OPEN ACCESS

GAVIN PUBLISHERS

Infectious Diseases Diagnosis & Treatment

Research Article

Zhang N. et al. Infect Dis Diag Treat 3: 133.
DOI:10.29011/2577-1515.100133

Quantitative Detection of Rubella Virus, Cytomegalovirus, and Toxoplasma Gondii Serum Antibodies During Pregnancy Using LIAISON®XL

Ning Zhang*[1], Xin Cui[3], Wei Wei[3], Xingchi Liu[3], Yuexin Yu*[2]

[1,2]Center of Reproductive Medicine, General Hospital of Northern Threater Command, No 83. Wenhua Road, Shenhe District, Shenyang, China

[3]Center of Reproductive Medicine, General Hospital of Northern Threater Command, China

Corresponding author(s): Ning Zhang, Center of Reproductive Medicine, General Hospital of Northern Threater Command, No 83. Wenhua Road, Shenhe District, Shenyang, China

Yuexin Yu, Center of Reproductive Medicine, General Hospital of Northern Threater Command, No 83. Wenhua Road, Shenhe District, Shenyang, China

Citation: Zhang N, Cui X, Wei W, Liu XC, Yu YX (2019) Quantitative Detection of Rubella Virus, Cytomegalovirus, and *Toxoplasma Gondii* Serum Antibodies During Pregnancy Using Liaison®Xl. Infect Dis Diag Treat 3: 133. DOI:10.29011/2577-1515.100133

Received Date: 22 November, 2019; **Accepted Date:** 05 Deceember, 2019; **Published Date:** 11 Deceember, 2019

Abstract

To provide reliable results and outline the decision process involved in quantitative antibody detection for rubella virus, Cytomegalovirus (CMV) and *Toxoplasma gondii*, eliminate inappropriate clinical intervention, and provide effective prevention and treatment during pregnancy.

Serum samples from pregnant women and from women before pregnancy were collected from Aug. 2013 to Jan. 2016, in General Hospital of Northern Threater Command , Shenyang, China. The total number of samples was 14,852 for rubella virus, 14,190 for CMV and 12,951 for *Toxoplasma gondii* respectively. The samples which returned a positive result were analyzed using dynamic analysis of antibody concentration in sequential samples, combined with the algorithm set out in China's ToRCH guidelines.

After using the Chinese algorithm, of the women who were both IgG and IgM positive for rubella or CMV or *Toxoplasma*, 44 cases of IgG and IgM-positive rubella were reported. 33 fetuses from these women were completely normal after birth, 6 were aborted for the traditional reason (if IgM is positive, then abortion is suggested), 1 was born with a chromosome abnormality, 1 presented with fetal dysplasia and 3 were lost at follow-up. There were also 4 positive cases of CMV; of these, 3 fetuses were completely normal after birth and 1 fetus was lost at follow-up. 3 women tested positive for *Toxoplasma*, and all of their fetuses were normal after birth. Out of the 22 positive cases for both rubella IgG and IgM before pregnancy, 5 women decided to initiate pregnancy and no abnormal consequences were found, whilst the other 17 refused to initiate pregnancy due to fear of birth defects. 19 other cases with only Toxoplasma IgM-positive result were proven as false positives using dynamic quantitative analysis. They continued their pregnancy, and the fetuses were born with no abnormalities.

Dynamic quantitative detection combined with the algorithm given in the Chinese ToRCH guidelines can eliminate inappropriate clinical intervention.

Keywords: CMV; LIAISON ®XL; Pregnancy; Quantitative analysis; Rubella; *Toxoplasma gondii*

NLC classification number: R714.5 Document code: C

Introduction

ToRCH screening during pregnancy was proposed in the 1960s [1], but it led to controversy for many reasons. Chief amongst these was that the detection methods cannot give the results of quantitative testing, yielding false results when there are positive screening indexes. With the progress of prenatal diagnosis techniques, especially improvements in fetal ultrasonic diagnosis and the introduction of the nuclear magnetic resonance technique, congenital fetal malformation has increasingly been found to be related to virus infection [2,3]. In recent years, European countries and Canada have established screening guidelines on rubella, CMV and *Toxoplasma* during pregnancy. The Guidelines on ToRCH Screening during Pregnancy, issued by the Family

