasmosis

jabbar S. Hassan*  BSc, MSc
Haider f. Ghazi*  BSc, MSc
Abid Al-Razaq H.Ahmed**  BSc,PhD

Summary:

Background: there are different procedures for the diagnosis of females suspected with toxoplasmosis. However, time, cost, and accuracy of the test should meet patient's needs.

Material and methods: one hundred and eleven female with suspected toxoplasmosis were under go three different procedures for the diagnosis of Toxoplasma gondii infection. Latex agglutination test, rapid chromatographic (immunoblot) and ELISA test were done for all patients. The results were described as frequency and percentage of positivity; also, specificity and sensitivity of immunoblot were assessed according to the result of other tests.

Results: The Latex test has shown 80% and 61.54% sensitivity and specificity respectively with IgG measured by ELISA while it has shown 100% sensitivity and specificity with IgM measured by ELISA. IgM immunoblot give a relatively higher sensitivity and specificity (95% and 98.89%) respectively than IgG immunoblot (88% and 89.29%) made with IgM and IgG ELISA respectively.

Conclusion: Rapid chromatographic test considered as a good test for detection of IgG and IgM anti Toxoplasma gondii antibodies in both acute and chronic Toxoplasmosis.

Key words: Immunoblot test and Toxoplasmosis.

Introduction:

In spite of the advance techniques in diagnosis of bacterial, viral and protozoan disease; diagnostic methods have to be renewed to be more rapid and specific. During the past few years, there has been an increased interest in the diagnosis of parasitic diseases using techniques, which are rapid, simple and inexpensive as well as sensitive and specific(1). Old serological procedures such as indirect haemagglutination (2), complement fixation test (3) and immuno-flourescence are tedious and difficult to standardize, conduct and interpret. Also the reagents are consumptive and require highly trained technicians as well as expensive instruments. (4)Toxoplasma gondii is a coccidian parasite of the cat and its infection may lead to major public health problems (5). The disease exhibits various clinical manifestations and therefore, poses difficulty in diagnosis (6). Serological methods have been employed in aid of diagnosis of this disease. Detection of antitoxoplasma IgG and IgM antibodies have been routinely used in many clinical laboratories to determine the probable immune status of individuals (1).

The most used assay today is indirect enzyme linked immunosorbent assay (ELISA); require highly trained and expensive instruments (1). So this research was planned, in parallel with rapid latex agglutination test and enzyme linked immunosorbent assay (ELISA), to standardize the commercially available Rapid chromatographic immunoassay (immunoblotting) technique, which is simple to perform and doesn’t need expensive equipment to detect IgG and IgM specific antibodies against Toxoplasma gondii.

Patient and Methods:

This study included one hundred and eleven female patients with suspected Toxoplasmosis, the age range from (18) years to (39) years with mean (28.57). All patients were outpatient visitor to the private gynecology clinic in Baghdad during March 2008-march 2009, clinical details at presentation were records, and sera were collected at the first visit and stored in aliquots at -20 °C till analyzed.

Three methods were used in this study, which is rapid latex agglutination test (qualitative) (Biokit®), this kit contains toxocell latex reagent which composed from latex particle coated with soluble Toxoplasma gondii antigen, if the reaction takes place, due to the presence of Toxoplasma antibodies in the serum, the latex suspension changes its uniform appearance and clear agglutination becomes evident. The second method is...
Toxo IgG and IgM Rapid test provided by (ACON®), the toxo IgG& IgM antibodies rapid test devices is a qualitative, chromatographic immunosassay for detection of IgM, IgG antibodies to *Toxoplasma gondii* in serum of patients with toxoplasmosis. In this test, antigen of *Toxoplasma gondii* are coated in the test line of membrane. During testing, the serum specimen reacts with Goat anti-human IgM or IgG coated particles in the test strip. The mixture then migrates forward on the membrane by capillary action and react with Toxoplasma specific antigen on the membrane on the test line region indicates a positives resulet for Toxoplasma infection (presence of a colored line), the procedure was performed in accordance with the manufactured instruction and the expected results are as follow:

**Negative control:** Only the control band (C band) show color development. The two test bands (T1 and T2) show no color development

**Positive control:** The C band and two T bands (T1 and T2) show color development.

**Interpretation of assay result:**

1. **Negative result:** If only the C band is present, the absence of color in both T bands (T1 and T2) indicates that no anti-Toxoplasma antibodies are detected (result is negative)

2. **Positive result:** In addition to the presence of C band, if only T1 band color is developed indicate the IgM anti-Toxoplasma is present in the specimen (IgM positive). While if only T2 band is developed indicate the (IgG positive) and if both T1 and T2 bands are developed in addition to the presence of C band that means both (IgM and IgG is positive), if no C band is developed the assay is invalid regardless of any color in the T bands. The third method is indirect enzyme linked immunosorbent assay (ELISA), the test kit is provided by (Biocheck®), the diluted patient serum is added to the purified Toxoplasma antigen coated on the surface of microwells, the Toxoplasma IgG, IgM –specific antibody, if present, binds to the antigen, all unbound material are washed away, Horse reddish peroxidase (HRP) conjugate is added, which binds to the antibody-antigen complex. After washing the solution of Tetramethylbenzidine (TMB) reagent is added, the enzyme conjugate catalytic is stopped at a specific time. The results are read by ELISA reader.

