

## Research Article

# Study of seroprevalance of *Toxoplasma gondii*, Rubella virus and Cytomegalovirus (ToRC) infections in antenatal women presented with bad obstetric history and comparative evaluation of Nanoplex ToRCH screen ELISA kit with VIDAS

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## ABSTRACT

**Background:** Infections caused by *Toxoplasma gondii*, Rubella virus and Cytomegalovirus are major causes of Bad Obstetric History (BOH). Cause of BOH may be genetic, hormonal, abnormal maternal immune response, and maternal infection. Women affected with any of these diseases during pregnancy are at high risk for miscarriage, stillbirth, or for a child with serious birth defects and/or illness and also a hazard to attending staff nurses.

**Methods:** A total 96 serum samples were collected from antenatal women with BOH attending the out-patient services of department of gynaecology at NRI general hospital, Chinakakani, Guntur district, Andhra Pradesh. Serum samples were obtained and were subjected to screening for Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies of *Toxoplasma gondii*, Rubella Virus and CMV infections by VIDAS (bioMerieux, France) and Nanoplex ToRCH Screen kit [Lilac Medicare (P) Ltd, Maharashtra, India].

**Results:** Majority of cases with BOH were found in females aged 18-23 years (25, 52.08%) followed by 24-29 years (18, 37.5%). Congenital anomalies and other complications were found to be more in age group 18-23 years followed by 24-29 years. The disease prevalence as studied with respect to IgM antibodies was found to be 31.25% for Cytomegalovirus Infections, 23.96% for *Toxoplasma gondii*, 21.88% for Rubella virus infections. The overall agreement in the Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) between VIDAS and Nanoplex ToRCH Screen kit for the detection of specific IgM and IgG antibodies in our study was excellent with sensitivity ranging from 90.91%-96.00% and specificity ranging from 89.47%-95.59% for the detection of IgM & IgG antibodies. The discrepancies were relatively less with 8.3% for CMV IgM, 6.2% for CMV IgG, 5.20% for Rubella IgM, 6.25% for Rubella IgG, 6.25% for Toxo IgM and 5.20% for Toxo IgG.

**Conclusion:** Nanoplex ToRCH Screen Kit is a cheap, cost effective, efficient and innovative alternate for the diagnosis of ToRCH infections. As the technology is new, it needs to be further explored bring out various multiplex kits for diagnosing other infectious diseases.

**Keywords:** BOH, TORCH, VIDAS, Nanoplex ToRCH Screen Kit

## INTRODUCTION

Bad Obstetric History (BOH) implies to previous unfavourable foetal outcome in terms of two or more consecutive abortion, history of intrauterine foetal death, intrauterine growth retardation, still birth, early neonatal death and/or congenital anomalies. Cause of BOH may be genetic, hormonal, abnormal maternal immune response, and maternal infection.<sup>1</sup> Recurrent pregnancy wastage due to maternal infections transmissible in utero at various stage of gestation can be caused by a wide array of organisms which include the ToRCH complex (*Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus) and other agents like Chlamydia trachomatis, *Treponema pallidum*, *Neisseria gonorrhoeae*, HIV etc.<sup>2</sup>

Toxoplasmosis acquired during pregnancy may cause damage to the fetus.<sup>2,3</sup> Sero epidemiological studies have shown that 10-20 percent of women in childbearing age in India are susceptible to Rubella infection.<sup>2,4</sup> Infection with Rubella during pregnancy may lead to congenital malformation in 10-54 percent of cases.<sup>2,5</sup> The infection caused by CMV in adult is usually asymptomatic but its significance is many times increased when it occurs during pregnancy. However the rate of primary CMV infection is significantly higher for pregnant women from low socioeconomic group.<sup>2,6</sup>

Women affected with any of these diseases during pregnancy are at high risk for miscarriage, stillbirth, or for a child with serious birth defects and/or illness and also a hazard to attending staff nurses.<sup>7,8</sup> Thus these tests are performed before or soon as pregnancy is diagnosed to determine the mother's exposure to *Toxoplasma*, Rubella virus, cytomegalovirus and Herpes Simplex virus and the necessary precautions be taken.<sup>7</sup>

This study was selected and done primarily to calculate the seroprevalance of *Toxoplasma gondii*, Rubella and CMV infections and secondarily to evaluate the performance of Nanoplex ToRCH screen IgG/IgM NanoELISA (Nanoplex ToRCH Screen kit) for the demonstration of IgM and IgG antibodies (Abs) in comparison with Vitek Immuno-Diagnostic Assay System (VIDAS).

## METHODS

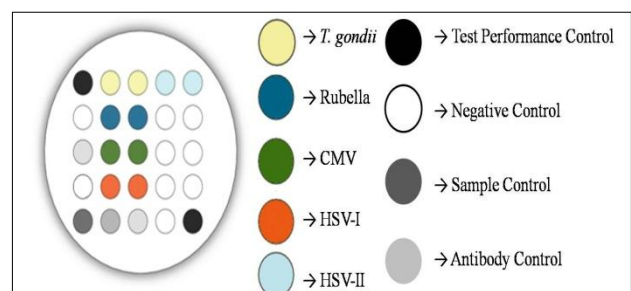
A total 96 serum samples were collected from antenatal women with BOH attending the out-patient services of department of gynaecology at NRI general hospital, Chinakakani, Guntur district, Andhra Pradesh. Patients included in our study were those who had history of 2-3 pregnancy wastages due to intrauterine deaths, preterm deliveries, intrauterine growth retardation and unexplained early neonatal deaths.

3 ml of venous blood was collected aseptically from the enrolled patients after duly collecting their informed consent. Serum samples were obtained and were subjected to screening for Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies of *Toxoplasma gondii*, Rubella virus and CMV infections by VIDAS (bioMerieux, France) and Nanoplex ToRCH screen kit (Lilac Medicare (P) Ltd, Maharastra, India).

All the samples collected were studied to evaluate the performance of Nanoplex ToRCH Screen kit in comparison with VIDAS, considering VIDAS as the reference standard as evidenced by earlier studies.<sup>9,10</sup> All the discrepant results between the assays were retested by collecting a fresh sample after 4 weeks from the date of first sample. The results obtained on retesting were considered final for the analysis. The reasons for discrepant results could be attributed to the different nature of antigens used in the assays.

VIDAS system is an Enzyme Linked Fluorescent Assay (ELFA) and it combines a two-step enzyme Immunoassay for IgG and an Immunocapture method for IgM with a final fluorescent detection. The VIDAS IgG assay uses a solid phase receptacle coated with membrane and cytoplasmic antigen (RH sabin stain grown in mice). The conjugate uses alkaline phosphatase labelled mouse monoclonal antihuman IgG antibody and 4-methyl umbelliferyl phosphate as a substrate.<sup>9,11,12</sup> The VIDAS IgM assay uses a solid phase receptacle coated with polyclonal anti-human IgM antibody (goat). The conjugate contains an immune complex and alkaline phosphatase labelled anti- P30 mouse monoclonal antibody and 4-methyl umbelliferyl phosphate is used as a substance.<sup>9,11,12</sup>

Nanoplex ToRCH Screen kit is a NanoELISA based multiplex immunoassay system designed and developed for detection of IgG and/or IgM antibodies in human sera against *Toxoplasma gondii*, Rubella, CMV, Herpes Simplex Virus (HSV) I & II. This in-vitro test is intended as an aid in detecting exposure and infection with any of the five ToRCH Pathogens. The multiplex immunoassay format allows the user of this test system to report individual analytes as well as ToRCH panel (Figure 1). The detection of antibodies to pathogenic antigens is based on the principle of ELISA.



**Figure 1: Figure showing the arrangement of antigen coated spots in Nanoplex ToRCH screen kit along with its controls.**

## RESULTS

**Table 1: Age wise distribution of cases with bad obstetric history.**

Age group (years)	Spontaneous abortion	Early neonatal death	Congenital Abnormality	Cases with more than 2 complications*	Total No. of patients
18-23	36	-	8	6	50
24-29	24	2	3	8	37
30-35	6	-	1	2	9
>35					Nil

\*Includes cases with intrauterine growth retardation, intrauterine foetal death along with any of the other specified in the Table.

All the 96 serum samples obtained from the patients were tested to study the seroprevalance of ToRC infections by VIDAS. The disease prevalence as studied with respect to IgM antibodies was found to be 31.25% for Cytomegalovirus Infections, 23.96% for Toxoplasma gondii, 21.88% for Rubella virus infections.

### **Comparison of Nanoplex ToRCH screen kit with VIDAS**

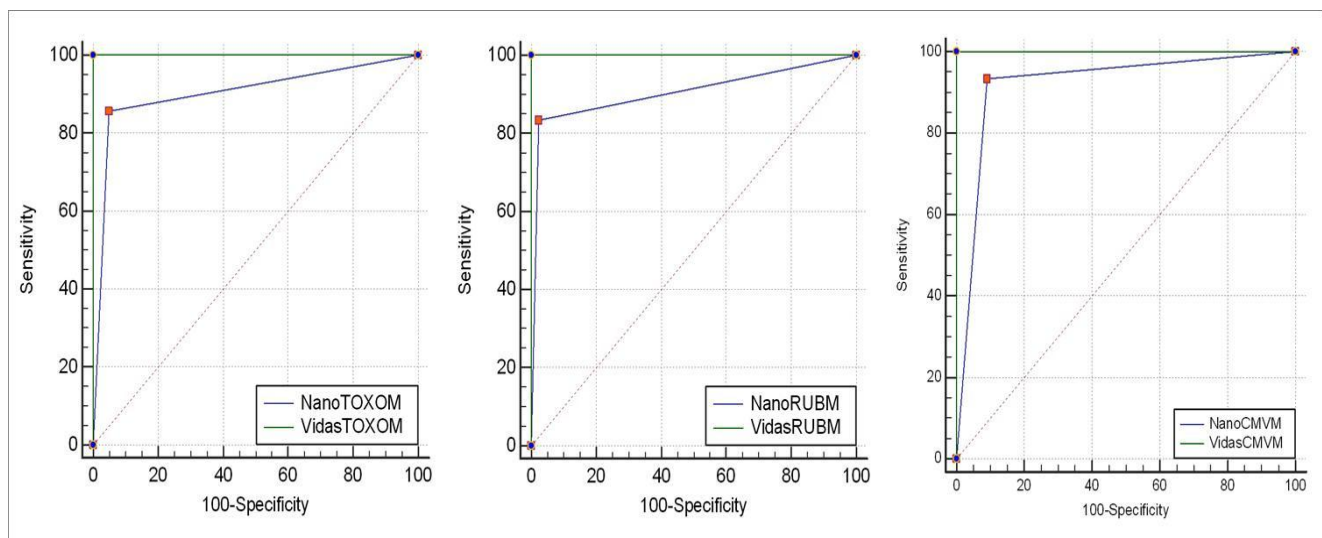
The results obtained were compared and statistically evaluated using Receiver Operating Characteristic (ROC) curve analysis. Area Under Curve (AUC) was compared between the kits and sensitivity and specificity of the Nanoplex ToRCH screen kit was obtained.

AUC - ROC Analysis of evaluation of IgM Antibodies: (Figure 2).

The sensitivity and specificity of Nanoplex ToRCH Screen kit for CMV IgM antibodies was 93.33% & 94.74% respectively with an AUC of 0.921.

The sensitivity and specificity of Nanoplex ToRCH screen kit for Rubella IgM antibodies was 90.48% & 94.81% respectively with an AUC of 0.905.

The sensitivity and specificity of Nanoplex ToRCH screen kit for Toxoplasma gondii IgM antibodies was 91.30% & 94.52% respectively with an AUC of 0.904.



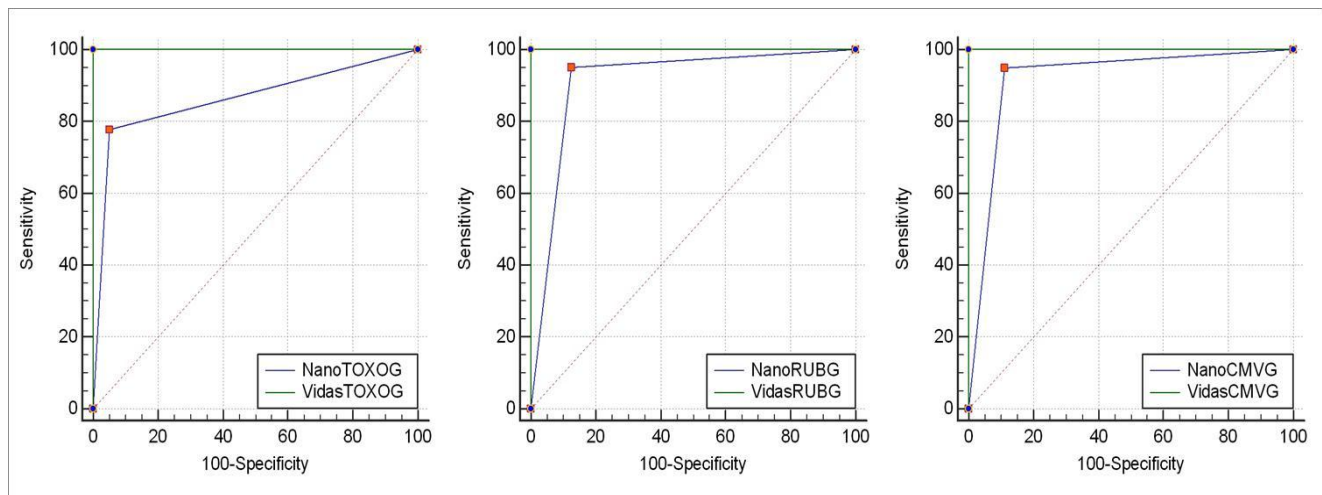
**Figure 1: AUC showing correlation between VIDAS and Nanoplex ToRCH screen kit for IgM antibody detection.**

AUC - ROC Analysis of evaluation of IgG Antibodies: (Figure 3).

The sensitivity and specificity of Nanoplex ToRCH screen kit for CMV IgG antibodies was 90.91% & 90% respectively with an AUC of 0.919.

The sensitivity and specificity of Nanoplex ToRCH screen kit for Rubella IgG antibodies was 96% & 89.47% respectively with an AUC of 0.913.

The sensitivity and specificity of Nanoplex ToRCH screen kit for Toxoplasma gondii IgG antibodies was 92.86% & 95.59% respectively with an AUC of 0.863.



**Figure 2: AUC showing correlation between VIDAS and Nanoplex ToRCH screen kit for IgG antibody detection.**

## DISCUSSION

Infection with one of the ToRCH pathogens contracted during pregnancy may be passed through placenta to the fetus affecting the fetus and new-born potentially causing serious birth defects. Asymptomatic infants may develop abnormalities later in life.<sup>13</sup> The infections caused by TORCH organisms are grouped together because they all result in serious birth defects when transmitted from an infected mother to her foetus during pregnancy.<sup>13</sup> Maternal infections play a critical role in pregnancy wastage and their occurrence in patients with BOH or complicated pregnancy is a significant risk factor.<sup>4,14</sup> These infections cause fetal and neonatal mortality and an important contributor to early and later childhood morbidity.<sup>4,14</sup> Previous history of pregnancy wastage and the serological reactions for TORCH infections during

current pregnancy must be considered while managing BOH cases to reduce the adverse foetal outcome.<sup>15</sup>

In the present study the disease prevalence was found to be 31.25% for Cytomegalovirus Infections, 23.96% for Toxoplasma gondii, 21.88% for Rubella virus infections. This is almost comparable with the seroprevalence of TORCH infection in BOH patients (Table 2). Toxoplasma gondii, a known etiological agent for recurrent pregnancy wastage showed true positivity of 23.9% in IgM and 29.1% true positivity for IgG Antibody in our study. Primary CMV infection causes symptomatic congenital infection and fetal loss. IgM antibodies of CMV showed 31.2% true positivity and 79.1% of IgG antibodies true positivity. For Rubella antibodies 21.8% of IgM antibodies and 80.2% of IgG antibodies are considered true positive.

**Table 2: Comparison of seroprevalance of ToRC infections.**

Agent	Turbadkar et al. <sup>2</sup>	Padmavathy et al. <sup>7</sup>	Sashi Chopra et al. <sup>16</sup>	Present study
Toxoplasma gondii	10.5 %	8.3 %	42.5 %	23.96 %
Rubella	26.8 %	16.7 %	17.5 %	21.88 %
Cytomegalovirus	8.4 %	33.3 %	29.5 %	31.25 %



In our study, due to lack of molecular diagnosis, VIDAS results were taken as reference standard as it has a variable sensitivity ranging from 90.24%-100% and specificity of 99.25%-100% as evident from the kit literature. VIDAS has been evaluated by many previous studies and studies have shown for Toxoplasma IgG a sensitivity of 99.59% and specificity of 98.57% and for Toxoplasma IgM a sensitivity of 76.74% and specificity of 99.76%.<sup>3</sup>

The overall agreement in the Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) between VIDAS and Nanoplex ToRCH screen kit for the detection of specific IgM and IgG antibodies in our study was excellent (Table 3). The discrepancies were relatively less with 8.3% for CMV IgM, 6.2% for CMV IgG, 5.20% for Rubella IgM, 6.25% for Rubella IgG, 6.25% for Toxo IgM and 5.20% for Toxo IgG.

**Table 3: Sensitivities, specificities of Nanoplex ToRCH screen kit for ToRC specific antibody detection.**

		Sensitivity		Specificity		Positive predictive value	Negative predictive value
		%	95% CI	%	95% CI		
Toxoplasma gondii antibody	IgM	91.30	71.92 - 98.68%	94.52	86.55 - 98.45%	84.00%	97.18%
	IgG	92.86	76.46 - 98.92%	95.59	87.63 - 99.03%	89.66%	97.01%
Rubella antibody	IgM	90.48	69.58 - 98.55%	94.81	88.74 - 99.12%	86.36%	97.30%
	IgG	96.00	87.22 - 98.53%	89.47	66.82 - 98.39%	97.33%	80.95%
Cytomegalovirus antibody	IgM	93.33	77.89 - 98.99%	94.74	81.25 - 96.57%	82.35%	96.77%
	IgG	90.91	87.06 - 98.52%	90.00	68.26 - 98.47%	97.30%	81.82%

Nanoplex ToRCH screen kit also performed well when compared with each established assay individually. The assay instrument has many advantages like, the sample volume required is very low (5 µL) for testing all parameters of the ToRCH panel, it tests all the samples in duplicate in one well, it is very cost effective with pricing below 100 rupees per panel, and it's software based data analysis and inclusion of internal controls to monitor the individual assay performance provides confidence in testing and interpretation of results.

To conclude, Nanoplex ToRCH screen kit is a cheap, cost effective, efficient and innovative alternate for the diagnosis of ToRCH infections. As the technology is new, it needs to be further explored to bring out various multiplex kits for diagnosing other infectious diseases.

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## REFERENCES

1. Sabreen AA, Kamal, Ruqaya MJ, Awadh, Ali HM, Al-Marzoqi. Genetic study of ToRCH Infections in women with bad obstetric history: multiplex polymerase chain reaction for detection of common pathogens and agents of congenital infections. J Biol Agriculture Healthcare. 2013;3(18):49-53.
2. Turbadkar D, Mathur M, Rele M. Seroprevalence of torch infection in bad obstetric history. Indian J Med Microbiol. 2003;21:108-10.
3. Sharma P, Gupta T, Ganguly NK, Mahajan RC, Malla N. Increasing Toxoplasma seropositivity in women with bad obstetric history and in new-borns. Natl Med J. 1997;10:65-6.
4. Seth P, Manjunath N, Balaya S. Rubella infection: the Indian scene. Rev Infect Dis. 1985;7(Suppl 1):S64-7.
5. Peekam C. Congenital infections in the United Kingdom before 1970; the prevaccine era. Rev Infect Dis. 1985;7(Suppl 1):S11.
6. Stagno S, Pass RF, Cloud G, Britt WJ, Henderson RE, Walton PD, et al. Primary Cytomegalovirus infection in pregnancy: incidence, transmission to fetus, and clinical outcome. JAMA. 1986;256:1904-8.
7. Padmavathy M, Mangala Gowri, Malini J, Umapathi BL, Navaneeth BV, Mohit Bhatia, et al. Seroprevalence of ToRCH infections and adverse reproductive outcome in current pregnancy with bad obstetric history. J Clin Biomed Sci. 2013;3(2):62-71.

8. Young AB, Reid D, Grist NR. Is Cytomegalovirus a serious hazard to female hospital staff? *Lancet.* 1983;321(8331):975-6.
9. Bernard Weber, Marisol Badiel, Yanet Alvarez-Otero, Philippe Thulliez, José G. Montoya. Comparative evaluation of AxSYM, VIDAS and VIDIA toxoplasmosis reagent performance in a high seroprevalence Latin American country. *Sientia Medica (Porto Alegre).* 2010;20(1):27-34.
10. Hopson DK, Niles AC, Murray PR. Comparison of the Vitek immunodiagnostic assay system with three immunoassay systems for detection of cytomegalovirus-specific immunoglobulin G. *J Clin Microbiol.* 1992;30(11):2893-5.
11. Pelloux H, Ciapia P, Goullier-Fleuret A, Ambroise-Thomas P. Evaluation dd system Vidas pour le diagnostic serologique de la toxoplasmose. *Ann Biol Clin.* 1993;5:875-8.
12. Dave J, Balfour AH, Perkin J. Evaluation of the VIDAS toxo competition assay for the detection of antibodies specific to *Toxoplasma gondii* in human sera. *Sero diagn immunother Infect Disease.* 1994;6:41-5.
13. Sadik MS, Fatima H, Jamil K, Patil C. Study of TORCH profile in patients with bad obstetric history. *Biol Med.* 2012;4(2):95-101.
14. Binnicker MJ, Jespersen DJ, Harring JA. Multiplex detection of IgM and IgG class antibodies to *Toxoplasma gondii*, Rubella virus, and Cytomegalovirus using a Novel Multiplex flow immunoassay. *Clin Vaccine Immunol.* 2010;17(11):1734-8.
15. Kumari N, Morris N, Dutta R. Is screening of ToRCH worthwhile in women with bad obstetric history: an observation from Eastern Nepal? *J Health Popul Nutr.* 2011;29(1):77-80.
16. Shashi C, Usha A, Aruna A. Prevalence of IgM antibodies to *Toxoplasma*, Rubella and Cytomegalovirus infections during pregnancy. *JK Sci.* 2004;6(4):190-3.

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