Planning and Sound Child Rearing Specialized Committee of the Chinese People's Liberation Army (PLA) in 2014, clearly state that "Quantitative analysis is the progress and best choice for ToRCH screening" [4]. Specialist Consensus on the Screening, Diagnosis and Intervention Principle and Work Flow for ToRCH infections, which was issued in 2016, further provides that "The quantitative technique should be used in the detection of ToRCH IgM and IgG antibodies". However, at present, it is general practice to report only positive or negative detection results, and to use the traditional interpretation procedure, where IgM positivity is identified as a recent infection, while IgG positivity is identified as previous infection. Due to the IgM false positive and persistence problems that exist in immunoassay, this traditional interpretation procedure has had a serious bearing on clinical diagnosis, and resulted in improper clinical interventions. In this paper we summarized the results of a prospective study to underline the importance of TORCH quantitative serological application. The traditional methods available in China on ToRCH serological application are only qualitative . With qualitative assays it is difficult to follow up the increase of antibodies during acute infection. In this study we used the quantitative analysis method to detect IgG and IgM antibodies of rubella, CMV and *Toxoplasma gondii*; established the result assessment method and process; followed up the pregnancy outcomes; summarized the significance and role of quantitative detection of antibodies, and reported consequently. (As there are no quantitative detection methods and reagents for HSV antibodies at present, they are not discussed here) [5].

Materials and Methods

Patients

In this prospective study the number of patients screened for rubella, CMV and *Toxoplasma gondii* before and during pregnancy in the Reproductive Center laboratory of Shenyang Northern Theater General Hospital from August 1, 2013 to January 1, 2016, was 14,852, 14,190 and 12,951 respectively. In two group of women with IgM positive antibodies. the dynamic analysis of antibody concentration in sequential samples was performed

In a first group of 70 pregnant women, 66 patient have been successfully followed up, and 4 patients were lost during the following up.In a second group of 22 women not pregnant at the beginning of the follow-up with IgM positive, during the follow -up, 5 decided to become pregnant, and 17 did not

All patients gave their informed consent, and all the experimental processes were reviewed by the hospital's ethics committee.

Instrument and reagents

During infectious diseases, the immune system is stimulated to produce antibodies (IgM at first and then IgG) against the infectious pathogen. To follow the increase of antibodies during ToRCH acute infection we used chemiluminescence quantitate immunoassays.

The chemiluminescence immunoassay analyzer and reagents we used were: LIAISON®XL Rubella, CMV, Toxoplasma IgG and IgM chemiluminescence immunoassay manufactured by DiaSorin (Saluggia, Italy) for the quantitative detection of IgG and IgM antibodies to rubella virus, cytomegalovirus and *Toxoplasma gondii*. The assays were used according to the instruction for use. LIAISON® XL assays for detection of Toxoplasma IgG, CMV IgG and IgM and Rubella IgG use two-step, indirect, chemiluminescent immunoassays. In the first incubation step, pathogen-specific antibodies in samples/controls bind to the solid phase (pathogen-coated magnetic particles); in the second step, an antibody conjugate (containing a murine monoclonal antibody linked to an antibody-isoluminol conjugate) reacts with the solid phase-bound pathogen-specific IgG and IgM antibodies. Unbound material is removed by washing after each incubation. Starter reagents are then added to induce the chemiluminescent reaction, which is measured as relative light units by a photomultiplier device. LIAISON® XL assays for detection of Toxoplasma IgM and Rubella IgM are antibody capture chemiluminescence immunoassays. In the first incubation step, IgM antibodies in samples/controls bind to the solid phase (mouse monoclonal IgG to human IgM-coated magnetic particles); in the second step, an antibody conjugate (containing a murine monoclonal antibody linked to an antibody-isoluminol conjugate) reacts with the solid phase-bound pathogen- specific IgG and IgM antibodies. Unbound material is removed by washing after each incubation. During the third incubation, the isoluminol-antibody conjugate reacts with the immune complex formed during the second incubation, thus revealing that the immunological reaction has taken place. After the third incubation, the unbound material is removed with a wash cycle. Starter reagents are then added to induce the chemiluminescent reaction, which is measured as relative light units by a photomultiplier device. The LIAISON®XL analyzer automatically calculates the concentration of IgG and IgM antibodies to CMV, Toxoplasma and Rubella, expressed as IU/ml or AU/ml. All the cut-offs used, and the ranges for the tests under evaluation, are given in Table 1.

	Unit	Range of reference value			Assay range
		Negative	Equivocal	Positive	
Toxoplasma IgG	IU/ml	＜7.2	7.2-8.8	≥8.8	3-400
Toxoplasma IgM	AU/ml	＜6	6-8	≥8	3-160
Rubella IgG	IU/ml	＜5	5-10	≥10	3-350

Rubella IgM	AU/ml	＜20	20-25	≥25	10-400
CMV IgG	U/ml	＜12	12-14	≥14	5-180
CMV IgM	U/ml	＜18	18-22	≥22	5-140

Table 1: Reference values and assay range for antibody quantitative detection of CMV, Toxoplasma and Rubella.

Two time periods (T1 and T2) were chosen for detecting IgG or IgM concentration (C1, C2) and calculating the multiple of the concentration change within a period of time, but as yet there is no reference value. One relatively commonly multiple is IgG C2 / C1 ＞4 times (Figure 1).

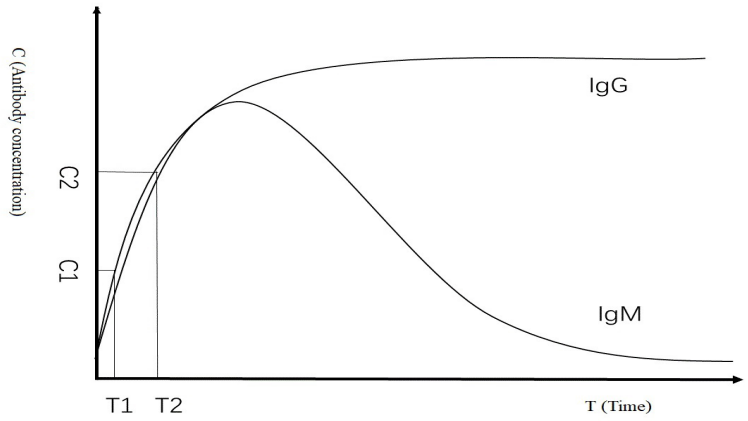

Figure 1: Dynamic quantitative analysis method.

According to Document [4], the result assessment process for antibody quantitative detection was plotted (Figure 2).

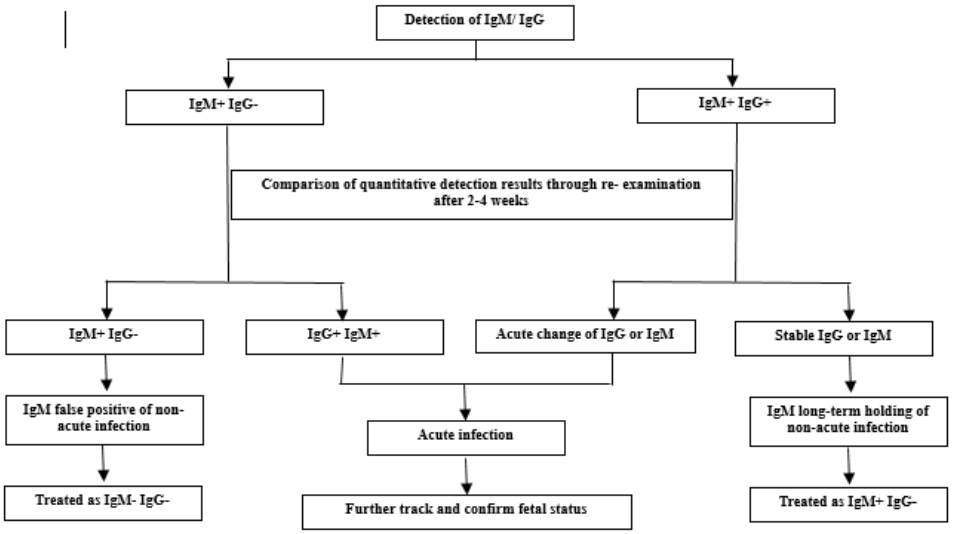

Figure 2: Interpretation Algorithm for serology testing.

Data processing: SPSS 22.0 software was used for statistical analysis.

Results

Table 2 shows the infection rate of CMV, Toxoplasma and Rubella in pregnant women in Shenyang.

	IgG			IgM		
	Number of patients	Number of positive patients	Positive rate (%)	Number of patients	Number of positive patients	Positive rate (%)
Rubella	14,852	12,598	84.82	16,527	271	1.64
CMV	14,190	13,486	95.04	16,339	70	0.43
Toxoplasma	12,951	46	0.36	15,366	73	0.48

Table 2: Infection rate of CMV, Toxoplasma and rubella in pregnant women in Shenyang.

In order to understand the antibody level (IgG and IgM) of CMV, Toxoplasma and rubella before and during pregnancy, the antibody concentration was used as the horizontal coordinate, and the relative frequency in appearance of the antibodies at different concentrations was used as the vertical coordinate to draw a histogram showing antibody concentration distribution (Figure 3).

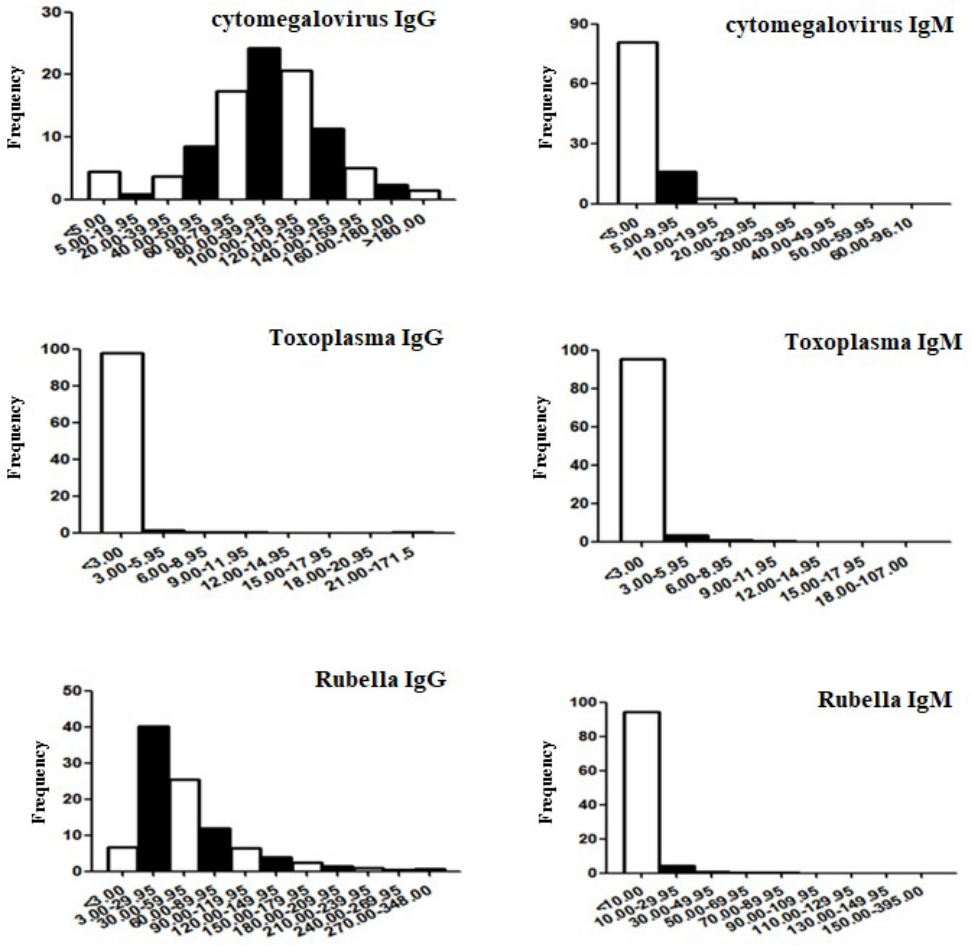

Figure 3: Distribution of quantitative results of CMV, Toxoplasma and Rubella antibodies (IgG and IgM) during pregnancy.

According to the dynamic quantitative analysis method as shown in Figure 1, pregnant women with both IgG and IgM positive antibodies to CMV, Toxoplasma and Rubella during pregnancy were advised to continue with pregnancy. See Table 3 for the follow-up outcome.

	Rubella IgG and IgM positive		CMV IgG and IgM positive		Toxoplasma IgG and IgM positive	
	Number of persons	%	Number of persons	%	Number of persons	%
Normal labor	33	75.0	3	75	3	100
Iatrogenic intervention (abortion as per clinician's advice)	6	13.6				
Abortion due to chromosomal abnormality	1	2.3				
Premature delivery due to Poor Intrauterine Fetal Growth	1	2.3				
Lost at follow-up	3	6.8	1	25		
Total	44	100	4	100	3	100

Table 3: Follow-up result of pregnancy outcome in pregnant women whose IgM and IgG antibodies of CMV, Toxoplasma and rubella were both positive

19 further persons had positive IgM for Toxoplasma only. This was identified as false positive through dynamic quantitative analysis. They continued with pregnancy and their children showed no abnormality after delivery.

In addition, 17 persons had positive IgM and IgG for Rubella in the pre-pregnancy detection, and did not conceive due to the influence of traditional interpretation methods.17 women who tested positive for both rubella virus IgM and IgG before pregnancy were not pregnant due to the influence of traditional interpretation methods.

Discussion

The quantitative analysis of the antibody level of CMV, Toxoplasma and Rubella during pregnancy.

The quantitative analysis of the antibody level of CMV, Toxoplasma and Rubella during pregnancy may not only help doctors understand the spread of infection in pregnant women in the local region (Table 2) and guide women with negative IgG antibodies to prevent infection during pregnancy, but may also help to understand antibody levels during pregnancy through the histogram of quantitative results. In Figure 3 we may see that, with the exception of IgG results for CMV which feature normal distribution, other results see skewed distribution. Judging by the highest point of occurrence frequency, IgG for CMV is 80.00-99.95IU/mL, Toxoplasma IgG is ＜3 IU/ml, Rubella IgG is 3.0-29.95 IU/ml, CMV IgM is <5.00 AU/ml, Toxoplasma IgM is ＜3.0 AU/ml, and Rubella IgM is <10.0 AU/ml. As far as CMV IgG is concerned, it would be worth further analyzing whether the quantitative detection value can be used as the index value of recurrent infection when it is 4 times higher than the highest point of the distribution frequency.

Dynamic analysis method and interpretation.

As regards the dynamic quantitative analysis method and interpretation algorithm for serology testing shown in Figure 1, we chose two time nodes (T1 and T2) to detect IgG or IgM concentration (C1 and C2) and calculate the gradient of the concentration change within a period of time (10 days-20 days), and we were effectively able to discover the specific immune reaction that occurs in the body when it is attacked by viruses. However, there are no reference values at present, and the most commonly-used value is IgG C2 / C1＞4 times [6]. This method may help us discover the active stage of infection. IgG or IgM, which is produced in the body in the event of a primary infection or recurrent infection during pregnancy, are part of a rapidly changing process, and can only be detected through quantitative analysis of concentration change [7]. The human body has different immune responses to virus infection, and the antibody levels vary greatly from one individual to another. Infection is a maternal-fetal dynamic process, and there are no distinct standards for each period of time. The cut-off value of the IgG or IgM concentration is the criterion of judgment for infection, but has limitations, and individual concentration gradient changes are of greater clinical significance. The range of the reference values for virus quantitation is determined based on a number of measurements of known negative and positive specimens. The reference values for different instruments vary. They are only used to judge the negative and positive results, and are not comparable. When there is a discrepancy in the result, the outcome of quantitative detection of antibodies in the person should be subject to longitudinal dynamic observation, so that a reasonable and correct evaluation can be made.

The dynamic quantitative analysis method can help identify both IgM false positive and IgM long-lasting antibodies. When the test result shows IgM positivity, it cannot be blindly considered as recent infection as per conventional methods, because the IgM positivity problem of non-acute infection exists in virus detection. The reasons mainly involve two circumstances: one is that after a patient is infected and expresses IgM constantly over many years, and repeated quantitative detection shows that the IgM level is kept at a low stable level while the IgG level does not increase four-fold, the IgM-positive status is normally termed long-lasting, and varies among individuals. The other circumstance is pure IgM positivity, where repeated quantitative detection shows that the IgM level remains unchanged, and IgG is not detected, termed a false positive. This mainly results from rheumatoid factor interference, immunological cross-reaction and polyclonal stimulation, amongst others.

