Original Research Article

Comparison of immunochromatogenic method of antigen detection and Giemsa staining with polymerase chain reaction (PCR) for detection of chlamydia trachomatis infection in infertile women

K M Lohi[1], C A Kumar[2*], J M Pramanik[3], S M Baveja[4]

[1]Ex-post graduate student, [2]Associate Professor, [4]Professor and HOD, Department of Microbiology LTMMC and LTMGH, Sion, Mumbai, Maharashtra, INDIA.
[3]Scientist F and HOD, Department of Infectious Diseases Biology, National Institute for Research in Reproductive Health, Parel, Mumbai, Maharashtra, INDIA.
Email: dr.kmlohi@gmail.com

Abstract

Context: Chlamydial infection is a silent epidemic. The varied prevalence of chlamydial infection in India, leads to an urgent need to look for simple, inexpensive and sensitive tests for early diagnosis. **Aims:** To determine the utility of a rapid Chlamydia antigen detection test and Giemsa staining for screening. **Settings and design:** Prospective cross-sectional hospital based study, approved by Institutional Ethics Committee. **Material and methods:** Three endocervical specimens were collected aseptically from 119 women aged 18-45 years. Of these, 89 patients presenting with primary and secondary infertility were taken as study group and 30 healthy term pregnant women as control group. Rapid antigen detection test and Giemsa stain were done as per standard protocol. PCR was the gold standard for comparison. **Result:** Out of 119 subjects, one infertile case was positive for *C. trachomatis* infection by rapidantigen detection test and was confirmed by PCR and none was positive by Giemsa test. Sensitivity and specificity of rapid test was 100% with100% positive and negative predictive value on comparison with PCR. For Giemsa staining specificity was100% with a 99.16% negative predictive value. **Conclusion:** In the absence of facilities for gold standard tests, screening patients with an efficient rapid antigen detection testcan play a significant role in screening of *C. trachomatis* and should beincorporated in routine infertility investigations.

Key Words: Chlamydial antigen detection, infertility, *Chlamydia trachomatis.*

[*]**Address for Correspondence:**
Dr. Chaya A. Kumar, Associate Professor, Department of Microbiology, LTMMC and GH, Dr. Babasaheb Ambedkar Road, Sion West, Mumbai-400022, Maharashtra, INDIA.
Email: dr.kmlohi@gmail.com
Received Date: 13/08/2017 Revised Date: 24/09/2017 Accepted Date: 20/10/2017
DOI: https://doi.org/10.26611/1008412

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 23 October 2017

INTRODUCTION

Chlamydial infection is a silent epidemic. *Chlamydia trachomatis* is one of the most frequently detected sexually transmitted bacterial pathogen. The World Health Organization(WHO) estimated in 2008 that per year 105.7 million new cases of *Chlamydia trachomatis* infection occur worldwide.[1] With increased testing and more sensitive tests, the incidence of reported *Chlamydia trachomatis* infection has been increasing steadily since reporting began in 1984 reaching an all-time peak of 457.6 cases per 100,000 in 2011, as per CDC (Centre for Disease Control and Prevention, Atlanta).[2] Chlamydial infection in women has major clinical and epidemiological significance. Asymptomatic and untreated genital infections due to *Chlamydia trachomatis* have serious ramifications for the reproductive health of women like ectopic pregnancy, pelvic inflammatory disease, salphingitis and infertility.[3] Laboratory confirmed

How to cite this article: K M Lohi, C A Kumar, J M Pramanik, S M Baveja. Comparison of immunochromatogenic method of antigen detection and Giemsa staining with polymerase chain reaction (PCR) for detection of chlamydia trachomatis infection in infertile women. *MedPulse International Journal of Microbiology.* October 2017;4(1): 04-08. https://www.medpulse.in/Microbiology/

incidence and prevalence of chlamydial infection in otherwise healthy males and females in India is limited. Initial prevalence range from 3.3% to 33% but most of these studies have focussed on high risk groups like STIs[4]. Presently, syndromic case management of sexually transmitted infections as per WHO further precludes the true prevalence and treatment of the pathogenic causative agent. Considering the varied prevalence of chlamydial infection in India, there is an urgent need to look for tests that are simple, inexpensive and sensitive to improve diagnosis so that accurate treatment and management can be done. Hence, the present study was undertaken to study the utility of a rapid Chlamydia antigen detection test and Giemsa stain as a potential diagnostic tool for screening.

MATERIAL AND METHODS

The study was conducted in Department of Microbiology at a tertiary care hospital in Mumbai as a cross-sectional study to study the utility of a rapid Chlamydia antigen detection test and Giemsa stain as a potential diagnostic screening test. The study was approved by Institutional Ethics Committee. It was carried out for a period of one year (May 2014 to April 2015).

Data and sample collection: Three endocervical specimens were collected aseptically from 119 women aged 18-45 years., attending obstetrics and gynaecology (OBGY) Out-Patient department(OPD) at a tertiary care hospital in Mumbai. Of these, 89 patients presenting with infertility (primary or secondary) were taken as study group and 30 healthy term pregnant women of similar age group attending antenatal clinic as control group. Infertility was defined as one year of unprotected intercourse without pregnancy and it was termed as primary if conception had never occurred and as secondary when patient failed to conceive after having achieved a previous conception. [5] Women on antibiotic therapy within a month of reporting to OBGY OPD were excluded. Rapid antigen detection test and Giemsa stain were done as per standard protocol. Polymerase chain reaction was the gold standard for comparison.

Rapid antigen detection test: The rapid antigen detection test for *C. trachomatis* used was SD BIOLINE Chlamydia Rapid Test (Standard Diagnostic Inc., Korea, Lot No. 094014002, Expiry date 12.11.2015). It worked on the principle of solid phase immunochromatographic assay. Test results were interpreted at 15 minutes. The positive result was indicated by the presence of two color bands ("Test Line" and "Control Line") within the result window. The presence of only one purple band within the result window indicated a negative result. If the purple color band was not visible within the result window after performing the test, the test was considered invalid.

Polymerase chain reaction (PCR): One endocervical swab was sent as per protocol for PCR reaction to National Institute for Research in Reproductive Health, Mumbai. The test was performed in the Department of Infectious Diseases Biology, on extracted DNA using primers designed from the conserved region of MOMP gene of *C. trachomatis* with sense primer: 5' GCC GCT TTG AGT TCT GCT TCC 3' and anti-sense primer: 5' GTC GAA AAC AAA GTC ACC ATA GTA 3' to amplify a 180 bp DNA fragment common to all serotypes. DNA was isolated from cervical specimen using rapid non-enzymatic method. The cells were pelleted and re-suspended in Tris-MgCl2-KCl buffer (pH 7.4) and treated with 10% Sodium Dodecyl Sulphate at 55ºC for 10 minutes to lyse the cells. The proteins were precipitated using saturated Sodium Chloride solution. DNA was precipitated with 100% ethanol and eluted in Tris EDTA buffer. [6] The quantity and quality of DNA was estimated spectrophotometrically and by loading an aliquot of DNA on 0.8% Agarose gel. PCR for β-globin gene was also performed for each sample as an internal control to rule out the presence of inhibitory factors in the extracted specimen. The amplified products were run on 2% Agarose gel, observed under a UV transilluminator and the results were documented.[7]

Microscopy: One endocervical swab was used to perform Giemsa staining. Staining was done as per standard protocol.[8] The presence of chlamydial inclusion bodies in Giemsa stain under oil immersion was observed on microscopic examination.

Statistical Analysis: The data was statistically analyzed using SPSS version 22. Fisher's exact test and unpaired t-test was applied to test the significance wherever necessary. A p value of < 0.05 was considered significant for the study.

OBSERVATIONS AND RESULTS

Of 119 women aged 18-45 years, attending OBGY OPD at a tertiary care hospital 89 patients presenting with infertility were taken as study group and 30 healthy term pregnant women of similar age group as control. The mean age of the cases presenting with infertility was 26.6 ± 3.5years (mean ± S.D) and of control group was 27 ± 2.5years, no statistical difference was found (p=0.89). Endocervical swabs from 119 patients were subjected to PCR. One case tested positive by rapid antigen detecting test was found to be positive by Polymerase chain reaction (Figure 1 and 2) (Table 1). Taking PCR as a gold standard test for diagnosis of *C. trachomatis* infection, sensitivity and specificity of rapid diagnostic test was found 100% and 100% respectively with 100% positive predictive value and 100% negative predictive value (Table 2). Out of total 119 samples, none was positive for

C. trachomatis infection by Giemsa test though 1 case was positive by PCR (Table 3). When it was compared with results of PCR the specificity was found 100% with a 99.16% negative predictive value (Table 4).

Figure 1: Positive (50) and negative (21) tests of rapid Chlamydia antigen detection test

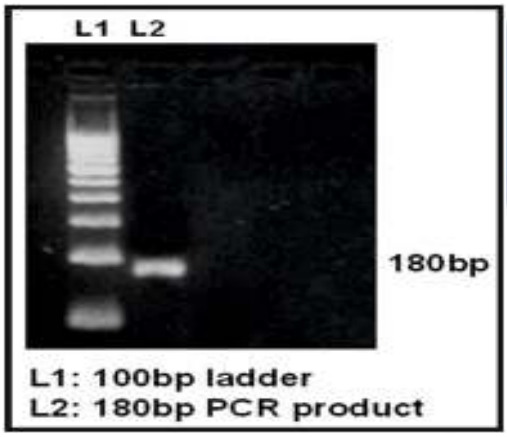

Figure 2: Agar gel electrophoresis of C. Trachomatis

Table 1: Comparison of PCR test with rapid antigen detection test

RDT	PCR (n=119)		Total
	Positive	Negative	
Positive	1	0	1
Negative	0	118	118
Total	**1**	**118**	**119**

Table 2: Prediction analysis of Rapid antigen detection test

Test	Sensitivity	Specificity	Positive Predictive value	Negative predictive value
Rapid antigen detection test	100%	100%	100%	100%

Table 3: Comparison of PCR test with Giemsa staining

Giemsa staining	PCR (n=119)		Total
	Positive	Negative	
Positive	0	0	0
Negative	1	118	119
Total	**1**	**118**	**119**

Table 4: Prediction analysis of Giemsa staining

Test	Sensitivity	Specificity	Positive Predictive value	Negative predictive value
Giemsa staining	0%	100%	-	99.16%

DISCUSSION

Out of a total 119 study cases, 1 was positive for *Chlamydia trachomatis* infection by rapid antigen detection test and it was confirmed by PCR. Hence, sensitivity and specificity of the rapid antigen detection test was found to be 100% and 100% respectively with 100% positive predictive value and 100% negative predictive value. In a study reported from Netherland by J. A. J. W. Kluytmans *et al.*[9], Clear view Chlamydia rapid detection test was compared with PCR as gold standard on 724 endocervical specimens. The rapid test gave a positivity of 35 out of 724 cases (7.5%) with 67.3% sensitivity, 99.7% specificity, 89.5% positive predictive value and 99% negative predictive value. In a study by Martin Skulnick *et al*[10], oncomparison of the Clearview Chlamydia Test and Cell Culture on 965 patients for detection of *C. trachomatis* in women in endocervical specimen, Clear view Chlamydia rapid detection test was compared with cell culture as gold standard and its sensitivity was found to be 80.4 %, specificity was 99.9%, positive predictive value 94.6% and negative predictive value 97.5%. In a study reported from China by Y-P Yin *et al* [11], 1497 cervical swabs from patients were analyzed with Clearview Chlamydia rapid detection test and was compared with PCR as gold standard. Its sensitivity was found to be 49.7%, specificity was 97.9%, positive predictive value 78.4% and negative predictive value 92.8%. In a study reported by Mahilum Tapay L. *et al*.[12] 1349 samples were analysed with *Chlamydia* Rapid test and compared with strand displacement amplification assay, sensitivity and specificity of the Chlamydia Rapid Test were 83.5% and 98.9%, positive predictive value 86.7% and negative predictive value 98.6%. In a Jannie J. van der Helm *et al*.[13] study undertaken at Netherlands, Chlamydia Rapid Test (CRT) (Diagnostics for the Real World(Europe), Cambridge, UK) compared to NAAT (Aptima, Gen-Probe, San Diego, USA) showed a sensitivity and specificity of 41.2% (95% CI, 31.9%–50.9%) and 96.4% (95% CI,95.0%–97.5%), respectively. PPV and NPV were 59.2% (95% CI, 47.5%–70.1%) and 92.9% (95% CI, 91.0%–94.5%), respectively. Thus, by comparing various studies with larger sample sizes, the rapid antigen detection tests gave a specificity of greater than 97% and above but the sensitivity ranged from 50-80%. More studies need to be done with the present rapid Chlamydia antigen detection kit to confirm the high sensitivity and specificity as the number of the patients

enrolled in the present study may not have been sufficient to give the actual performance of the test. Chlamydial rapid antigen detection test should beused as point of care test for the diagnosis of *C. trachomatis* infection to prevent various morbidities. According to a study conducted by P Vickerman *et al*.[14], the required sensitivity of a rapidpoint of care (POC) test for *C. trachomatis* is 50% (gold standard test sensitivity is 90%) if either 55% return for treatment and there is no STI transmission, or 80% return for treatment and 50% of infected women infect their partner during the delay in treatment. Furthermore, in these settings a POC test of even moderate sensitivity can lead to significantly more STI being averted than the gold standard test. These findings are implied for STI control inresource poor settings, where laboratory facilities are limited and cheap POC tests could be a useful addition to the limitations of current syndromic management approaches.[15] In the present study, Giemsa stain was used to detect the inclusion bodies of *C. trachomatis* microscopically. Out of a total 119 subjects, none was positive for *C. trachomatis* infection by Giemsa staining, though 1 case was positive by PCR. Hence the specificity of Giemsa staining for detecting inclusion bodies of *C. trachomatis* was found 100% and 99.17% negative predictive value with 0% sensitivity on comparing with PCR which was taken as the gold standard. Max A Chernesky in his review on the laboratory diagnosis of *Chlamydia trachomatis* infections (2005)[16] has stated that cytological testing by Giemsa staining to detect inclusions is particularly useful in diagnosing acute inclusion conjunctivitis of the newborn. He has given a sensitivity exceeding 90% for this method. He has also said that cytological testing is relatively insensitive when diagnosing adult conjunctival and genital tract infections. More studies with larger sample size need to be done to find the usefulness of microscopy for diagnosing Chlamydial infection as the present study could not detect chlamydial infectionon microscopy. Based on present study observations rapid test for *C. trachomatis* detection can be considered as a better screening test than compared to Giemsa staining.

CONCLUSION

C. trachomatis can cause infection at any point in the reproductive life leading to long term complications like tubal factor infertility, so screening of the infertile patients in the initial stages of infertility is recommended for an early therapeutic intervention and to prevent further complications. In the absence of facilities for culture, direct fluorescent antibody test, and PCR which have a high sensitivity and specificity in the diagnosis of *C. trachomatis*, screening patients with an efficient rapid antigen detection test can play a significant role in screening of *C. trachomatis* and should be incorporated in routine infertility investigations. Further studies with a larger sample size would more accurately determine the prevalence, risk factors and extent of infertility caused by *Chlamydia trachomatis*.

REFERENCES

1. Jane R, Igor T, Ndowa F. Global incidence and prevalence of selected curable sexually transmitted infections – 2008. In World Health Organization; 2012. p. 1–5.
2. Charlotte A. Gaydos, Thomas C. Quinn. Chlamydial infections. In: Harrison's Principles of Internal Medicine. 19th ed. Mc Graw Hill; 2015. p. 1165–74.
3. Patel AL, Sachdev D, Nagpal P, Chaudhry U, Sonkar SC, Mendiratta SL, Saluja. Prevalence of Chlamydia infection among women visiting a gynaecology outpatient department: evaluation of an in-house PCR assay for detection of Chlamydia trachomatis. Annals ClinMicrobiolAntimicrob 2010; 9-24.
4. Vidwan NK, Regi A, Steinhoff M, Huppert JS, Staat MA, et al. Low Prevalence of Chlamydia trachomatis Infection in Non-Urban Pregnant Women in Vellore, S. India. PLoS ONE 2012; 7(5): e34794.
5. Padubidri VG DSN, editor. Infertility and sterility. In: Shaw's Textbook of Gynaecology. 16th ed. Elsevier; 2015. p. 237–62.
6. Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for thepreparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 1991;19:5444.
7. Mania-pramanik J, Potdar ÃS, Kerkar S. Diagnosis of Chlamydiatrachomatis Infection. J Clin Lab Anal. 2006;20:8–14.
8. Cheesbrough Monica. Microscopical techniques used in microbilogy. In:District Laboratory Practice in Tropical Countries. 2nd ed. 2006. p. 35–45.
9. Kluytmans JA, Goessens WH, Mouton JW, van Rijsoort-Vos JH, Niesters HG, et al. Evaluation of Clearview and Magic Lite Tests, Polymerase Chain Reaction, and Cell Culture for Detection of Chlamydia trachomatis in Urogenital Specimens. JClin Microbiol. 1993;31(12):3204–10.
10. Skulnick M, Small GW, Simor AE, Low DE, Khosid H, Fraser S, et al. Comparison of the Clearview Chlamydia test, Chlamydiazyme, and Cell Culture for Detection of Chlamydia trachomatis in Women with a Low Prevalence of Infection. J Clin Microbiol. 1991;29(9):2086–8.
11. Yin YP, Peeling RW, Chen XS, Gong KL, Zhou H, Gu WM, et al. Clinic-based evaluation of Clearview Chlamydia MF for detection of Chlamydia trachomatis in vaginal and cervical specimens from women at high risk in China. Sex Transm Infect. 2006;82(Suppl V):v33–7.
12. Mahilum-Tapay L, Laitila V, Wawrzyniak J, Lee H, Alexander S, Ison C et al. New point of care Chlamydia Rapid Test bridging the gap between diagnosis and treatment: performance evaluation study. BMJ. 2007;335(7631):1190-1194.
13. Van der Helm JJ, Sabajo LOA, Grunberg AW, Morre´ SA, Speksnijder AGCL, et al. (2012) Point-of-Care Test

for Detection of Urogenital Chlamydia inWomen Shows Low Sensitivity. A Performance Evaluation Study in Two Clinics in Suriname. PLoS ONE 7(2): e32122. doi:10.1371/journal.pone.0032122
14. Vickerman P. Sensitivity requirements for the point of care diagnosis of Chlamydia trachomatis and Neisseria gonorrhoeae in women. Sex Transm Infect. 2003;79(5):363-367.
15. Ward H. Validity and utility of screening tests for STIs. Sex Transm Infect. 2003;79(5):356-357.
16. Chernesky MA. The laboratory diagnosis of Chlamydia trachomatis infections. Can J Infect Dis Med Microbiol. 2005;16(1):39–44.

Source of Support: None Declared
Conflict of Interest: None Declared