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COMPARISON OF MAT WITH ELISA AND LAT TESTS IN DETECTING *TOXOPLASMA GONDII* ANTIBODIES IN HUMAN SERA

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ABSTRACT:
The modified agglutination test (MAT) has been widely used for the detection of *Toxoplasma gondii* infection in numerous animal species. For the standard protocol of MAT test, *T. gondii* whole cell antigens were produced in the laboratory mice, which was a tedious process. The produced antigen in cell culture was used to assure its capability for MAT test. For detecting the antibodies of *T. gondii* in human sera, comparison was made between MAT, enzyme linked immunosorbent assay (ELISA) and latex agglutination test (LAT). A total of 96 human serum samples were tested. The anti-*Toxoplasma* IgG antibodies were found in 25.0% (24/96), 20.8% (20/96) and 13.5% (13/96) samples by MAT (cutoff 1:25), ELISA and LAT tests, respectively. The MAT and ELISA tests matched 95.8% in detection of IgG antibodies, with the positive percent agreement of 100% (20/20) and negative percent agreement of 94.7% (72/76). For the MAT versus LAT tests, the overall agreement was 88.5% (85/96), with the positive percent agreement of 100% (13/13) and negative percent agreement of 86.7% (72/83). These results suggest a strong correlation between the MAT and ELISA tests in detecting serum IgG to *T. gondii* in human sera. In conclusion, *T. gondii* prepared in cell culture provides an alternative solution to produce antigens for MAT test.

KEYWORDS: Toxoplasma gondii, MAT, LAT, ELISA.

1. INTRODUCTION

Toxoplasma gondii is a common parasite that infects mammals and birds (Dubey, 2010). Felines (domestic and wild cats) are the main animal hosts in transmission of *T. gondii* by shedding oocysts in feces and then contaminate environment. Warm blooded animals can be infected by ingesting oocysts from contaminated environment and then often establish chronic infection in which the parasite form tissue cysts in brain and muscle tissues of these animals. One third of the human population in the world is chronically infected with this parasite. Infection in human can cause ocular and congenital toxoplasmosis in healthy individuals, and encephalitis in immunocompromised patients (Montoya and Liesenfeld, 2004). *Toxoplasma gondii* is considered one of the major foodborne pathogens in the United States (Batz *et al*., 2011). In the United states of America it *is* estimated that 750 people die of toxoplasmosis each year (Mead *et al*., 1999) and the annual incidence of congenital infection in USA ranges from 400 to 4,000 (Jones et al., 2001).
To understand the epidemiology of *T. gondii* transmission, it is important to diagnose the infection in animal and human populations. A number of serological assays are used for this purpose. Among these tests, the modified agglutination test (MAT) has been widely performed in animals due to its ease of use. For the commonly used protocol of MAT test, *T. gondii* whole- cell antigens are produced in laboratory mice, which needs to use animals and the process is tedious. This antigen was used for MAT test of human serum samples and it showed excellent agreement with the Sabin-Feldman dye test(Desmonts and Remington, 1980). Recently, cell-culture produced MAT antigen was compared with the conventional mouse-derived antigens in MAT assay in animal serum samples, and showed excellent agreement(Al-Adhami *et al*., 2016) . However, it is not clear how cell-culture derived antigens perform for human serum samples in MAT test. In this study, we address the question by comparing it with the commonly used enzyme linked immunosorbent assay (ELISA) and latex agglutination test (LAT) for the detection of IgG antibodies to *T. gondii* in human sera.

2. MATERIALS AND METHODS

2.1 Sample collection

A total of 96 human serum samples were obtained from Southern Hills Medical Center and some walk-in clinics in Nashville, Tennessee, USA from November 2014 to March 2015. Blood samples were originally collected from patients for routine medical procedures. We obtained some of these serum samples for our study. Serum samples were stored at -20°C till use for serological testing process.

2.1.1 MAT test of IgG antibody to T. gondii:

The MAT tests were performed in Dr. Chunlei Su laboratory in the Department of Microbiology at the University of Tennessee at Knoxville. The *T. gondii* whole-cell antigens were prepared in the laboratory by growing the RH strain using human forehead fibroblast (HFF) cell culture. The tachyzoites were harvested and treated with 6% formaldehyde overnight at 4°C, washed with PBS and diluted in the alkaline buffer to $2x10^8$ tachyzoites/ml. The MAT test was carried out following the procedure described previously(Desmonts and Remington, 1980 ; Dubey and Desmonts, 1987). The MAT tests were carried out using 96 well U-bottom microtiter plates. Serum samples were serial diluted from 1:25 to 1:3200. Positive and negative controls were included for each microtiter plate. The cutoff for MAT titer is 1:25, titers >=1:25 is considered seropositive for *T. gondii* infection.

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2.1.2 ELISA IgG: The ELISA tests were performed in the laboratory of Biology Department at the Tennessee State University in Nashville, TN. The commercial ELISA kit for ELISA IgG (catalog# TXGT) tests were purchased from AMICO Laboratories, Inc. Nashville, TN, USA. Human serum samples were processed following manufactory's instruction. Briefly, serum specimens were prepared by mixing 10 µl of serum with 200 microliter of sample diluent. Then 100 microliters of each diluted sample was added to the microplate in duplicates. Both negative and positive controls were also included. Microtiter plates were incubated at room temperature for 20 minutes, washed three times with washing buffer. One hundred microliter of HRP-conjugated anti-human IgG was added and incubated at room temperature for 20 minutes. The microplate was washed three times with washing solution and blotted onto paper towels. One hundred microliters of TMB was added to each well and incubated at room temperature for 10 minutes. Then a 100 microliter of Stop Reagent was added to each well. Samples were read by ELISA reader at 450 nanometers with subtracting the blank value. The results were interpreted as positive, equivocal or negative by determining the immunoglobulin index. IgG index values <0.90, 0.90-0.99 and >=1.0 were considered negative, equivocal and positive, respectively.

2.1.3 LAT IgG: The LAT test was performed in the laboratory of Biology Department at the Tennessee State University in Nashville, TN. The commercial LAT kit for anti-*Toxoplasma* IgG (catalog number TXGLX) was purchased from AMICO Laboratory Inc. Nashville, TN, USA The samples were tested following the manufactory's instruction. Briefly, the latex reagent bottle was shaken gently and one drop was added to each of the three circles on the slide. One drop of serum was added to one circle, to the other two circles one drop each of the positive and negative controls were added. Then slide was rotated for two minutes on a mechanical rotator. The results were read under high intensity lamp. Appearance of agglutination indicates positive reaction, whereas, lack of agglutinations indicates negative reaction (Balfour *et al*., 1982).

2.2 Data analysis

The data were analyzed by Cohen's Kappa (Cicchetti and Feinstein, 1990) and Pearson Correlation Coefficient with the Graphpad prism v.5.01 Package (GraphPad Software, Inc. USA).

3. RESULTS

The results of MAT, ELISA and LAT tests are summarized in Table 1. From the total of 96 serum samples, 25.0%, 20.8% and 13.5% were positive for MAT, ELISA and LAT IgG testes, respectively. The MAT titers and ELISA of IgG are summarized in Table 2. Linear regression analysis showed a good association between MAT titers and ELISA IgG index value (Fig. 1). The summary of MAT and ELISA tests is presented in Table 3. For these two tests, the overall percent agreement is 95.8%, the positive percent agreement is 100%, and the negative percent agreement is 94.7% (Table 3). For the MAT vs LAT tests (Table 4), the overall percent agreement is 88.5% with the positive agreement 100%, and negative percent agreemnent 86.7%.

Table 1. Summary of MAT, ELISA and Latex tests of human sera

	Positive		Negative	
	No.	%	No.	%
MAT (≥1:25)	24	25.0	72	75.0
ELISA IgG	20	20.8	76	79.2
Latex IgG	13	13.5	83	86.5

Table 2. Correlation between MAT titers and ELISA index of human sera

MAT titer	ELISA								
1:25	0.82	0.56							
1:50	0.82								
1:100	0.85								
1:200	1.72	2.30	1.97	2.32	1.18	1.59	2.40		
1:400	3.75	3.00	1.89	2.16	2.55	3.02	2.79	2.29	2.17
1:800	4.04	3.64	3.06						
>=1:1600	4.46								

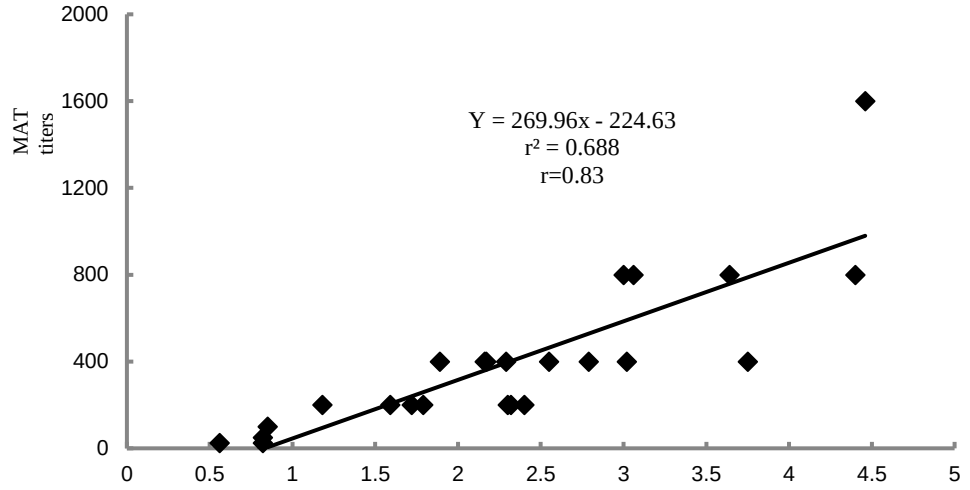

Fig. 1. Correlation between ELISA and MAT IgG titers in serum samples based on the Pearson correlation analysis. There is significant positive correlation between the ELISA index and MAT titers (P≤0.01).

Table 3. The agreement between MAT and ELISA tests

			ELISA	
		Positive	Negative	Total
MAT	Positive	20	4	24
	Negative	0	72	72
	Total	20	76	96

Overall percent agreement = (20+72)/96 =95.8%
Positive percent agreement = 20/20 = 100%
Negative percent agreement = 72/76 = 94.7%

Table 4. The agreement between MAT and LAT

			LAT	
		Positive	Negative	Total
MAT	Positive	13	11	24
	Negative	0	72	72
	Total	13	83	96

Overall percent agreement = (13+72)/96 =88.5%
Positive percent agreement = 13/13 = 100%
Negative percent agreement =72/83 = 86.7%

4. DISCUSSION

The present study evaluates the efficiency of MAT, ELISA and LAT tests for detection of anti-*T. gondii* IgG antibodies in human sera. For cell-culture derived whole cell antigen, the MAT test matched with the ELISA IgG tests well, suggesting the cell-derived antigen is adequate for MAT test for human sera. In addition, positive association of MAT titers with ELISA index is also obvious, allowing to predict antibody titers from the ELISA test. This method provides an alternative method to the commonly used ELISA test in diagnosing *T. gondii* infection in human. Particular advantage of MAT test is its low cost, simple to setup and easy to perform

The conventional MAT test has been widely used to determine *T. gondii* infection in animals (Dubey, 2010). The whole-cell antigen for this test is produced by using *T. gondii* RH strain cultivated along with mouse sarcoma cells in the peritoneal cavities of mice. Results of the MAT test showed high accordance with that of the gold-standard Sabin-Feldman dye test (Desmonts and Remington 1980) . The conventional MAT test was compared with other serological tests in different animal species and demonstrated good correlation with ELISA test in detecting *T. gondii* infection in pigs (Gamble *et al*., 2005; Hill *et al*., 2006; Sroka *et al*., 2008) and sheep (Mainar-Jaime and Barberán 2007). In one study of detecting antibodies to sheep and goats, the conventional MAT test seemed to be more sensitive that the ELISA test (Gebremedhin *et al*., 2013).

Recently cell-culture derived whole-cell antigen has been used for MAT test to detect *T. gondii* infection in pigs, cats and wildlife, it showed excellent agreement with the mouse-derived antigens (Al-Adhami *et al*., 2016). In this study, it is also demonstrated that the cell-culture derived antigen performed well in detecting anti-*T. gondii* IgG in human sera, therefore, it can be an alternative method to diagnose *T. gondii* infection in human.

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REFERENCES

Al-Adhami, B.H.; Simard, M.; Hernández-Ortiz, A.; Boireau, C., and Gajadhar, A.A. (2016) Development and evaluation of a modified agglutination test for diagnosis of Toxoplasma infection using tachyzoites cultivated in cell culture Food and Waterborne Parasitology, 2:15-21

Batz, M.; Hoffmann, S., and Morris, Jr. J. (2011) Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida.

Cicchetti, D.V., and Feinstein, A.R. (1990) High agreement but low kappa: II. Resolving the paradoxes. Journal of clinical epidemiology, 43:551-558

Desmonts, G., and Remington, J.S. (1980) Direct agglutination test for diagnosis of Toxoplasma infection: method for increasing sensitivity and specificity. Journal of clinical microbiology, 11:562-568

Dubey, J.P. (2010) Toxoplasmosis of animals and humans CRC Press is an imprint of Taylor & Francis Group, USA

Dubey, J.P., and Desmonts, G. (1987) Serological responses of equids fed Toxoplasma gondii oocysts. Equine veterinary journal, 19:337-339

Edwin, H.; Lennette, A.B.; William, J.; Hausler, Jr., and Jean Shadomy, H. (1985) Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.

Gamble, H.; Dubey, J, and Lambillotte, D. (2005) Comparison of a commercial ELISA with the modified agglutination test for detection of *Toxoplasma* infection in the domestic pig. Veterinary parasitology, 128:177-181

Gebremedhin, E.Z.; Abdurahaman, M.; Hadush, T., and Tessema, T.S. (2013) Comparison Between Enzyme Linked Immunosorbent Assay (ELISA) and Modified Agglutination Test (MAT) for Detection of *Toxoplasma gondi*i Infection in Sheep and Goats Slaughtered in an Export Abattoir at Debre-zeit, Ethiopia Global Veterinaria, 11 (6): 747-752.

Hill,D.E.; Chirukandoth, S.; Dubey, J.P.; Lunney, J.K., and Gamble, H.R. (2006) Comparison of detection methods for *Toxoplasma gondii* in naturally and experimentally infected swine. Vet. Parasitol., 141:9-17

Jones, J.L.; Kruszon-Moran, D.; Wilson, M.; McQuillan, G.; Navin, T., and McAuley, J. B. (2001) *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. American journal of epidemiology, 154:357-365

Mainar-Jaime, R., and Barberán, M. (2007) Evaluation of the diagnostic accuracy of the modified agglutination test (MAT) and an indirect ELISA for the detection of serum antibodies against *Toxoplasma gondii* in sheep through Bayesian approaches. Veterinary parasitology, 148:122-129

Mead, P.S. *et al.* (1999) Food-related illness and death in the United States. Emerging infectious diseases, 5:607-625 doi:10.3201/eid0505.990502

Montoya, J. G., and Liesenfeld, O. (2004) Toxoplasmosis Lancet 363:1965-1976

Sroka, J.; Cencek, T.; Ziomko, I.; Karamon, J., and Zwolinski, J. (2008) Preliminary assessment of ELISA, MAT, and LAT for detecting *Toxoplasma gondii* antibodies in pigs. Bull. Vet. Inst. Pulawy., 52:545-549

کورتیا لێکولینێ:

تێستی ئەگلوتینەیشنی دەستکاری کراو(MAT) کە لەبواریکی فراوان بەکارهاتوە بۆ دەستنیشانکردنی نەخۆشی پشیلە لە ئاژەلە جیاوازەکان، لە برۆتۆکۆلی تیستی ماتی ستاندەر کەتیایدا دژە پەیداکەری تۆکزۆبلازما گۆندی(T.gondii) بەرهەم هاتووە لەتاقیگەی مشکەکان کە بەکارێکی قورس و زەحمەت هەژماردەکریت، ئەو دژە تەنەی کەبەرهەم هینراوە لە کەلچەری خانەیی بەکارهاتوە بۆدڵنیابوون لەبەکارهینانی لە تێستی مMAT . بۆدەستنیشان کردنی دژە تەنی تی گۆندی لە زەرداوی خوێنی مرۆڤدا بۆ ئەم مەبەستە بەراورد کراوە لەنێوان تێستی MATو ELISAو LAT بۆ ئەم مەبەستە 96 نموونە لەزەردواوی خوێنی مرۆڤ کەتیایدا رێژەی دژە پەیداکەری تی گوندی دریژخایەن بەرێژەی 25% واتە(24/96) وە رێژەی 20.8 واتە(20/96) وەرێژەی 13.5 واتە (13/96) بۆ هەریەك لە تێستی MAT(1:25) و ELISA وە هەروەها لLAT بەشیوەی یەك لەدوای یەك. لەلایەکی ترەوە تێستی MATو تێستی ELISA هاویەکن بەرێژەی 95.8% بۆ دەستنیشانکردنی دژە تەنی. T gondii ، وە هەردوو تێست بەرێژەی 100% واتە (20/20) ریکەوتەی ئەرینین وە بەرێژەی 94.7% واتە (72/76) بەرێکەوتەی نەرینین وە هەروەها لەبەراوردی نێوان MAT و LAT بەرێژەی 88.5% واتە(85/96) رێکەوتە ی تەواون وەبەرێژەی 100% واتە (13/13) رێکەوتەی ئەرێنین وە بەرێژەی 86.7% واتە (72/83) بۆ رێکەوتەی نەرێنی، ئەم ئەنجامانە ئاماژە بەوە دەکەن کەپەیوەندیەکی بەهێز هەیە لەنێوان تیستی MATو ELISA بۆ دۆزینەوەی دژەتەنی نەخوشی پشیلەی درێژ خایەن لەزەردواوی خوێنی مرۆڤدا، لەکۆتایدا ئەوە بەرجەستە دەکرێت کەT .gondi ئامادە کراو لەچاندنە خانەدا دەبێتە ئەلتەرناتیڤێکی باش بۆ دروست کردنی دژە پەیداکەر بۆ بەکارهێنانی لە تێستیMAT دا.

خلاصة البحث:

لقد استخدم اختبار التراص المعدلة (MAT) على نطاق واسع للكشف عن داء القطط في الحيوانات المختلفة.لاجراء اختبار الMAT القياسي ، أنتجت مستضدات الخلية الكاملة لـT.gondii في الفئران المختبرية، والتي كانت عملية شاقة . استخدم المستضد المنتج في المزرعة الخلوية لضمان قدرته في اختبار MATللكشف عن الأجسام المضادة من T.gondiiفي الأمصال البشرية، وقد تمت مقارنة بين الMATواختبار انزيم المرتبط المناعي (ELISA)واختبار تراص اللاتكس (LAT) تم اختبار 96 عينة من مصل الإنسان. وتم العثور على أجسام مضادة T.gondii المزمنة بنسبة25% (24/96)، 20.8% (20/96) و 13.5% (13/96) بواسطة MAT(تخفيف 1:25)، ELISA واختبارات LAT، على التوالي. وكان اختباري ال MAT وال ELISA مطابقا95.8% في الكشف عن الأجسام المضادة، مع اتفاق ايجابي من 100% (20/20) والاتفاق سلبيا من 94.7% (72/76). ل MAT مقابل اختبار LAT، كان اتفاق شامل 88.5% (85/96)، مع اتفاق ايجابي من 100% (13/13) والاتفاق سلبيا من 86.7% (72/83). وتشير هذه النتائج الى وجود علاقة قوية بين اختبار الMAT واختبار ELISA في الكشف عن مضاد داء القطط المزمنة في الأمصال البشرية. وفي الختام استنتج بان T.gondii التي تم اعدادها في المزرعة الخلوية توفر حلا بديلا لإنتاج المضادات لاختبار MAT.