The dynamic quantitative analysis method combined with the virus result assessment process (Figure 2) were used to conduct dynamic observation of women whose IgG was positive, or whose IgG and IgM were both positive; advice on continued pregnancy was given according to the observation result, and follow-up visits were scheduled (Table 3). 70 pregnant women were followed up for the outcome of the continued pregnancy after advice, and no disabled infants related to the target virus were born. Specifically, 44 patients whose Rubella IgG and IgM were both positive were given advice to continue their pregnancy. 33 of them gave birth to normal babies, 6 patients received surgical abortion under the impact of the traditional interpretation procedure, 1 baby had chromosomal abnormality, 1 baby suffered from Poor Intrauterine Fetal Growth, and 3 patients failed to be followed up. In addition, advice was also given to 4 persons with IgG and IgM positivity to CMV and 3 persons with IgG and IgM positivity to Toxoplasma during pregnancy. With the exception of 1 person, who was not followed up, the others gave birth to normal children. Prior to pregnancy, advice was given to 22 women; 5 became pregnant, and 17 of them decided not to conceive under the impact of the traditional interpretation procedure Other 19 person had positive Toxoplasma IgM only, identified as false positive through the dynamic quantitative analysis, and continued with pregnancy; their children showed no abnormality after delivery. As shown by these results, the dynamic quantitative determination process may reduce and eliminate improper clinical intervention.

The dynamic quantitative analysis method and pre-pregnancy baseline immunity status were unknown. As a result of health consciousness, it is difficult to popularize pre-pregnancy virus screening, which makes it impossible to explain positive screening results during pregnancy. The method we used involved conducting screening while determining pregnancy in order to understand the baseline immunity status of pregnant women. This is called the "screening at conception method". The screening at conception method is a remedy for no examination prior to pregnancy.

ToRCH screening during pregnancy diagnoses infection in pregnant women, based on which the possibility of fetal infection and developmental defects are inferred. Screening for certain infective diseases during pregnancy makes it possible to diagnose any infections involving the mother and/or the fetus, thereby allowing effective treatment of the fetus. Without a correct determination method, improper clinical intervention will doubtless be made, with serious consequences as a result. The significance and role of antibody quantitative detection are to provide reliable results and determination processes, reduce and eliminate improper clinical intervention, and provide the principle of effective in-pregnancy prevention and treatment.

References

1. Dollard SC, Staras SA, Amin MM, Schmid DS, Cannon MJ (2011) National prevalence estimates for cytomegalovirus IgM and IgG avidity and association between high IgM antibody titer and low IgG avidity. Clin Vaccine Immunol 18 :1895-1899.

2. Dontigny L, Arsenault MY, Martel MJ (2008) Rubella inpregnancy. J Obstet Gynaecol Can 30: 152-168.

3. Li Dongzhi (2009) Is TORCH screening still meaningful?. Chinese Journal of Prenatal Diagnosis (electronic version) 1: 1-2.

4. Cheeran MCJ, Lokensgard JR, Schleiss MR (2009) Neuropathogenesis of Congenital Cytomegalovirus Infection: Disease Mechanisms and prospects for Intervention Clin Microbiol Rev 22: 99-126.

5. Swanson Elizabeth C, Mark R Schless (2013) Congenital Cytomegalovirus Infection: New Prospects for Prevention and Therapy. Pediatr Clin North Am 60.

6. Zhang Jinman, Ruan Qiang, Zhang Ning et al. (2016) Expert consensus on the principle and work flow of TORCH infection screening, diagnosis and intervention. Chinese Journal of Practical Gynecology and Obstetrics 32: 535-540.

7. Zhang Ning, Yan Suwen, Feng Zhixiao (2014) Guideline of TORCH Screening during Pregnancy. Medical & Pharmaceutical Journal of Chinese People's Liberation Army 24: 102-116.