The procedure assay can be summary as follow:

1. Sample ,controls and calibrator are dilution 1:40 by adding 5µ /200µl
2. three incubations at 37°C

Stop with stop solution 100 µl and read at 450nm.all results above the cut-off value (10 IU/Ml) conceders positive.

**Statistical analysis:** All data were presented as frequency and percentage of positive and negative results and cutoff value was determined (7), sensitivity and specificity were determined for each procedure as the following equations:

\[
\text{Sensitivity} = \frac{a}{a + c} \\
\text{Specificity} = \frac{d}{b + d}
\]

a = True positive

b = False positive
c = False negative
d = True negative

**Results:**

In the present study, nearly 111 Toxoplasma-suspected human female samples were tested. According to testing method, 83 (76.16%) samples showed positive for Toxoplasma by Latex test (BIOKIT). When these samples tested by Rapid chromatographic method for IgM and IgG; 20 and 55 samples were positive respectively. While in ELISA test, 20 samples were IgM positive and 52 samples were IgG positive (Table 1).

**Table 1: Results of positive Toxoplasma gondii suspected cases measured by Latex, Immunoblot and ELISA kit.**

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Percentage</th>
<th>Negative</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td>83</td>
<td>76.15%</td>
<td>26</td>
<td>23.85%</td>
</tr>
<tr>
<td>IgG Immunoblot</td>
<td>55</td>
<td>49.55%</td>
<td>56</td>
<td>50.45%</td>
</tr>
<tr>
<td>IgM immunoblot</td>
<td>20</td>
<td>17.86%</td>
<td>92</td>
<td>82.14%</td>
</tr>
<tr>
<td>IgG ELISA</td>
<td>52</td>
<td>46.43%</td>
<td>60</td>
<td>53.57%</td>
</tr>
<tr>
<td>IgM ELISA</td>
<td>20</td>
<td>18.35%</td>
<td>89</td>
<td>81.65%</td>
</tr>
</tbody>
</table>

The evaluation results of Toxocell latex and immunoblot tests compared with ELISA test were shown in table (2)

The Latex test has shown 80% and 61.54% sensitivity and specificity respectively with IgG measured by ELISA while it has shown 100% sensitivity and specificity with IgM measured by ELISA.
Evaluation of Rapid Chromatographic Immunoassay with Latex Agglutination Test and ELISA for Diagnosis of Human Toxoplasmosis

Table 2: Results of sensitivity and specificity of Latex and Immunoblot (IgG and IgM) according to ELISA results.

<table>
<thead>
<tr>
<th>Latex</th>
<th>IgG Immunoblot</th>
<th>IgM Immunoblot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sensitivity</td>
<td>specificity</td>
</tr>
<tr>
<td>IgG ELISA</td>
<td>80.00%</td>
<td>61.54%</td>
</tr>
<tr>
<td>IgM ELISA</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Discussion:
In this study, the rapid chromatographic test (immunoblot) and ELISA method for the detection of IgM and IgG anti-Toxoplasma gondii antibodies were evaluated. In acute infections, IgG and IgM antibodies levels generally rise within one to two weeks of infection (8). The presence of elevated levels of T. gondii specific IgG antibodies indicates that infection has occurred but does not distinguish between recent infection and infection acquired in the distant past. Detection of T. gondii specific IgM has been used as an aid in determining the time of infection: a negative IgM test result with a positive IgG result usually indicates infection at least six months previously. However, the interpretation of T.gondii specific IgM-positive result is complicated by the persistence of IgM antibodies up to 18 months after infection (9), and by false-positive reactions in commercial tests (10). IgM immunoblot give a relatively higher sensitivity and specificity (95% and 98.89%) respectively than IgG immunoblot (88% and 89.29%) made with IgM and IgG ELISA respectively. This variability in sensitivity and specificity between IgG and IgM with latex may be due to that all latex positive cases were ELISA positive for IgM. By another mean, we can depend on the latex test for diagnosis of an acute cases with toxoplasmosis rather than chronic cases. This variability may be attributed to that IgM more potent agglutinin than IgG since it have pentamer structure (10). Thus, this study suggest that there is fairly good agreement between the results of Toxocell latex which is commercially available diagnostic kits for the detection of IgM antibodies to T. gondii. Hence we conclude and recommend that Toxocell latex is a very useful diagnostic aid for epidemiological studies of prevalence of toxoplasma acute infection. The early diagnosis of acute toxoplasmosis can be possible by the detection of IgM antibodies to T. gondii. The simultaneous determination of the IgG and IgM antibodies shall facilitate and help assessment of the actual immune status of the patients. False negative results are also reported in the presence of high concentration of antitoxoplasma IgG [11]. In order to rule out such interfering factor, we can depend on the immunoblot test for detection of IgG and IgM antitoxoplasma antibodies. In conclusion, our evaluation immunoblot test for detection of IgG and IgM antibodies to T. gondii. The kits can be used in the pathological laboratories for the diagnosis of toxoplasmosis as well as for the immune status of the patients.

References: