IJM Iranian Journal of Microbiology
Volume 6 Number 1 (February 2014) 8-13

Using recombinant *Chlamydia trachomatis* OMP2 as antigen in diagnostic ELISA test

Mahdieh Javaherian[1], Zarin Sharifnia[2], Robabeh Taheripanah[3], Mojgan Bandepour[2,4], Mohammad Soleimani[1], Bahram Kazemi[2, 4*]

[1]Microbiology Department, Islamic Azad University, Qom, Iran. [2]Cellular and Molecular Biology Research center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Fertility and Infertility Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]Biotechnology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Received: October 2013, **Accepted:** December 2013.

ABSTRACT

Background and Objectives: The obligate intracellular bacterium *Chlamydia trachomatis* causes sexually transmissible diseases in human. Timely and sensitive detection of this pathogen is very important. There are many cross-reactions in bacteriological and serological methods in detection of this type of pathogens. The aim of this study was to achieve a more specific antigen for serological tests.

Materials and Methods: Blood samples were taken from 192 women with suspected chlamydial infection and sera were isolated. ELISA plate wells were coated with recombinant *C. trachomatis* OMP2 as antigen. Cut-off system was determined with 40 negative sera. The final results of this research were compared with Euroimmun commercial kit.

Results: The ELISA system cut-off was calculated at 0.27 using negative sera samples. ODs of positive samples were higher than 0.27 and negative samples were lower than it. We obtained 30 samples (15.62%) as positive and 162 cases (84.37%) as negative. Sensitivity and specificity of the recombinant antigen were 90% and 86%, respectively. This antigen showed no cross-reactivity with sera of patients infected with Hydatid cyst, HCV, *Epstein barr* virus, HBV, *Helicobacter pylori*, *Toxoplasma gondii*, *Cytomegalovirus*, *Mycoplasma*, *Measles* and *Varicella zoster* virus.

Conclusion: The sensitivity and specificity of rOMP2 in ELISA for detection of *C. trachomatis* were 90% and 86%, respectively. Though the sensitivity was higher than results of Euroimmun commercial kit, its specificity was calculated lower than reference kit.

Keywords: *Chlamydia trachomatis*, ELISA, OMP2

INTRODUCTION

Chlamydia trachomatis is responsible for trachoma and sexually transmissible diseases in human. This bacterium is obligate intracellular parasite and use host cell energy system. Genital tract infection with chlamydia is common in some countries (1).

* Corresponding author: Bahram Kazemi
Address: Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences.
Tel/Fax: +98-21-22439956
E-mail: Kazemi@sbmu.ac.ir

Serotypes L3, L2, L1, D, and K of *C. trachomatis* cause genital tract infections. Some infections of genital tract of women are often asymptomatic. With the progresses of the disease, symptoms may appear (2). *C. trachomatis* is causative of cervicitis, endometritis, salpengitis, prehepatitis bartonellosis in women (3). Complications of untreated chlamydial infections may occur and these include pelvic inflammatory disease, ectopic pregnancy, fallopian tube obstruction, infertility, cervical dysplasia, and prehepatitis (4). Prevalence of chlamydial infections of genital tracts among pregnant women in Western countries is about 5-13 percent (5). 33-50% of infants

of infected mothers get chlamydial infections such as conjunctivitis, nasopharyngitis, otitis media and neonatal *Chlamydia pneumonia* during passage through the birth canal (6-9). Studies showed 35-50% of non-gonococcal urethritis (NGU) and a maximum of 70% of subsequent gonorrhea urethritis (post gonococcal urethritis = PGU) are caused by chlamydia (10).

Because of the importance of early and accurate diagnosis of chlamydial infection and its role in the health system, this study was aimed to use recombinant chlamydia OMP2 protein as an antigen in diagnostic ELISA Kit.

MATERIALS AND METHODS

Sampling. Blood samples from 192 women with suspected *C. trachomatis* infection refereed by physician and 40 healthy women (controls) were collected. Sera samples were separated and divided into small tubes and maintained at -20°C.

Preparation of antigen. BL21 *E. coli* bacterial colony containing *C. trachomatis* OMP2 gene construct (11) was cultured. When the turbidity of the culture reached to OD600 = 0.6 plasmid promoter was induced by 0.5mM IPTG (USA, Sigma) and bacteria were precipitated after 5 h and lysed by sonication. OMP2 *C. trachomatis* recombinant protein was purified by Ni-NTA affinity chromatography (12). The wells of ELISA plates were coated with recombinant protein as previously described (11).

Cut off determination. Forty negative *C. trachomatis* sera were examined by ELISA to determine system Cut-off**.** The graph and table of samples optical density at 1/200 dilution and standard deviation were calculated.

Evaluation of cross-reactivity with other infections. Possible cross-reactivity to sera of patients with other infections, such as: hydatid cyst (5 samples), *Hepatitis B virus* (5 cases), *Hepatitis C virus* (5 samples), *Helicobacter pylori* (5 samples), *Toxoplasma gondii* (5 cases), and *Mycoplasma spp.* (3 cases), *Cytomegalovirus* (3 samples), *Measles* (3 samples), *Herpes zoster* (1 case), *Epstein barr virus* (2 samples) were examined. Optical density (OD) of all samples at 1/200 dilution were obtained lower than of *C. trachomatis* Cut-off, demonstarting that they were negative and there are no cross reactivity between these sera samples and rOMP2 of *Chlamydia trachomatis*.

The samples evaluation by ELISA. 192 chlamydial infections suspected sera samples were tested by recombinant omp2 coated plate and Euroimmun commercial kit. The amount of antigen was 3 mg and sera were diluted at 1/400, but better results were taken at 1/200. The sensitivity and specificity of ELISA with recombinant protein and commercial kit were calculated using formula: Cut off = mean + 2SD.

The Cut off for 1/400 serum dilution was 0.244 (0.116 + 2 X 0.064) and for 1/200 serum dilution was 0.271(0.127 + 2 X 0.072). We used 0.271 as cut- off for ELISA test carry out.

Specificity and Sensitivity were determined using the formula:

Specificity = number of true negative/number of true negative + number of false positive X100

Sensitivity = number of true positive/number of true positive + number of false negative X 100

The T- student statistical method was used to analyse of the results.

RESULTS

Sera were collected from 192 suspected patients infected with *Chlamydia trachomatis*. *C. trachomatis* rOMP2 was purified by affinity chromatography and ELISA plate wells were coated with it. Forty seronegative samples were tested and 0.271 were considered as cut- off for this ELISA system.

For specificity of system, the sera with other infections were examined by this protein. There were no cross-reactivity between rOMP2 protein and sera with other infections (Fig. 1).

Fig. 2 shows comparison of Cut Off for 40 negative sera at 1/200 and 1/400 dilutions and 3 mg/ml rOMP2.

We tested antigen concentration for coating on microplate wells with 3 mg/ml and 2 mg/ml and used for serum concentration (Fig. 3). The OD of 192 *C. trachomatis* suspected serum were calculated by rOMP2 and Euroimmun commercial kit as shown in Figs. 4 and 5.

According to Table 1 and Fig. 3, 3 mg/ml of rOMP2 and serum dilution at 1/200 is the best in this ELISA system.

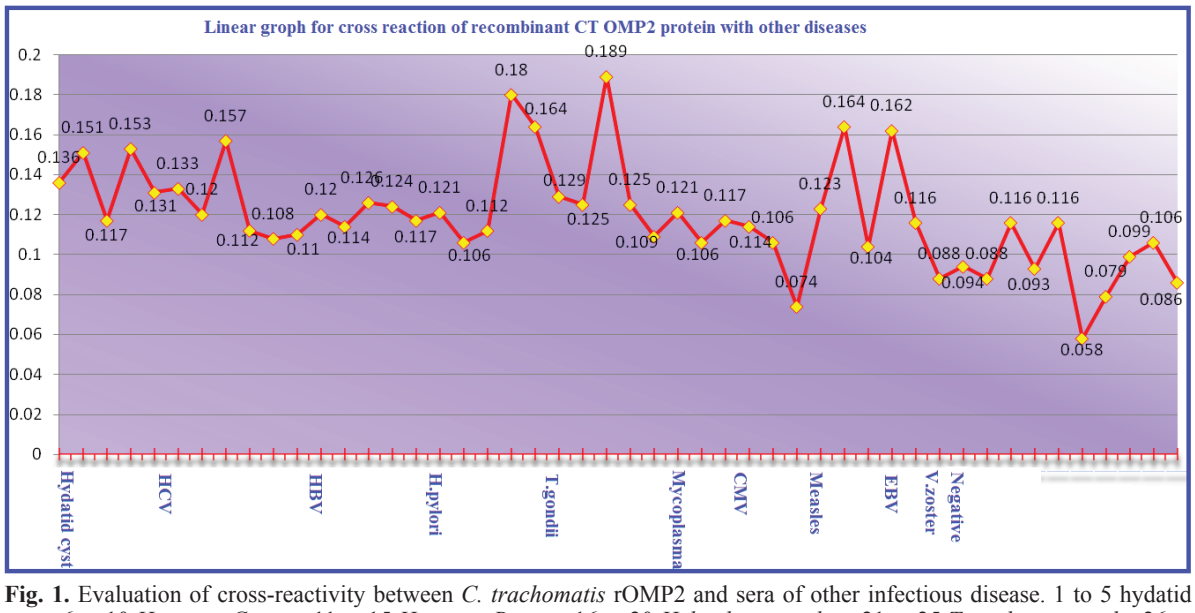


Fig. 1. Evaluation of cross-reactivity between *C. trachomatis* rOMP2 and sera of other infectious disease. 1 to 5 hydatid cyst, 6 to 10 *Hepatitis C virus*, 11 to 15 *Hepatitis B virus*, 16 to 20 *Helicobacter pylori*, 21 to 25 *Toxoplasma gondii*, 26 to 28, *Mycoplasma spp.*, 29 to 31 *Cytomegalovirus*, 32 to 34 *Measles*, 35 to 36 *Epstein barr virus* 37 *Varicella zoster virus*, 38 to 48 healthy serum samples.

The specificity and sensitivity of ELISA were calculated 86 and 90% for rOMP2 protein and 97.1 and 78.2 for Euroimmun commercial kit, respectively. Therefore rOMP2 protein for ELISA is more sensitive and less specific than Euroimmun commercial kit.

DISCUSSION

C. trachomatis is one of the main causes of urinary tract - genital infections (13). *C. trachomatis* is causative of 30-50% of non-gonococcal urethritis (NGU) and the most common causative of bacterial STD (14). The bacterial causative diseases such as trachoma, keratoconjunctivitis and lymphogranuloma syndrome (LGV) are *Chlamydia trachomatis*. Due to eye, neurological and genital diseases, timely and sensitive detection of *C. trachomatis* is most importance. *C. trachomatis* detection is done based on molecular and serological diagnosis, inoculation of suspected material into 8-7 day chick embryo yolk sac and culture in cells such as Hela229T, McCoy, BHK2, BGMK. Chlamydia LPS antibodies show cross reaction with *Salmonella* and *E. coli* LPS by immunoperoxidase and immunofluorescent techniques (14).

Recombinant cysteine-rich protein (OMP2) of

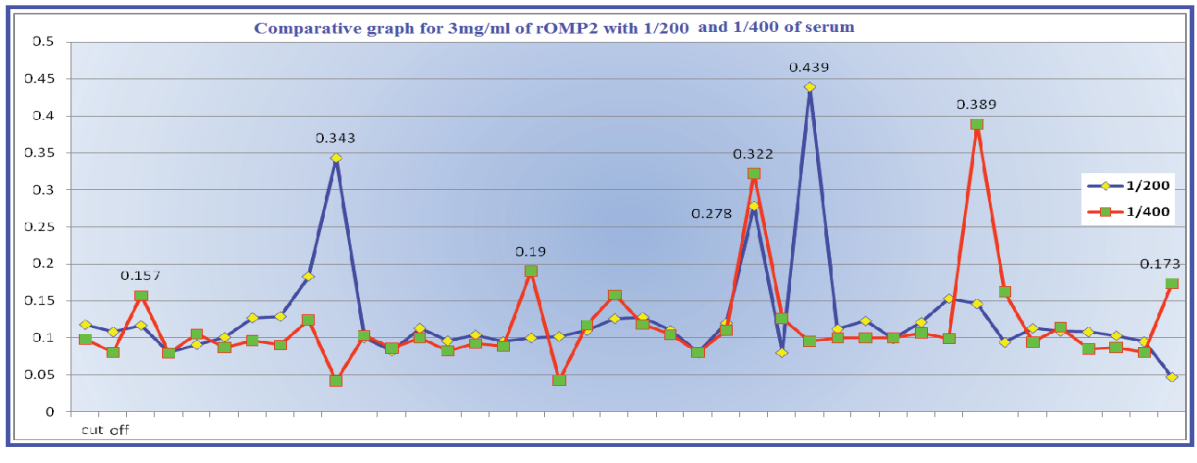


Fig. 2. Comparison of Cut off based on 1/200 and 1/400 serum dilutions. The best Cut off was obtained at 1/200 serum dilution.

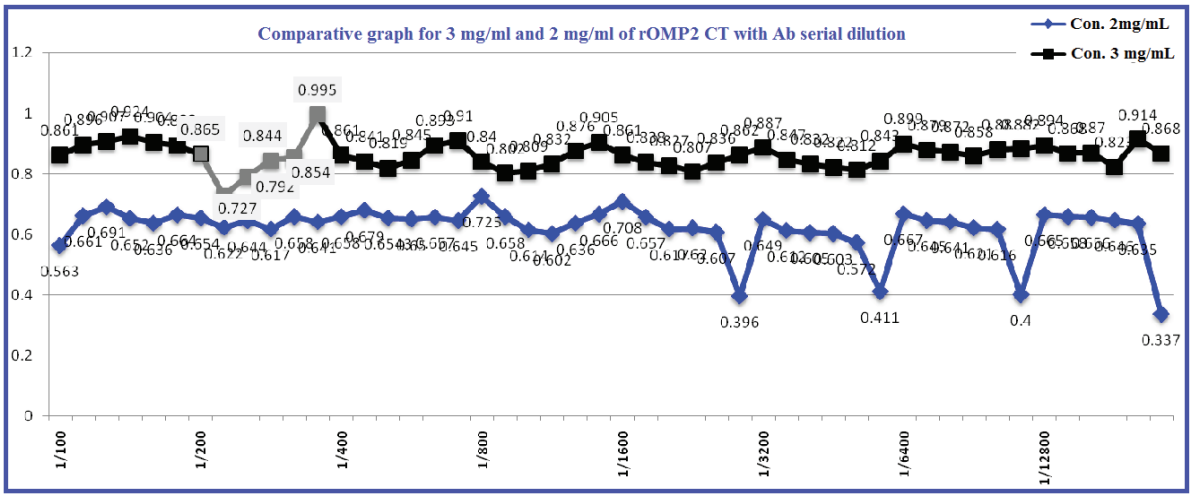
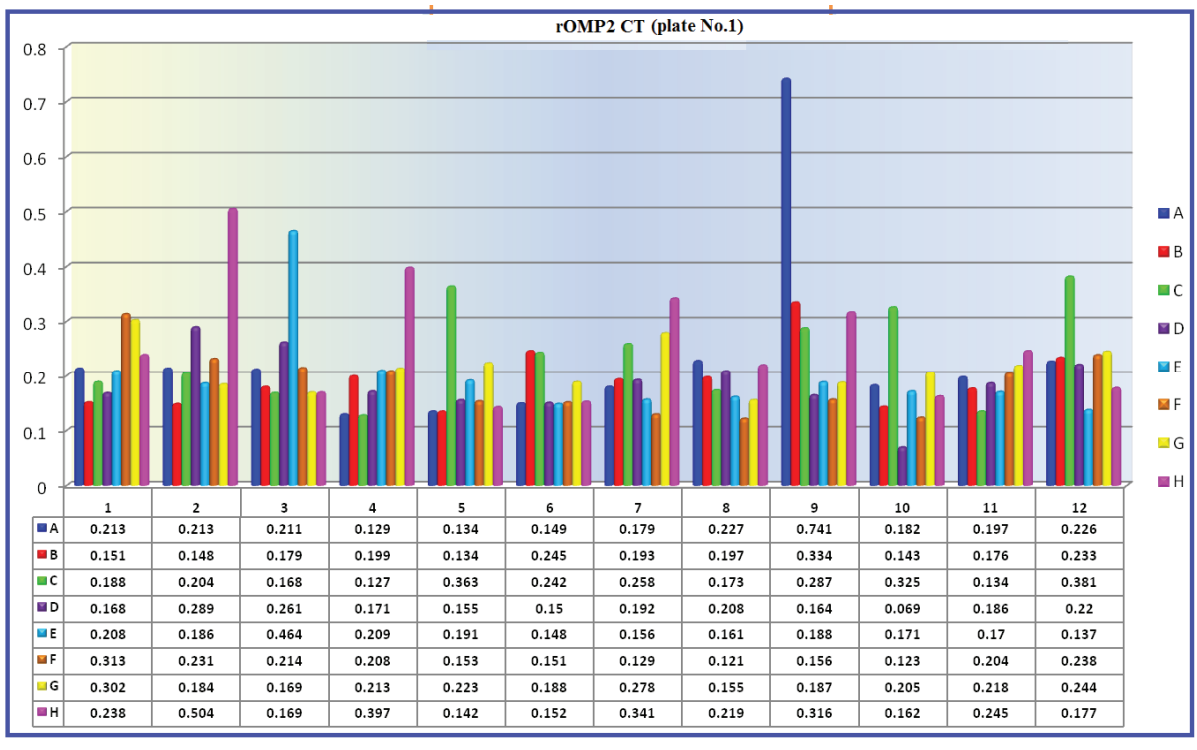


Fig. 3. Comparison of sera dilutions with 3 mg/mL and 2 mg/mL of rOMP2 protein.

C. trachomatis was prepared by Goodall *et al.* and detected activated immune response against OMP2 in peripheral blood and synovial fluid (14). Bas *et al.* used serological tests with OMP2 and MOMP antigens for detection of *C. trachomatis* and declared that OMP2 has more sensitivity and specificity than MOMP (15). Gdoura *et al.* used cell culture, PCR and ELISA methods for detection of *C. trachomatis* in men's semen and no difference was found between the methods (16).

Culture of this bacterium is difficult in the common media and it is not possible practically. Serological and molecular methods are the best tools for *C. trachomatis* detection in human fluid and tissues. Access the specific antigen is necessary for a successful serological test.

In this study, the protein of *C. trachomatis* named rOMP2 (11) was designed as specific antigen and coated on wells of ELISA microplates. Sera of patients with *Hepatitis C virus*, *Hepatitis B virus*, *Epstein barr virus*, *Helicobacter pylori*, *Toxoplasma gondii*, *Cytomegalovirus*, *Measles, Mycoplasma*, hydatid



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.213	0.213	0.211	0.129	0.134	0.149	0.179	0.227	0.741	0.182	0.197	0.226
B	0.151	0.148	0.179	0.199	0.134	0.245	0.193	0.197	0.334	0.143	0.176	0.233
C	0.188	0.204	0.168	0.127	0.363	0.242	0.258	0.173	0.287	0.325	0.134	0.381
D	0.168	0.289	0.261	0.171	0.155	0.15	0.192	0.208	0.164	0.069	0.186	0.22
E	0.208	0.186	0.464	0.209	0.191	0.148	0.156	0.161	0.188	0.171	0.17	0.137
F	0.313	0.231	0.214	0.208	0.153	0.151	0.129	0.121	0.156	0.123	0.204	0.238
G	0.302	0.184	0.169	0.213	0.223	0.188	0.278	0.155	0.187	0.205	0.218	0.244
H	0.238	0.504	0.169	0.397	0.142	0.152	0.341	0.219	0.316	0.162	0.245	0.177

Fig. 4. The OD of sera of *C. trachomatis* suspected patients in rOMP2 coated ELISA plate wells.

Table 1. Comparison of statistical T-test against 2 mg/ml of rOMP2: t = 55.1 ($p < 0.05$) and 3 mg/mL of rOMP2: t = 139.2 ($p < 0.05$). Therefore, using 2 mg/ml and 3 mg/ml of rOMP2 for ELISA are significant.

Test values / Concentration	Test Value = 0					
	t	df.	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Concentration 2	55.190	47	0.000	0.620652	0.59803	0.64328
Concentration 3	139.249	47	0.000	0.859917	0.84749	0.87234

Table 2. Results of ELISA test with rOMP2 on 192 sera samples of *C. trachomatis* suspected patients.

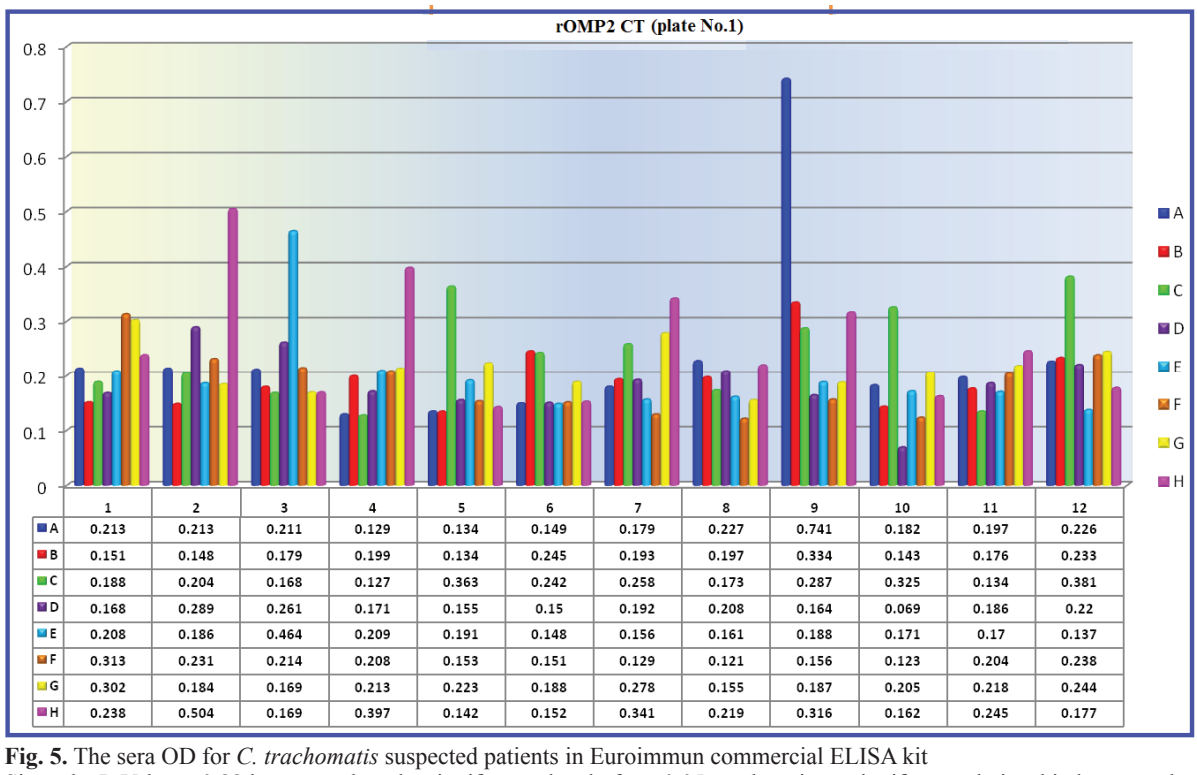
Total	**Negative**		**Positive**	
	Percent	Number	Percent	Number
192	84.37	162	15.62	30

Table 3. Results of ELISA test with Euroimmun commercial ELISA kit on 180 sera samples of *C. trachomatis* suspected patients.

Total	**Negative**		**Positive**	
	Percent	Number	Percent	Number
180	96.1	173	3.8	7

Table 4. Results of ELISA with Euroimmun commercial kit and *C. trachomatis* rOMP2.

rOMP2 ELISA(-)		rOMP2 ELISA (+)		rOMP2 ELISA2 (-)		rOMP2 ELISA (+)	
Euroimmun (+)		Euroimmun (-)		Euroimmun (-)		Euroimmun (+)	
Percent	Number	Percent	Number	Percent	Number	Percent	Number
45.4	169	54.3	102	89.7	173	9.9	37



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.213	0.213	0.211	0.129	0.134	0.149	0.179	0.227	0.741	0.182	0.197	0.226
B	0.151	0.148	0.179	0.199	0.134	0.245	0.193	0.197	0.334	0.143	0.176	0.233
C	0.188	0.204	0.168	0.127	0.363	0.242	0.258	0.173	0.287	0.325	0.134	0.381
D	0.168	0.289	0.261	0.171	0.155	0.15	0.192	0.208	0.164	0.069	0.186	0.22
E	0.208	0.186	0.464	0.209	0.191	0.148	0.156	0.161	0.188	0.171	0.17	0.137
F	0.313	0.231	0.214	0.208	0.153	0.151	0.129	0.121	0.156	0.123	0.204	0.238
G	0.302	0.184	0.169	0.213	0.223	0.188	0.278	0.155	0.187	0.205	0.218	0.244
H	0.238	0.504	0.169	0.397	0.142	0.152	0.341	0.219	0.316	0.162	0.245	0.177

Fig. 5. The sera OD for *C. trachomatis* suspected patients in Euroimmun commercial ELISA kit
Since the P-Value = 0.88 is greater than the significance level of a = 0.05, so there is no significant relationship between the rOMP2 and Euroimmun commercial ELISA kit.

cyst, *Varicella zoster virus* were examined and no cross reaction were found with ELISA by rOMP2 protein and Euroimmun commercial kit.

There were 7 positive samples (3.83%) and 173 negative samples (96.1%) of 180 suspected *C. trachomatis* sera by Euroimmun commercial ELISA kit. The specificity and sensitivity of Euroimmun ELISA kit were 97.1% and 78.2% respectively, whereas those of rOMP2 were 86% and 90%. That means ELISA assay with recombinant protein rOMP2 antigen is more sensitive but the commercial kit is more specific. Bas *et al.* reported 76% sensitivity and 85% specificity for recombinant protein and synthetic peptides in ELISA system (15).

OMP2 is an extra membrane protein of *C. trachomatis* and was described as cysteine-rich protein (containing 24 disulfide bonds) with 60 kDa by Allen in 1989 for the first time (17). Portig *et al.* declared that OMP2 is a properly antigen in ELISA system for detection of chlamydial infections (18).

In conclusion, the sensitivity and specificity of rOMP2 in ELISA for detection of *C. trachomatis* are 90% and 86%, respectively, which is more sensitive than Euroimmun commercial and its specificity is lower than Euroimmun commercial kit

REFERENCES

1. Anestad G, Lunde O, Moen M, Dalaker K. Infertility and chlamydial infection**.** *Fertil Steril* 1987; 48: 787-790.
2. Davis. B (1990), Microbiology, 4th edit. Lippincott.
3. Cliff SJ, Tabrizi S, Sullivan EA. Chlamydia in the Pacific region, the silent epidemic. *Sex Transm Dis* 2008; 35: 801-680.
4. Kane JL, Woodland RM, Forsey T, Darougar S, Elder MG. Evidence of chlamydia infection in infertile women with and withour fallopian tube obstruction. *Fertile Steril* 1984; : 843-848.
5. Binns B, Wiliams T, McDowell J, Brunham RC. Screening for *Chlamydia trachomatis* infection in pregnancy counseling clinic. *Am J Obstet Gynecol* 1988; 159: 1144-1149.
6. Lucisano A, Morandotti G, Marana R, Leone F, Branca G, Dell'Acqua S, Sanna A. Chlamydia genital infections and laparoscopic findings in infertile women. *Eur J Epidemiol* 1992; 8: 645-649.
7. Bekler C, Kultursay N, Ozacar T, Sayiner A, Yalaz M, Akisu M. Chlamydial infections in term and preterm neonates. *Jpn J Infect Dis* 2012; 65: 1-6.
8. Chojnacka K, Szczapa J, Kedzia W. [Perinatal transmission of *Chlamydia trachomatis* and its complication in preterm infants]. *Ginekol Pol* 2012; 83: 116-121.
9. Rours GI, Duijts L, Moll HA, Arends LR, de Groot R, Jaddoe VW, et al. *Chlamydia trachomatis* infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. *Eur J Epidemiol* 2012; 26: 493-502.
10. Stephens AJ, Aubuchon M, Schust DJ. Antichlamydial antibodies, human fertility, and pregnancy wastage. *Infect Dis Obstet Gynecol* 2011: 525182. doi: 10.1155/2011/525182.
11. Kazemi B, Seyed N, Bandehpour M, Sharifnia. Z, Pakzad. P. Cloning, expression and purification of truncated *Chlamydia trachomatis* outer membrane protein (OMP_2) and its application in an ELISA assay. *Iran J Immunol* 2008; 5: 148-155.
12. Bandehpour M, Khodabandeh M, Mosaffa N, Sharifnia Z, Ghazanfari T, Kazemi B. An efficient procedure for purification of recombinant human β heat shock protein 90. *DARU* 2010; 18: 64-68.
13. De Schryver A, Meheus A. Epidemiology of sexually transmitted disease: the global picture. *Bull WHO* 1990; 68: 639-654.
14. Goodall JC, Beacock-sharp H, Deane KH, Gaston JS. Recognition of the 60 kilodalton cysteine – rich outer membrane protein OMP_2 by CD_4(+) T cell from human infected with *Chlamydia trachomatis. Clin Exp Immunol* 2001; 129: 488-493.
15. Bas S, Genevay S, Schenkel MC, Vischer TL. Importance of specific antigens in the serodiagnosis of *Chlamydia trachomatis* reactive arthritis. *Rheumatology* 2002; 41: 1017-1020
16. Gdoura R, Daoudi F, Bouzid F, Ben Salah F, Chaigneau C, Sueur JM, Eb F, et al. Detection of *Chlamydia trachomatis* in semen and urethral specimens from male members of infertile couples in Tunisia. *Eur J Contracept Repord Health care* 2001; 6: 14-20.
17. Allen JE, Stephens RS. Identification by sequence analysis of two- site post translational of the cysteine- rich outer Membrane protein 2 of *Chlamydia trachomatis* serovar L2. *J Bacteriol* 1989; 171: 285-291.
18. Portig I, Goodall JC, Bailey RL, Gaston JS. Characterization of the humoral immune response to *Chlamydia* outer membrane protein 2 in chlamydia infection. *Clin Diagn lab Immunol* 2003; 41: 103-107.