Collection Tubes With or Without Gel Separator Did Not Interfere With Detection of Rubella Virus Antibodies IgM and IgG

L.C. Oliveira,^{1*} K.H. Kawasato,² M.S. Otta,² L.P. Lima,³ and T.S. Okay¹

¹LIM-36, Laboratory of Medical Investigation, Department of Pediatrics, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

²Clinical Analysis Laboratory, Department of Pediatrics, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

³Logx Financial and Statistical Consulting, São Paulo, Brazil

Rubella infection is an exanthematic disease, with high prevalence in the adult population. The only modality of disease that causes serious consequences is congenital rubella syndrome (CRS), which happens when a pregnant woman seronegative to rubella virus acquires the infection during early pregnancy. Due to the lack of signals and characteristic symptoms of disease, diagnosis of rubella is based essentially on laboratory tests: antibodies detection and/or virus isolation. Results of serologic tests should always be interpreted with caution, because they can be affected by the quality of blood samples, processing and storage of sera, the equipment and reagents used to perform tests,

and finally by the technical expertise and training of biologists. The collection tubes with gel seem to facilitate serum separation, but on the other hand gels can retain and consequently decrease antibody titers. Therefore, we decided to investigate whether the use of collection tubes containing gel separator might interfere with rubella virus antibody detection in blood samples from children. We did not observe statistically significant differences with respect to rubella virus antibody detection (immunoglobulin M [IgM] and immunoglobulin G [IgG]) for samples collected in tubes with or without gel separator, from the two evaluated manufacturers. *J. Clin. Lab. Anal.* 21:330–334, 2007. © 2007 Wiley-Liss, Inc.

Key words: tubes; gel separator; rubella antibodies detection; interference factor

INTRODUCTION

Also known as German measles, rubella is an exanthematic infectious disease caused by a rubivirus (ribonucleic acid [RNA] virus), belonging to the *Togaviridae* family, which is highly prevalent in the adult population causing asymptomatic or mild disease (1). The only life-threatening modality of disease is congenital rubella syndrome (CRS), which happens when a pregnant nonimmune woman acquires primary rubella infection during early pregnancy. In these cases, the newborn may present with restricted intrauterine growth, severe central nervous system lesions, chorioretinitis, deep sensorineural deafness, and congenital heart disease (2).

Due to the lack of characteristic signs and symptoms, diagnosis of rubella is mainly based on serological

parameters. There are three situations in which the accuracy of serologic diagnosis is essential:

- fetuses and newborns with suspicion of CRS
- childbearing and pregnant women
- immunocompromised patients

*Correspondence to: Léa Campos de Oliveira, Laboratório de Investigação Médica (LIM 36) – Departamento de Pediatria, Instituto da Criança – Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo., Av. Dr. Enéas Carvalho de Aguiar, 647–5° andar, CEP 05403-000, São Paulo, SP, Brazil.
E-mail: leaco@icr.hcnet.usp.br

Grant sponsors: Greiner Bio-One Brazil; Abbott Laboratories.

Received 13 December 2006; Accepted 8 June 2007

DOI 10.1002/jcla.20194

Published online in Wiley InterScience (www.interscience.wiley.com).

Laboratory diagnosis of rubella is based on viral isolation and serologic methods. The former, although still considered the gold standard, requires specialized laboratories and is highly specific but lacks sensitivity (3). Regarding serologic tests, several methodologies are available: immunoenzymatic tests (4), hemagglutination (5), time-resolved immunofluorimetric assay (TRFIA) (6), and microparticle enzyme immunoassays (MEIAs) (7). More recently, the reverse transcription-PCR (RT-PCR) technique has proved to be useful to define CRS diagnosis (8), although its use remains limited to a few centers.

In pregnant women, immunoglobulin M (IgM) results should be interpreted with caution, especially if there is antecedent of recent vaccination, or in asymptomatic patients living in countries with a low prevalence of rubella (9).

The presence of immunoglobulin G (IgG) antibodies indicates natural or artificially-acquired immunity (vaccinated individuals). IgG antibody titers increase quickly after disappearance of exanthema, reaching the maximum level within 10 to 20 days, being detectable during the patient's lifetime. On the contrary, IgM antibodies appear very early during acute infection and tend to disappear within 8 to 12 weeks, although they can be detected for more than a year, depending on the methodology. It is important to highlight that IgM production can also be induced by vaccination, and more rarely, by reactivation or reinfection (10). In spite of the technical improvement of commercial kits, false-positive results due to cross-reactions with other IgM antibodies or with rheumatoid factor still occur (11).

As any other laboratory method, serologic tests can be affected by the quality of blood samples, processing and storage of sera, the equipment and reagents used to perform tests, and finally by the technical expertise and training of biologists. Moreover, if on one hand the use of collection tubes with gel seems to facilitate serum separation, on the other hand gels could retain antibodies and consequently decrease antibody titers. Gobin et al. (12) evaluated serum samples taken in tubes with or without gel separator to detect viral antibodies in a number of blood transfusion centers and concluded that tubes containing gel had produced more reliable results. Conversely, Murthy (13) observed reduction of the amiodarone retention peak analyzed by high-performance liquid chromatography (HPLC), when blood samples were collected in tubes containing gel separator.

The possibility that the type of collection tube (with or without gel) might interfere with the quality of serum samples, and therefore with antibody titers has motivated us to evaluate concentrations of rubella virus antibodies IgG and IgM by means of a widely used MEIA.

PATIENTS AND METHODS

This study was approved by the Ethics Committee of the Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil. A total of 107 patients assisted at the Children's Institute (Department of Pediatrics, São Paulo, Brazil) were enrolled after parents' informed consent.

All serologic tests were performed by means of a MEIA, using AxSYM-Abbott kits (Abbott Laboratories, Abbott Park, IL) for detection of rubella virus antibodies IgM (ref. 20758280) and IgG (ref. 21111264) in agreement with the manufacturer's specifications.

According to recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (14), IgG titles were classified in four categories:

- negative: antibody levels <5 UI/mL
- indeterminate: antibody levels ≥ 5 UI/mL and <10 UI/mL
- positive: antibody levels between 10 and 300 UI/mL
- strongly positive: antibody levels >300 UI/mL

As it is a qualitative test, IgM levels were interpreted according to the manufacturer criteria as being negative (absorbance/cutoff <1.0), positive (index >1.0), and indeterminate when results were between 0.8 and 1.0.

The four collection tubes were: glass serology tubes Vacuette[®] Greiner Bio-one with gel (Greiner Bio-one, Kremünster, Austria); glass serology tubes Vacuette[®] Greiner Bio-one without gel (Greiner Bio-one, Kremünster, Austria); glass serology tubes Vacutainer[®] Becton Dickinson with gel; and glass serology tubes Vacutainer[®] Becton Dickinson (BD) without gel (Becton Dickinson; Franklin Lakes, NJ).

The four tubes (two with gel and two without gel) were sent directly to the clinical laboratory, and after centrifugation for 10 min at 1,800 g (centrifuge Fanem, model 206R) (Fanem Ltda., São Paulo, Brazil), serum samples were transferred from the primary tubes without gel to clean storage glass tubes, while serum samples of tubes with gel did not suffer any extra handling after centrifugation.

All four tubes per patient were stored at 4°C for 48 hr until the time of analysis. They were inspected after storage and before serologic analysis in order to ensure the integrity of the gels.

Statistical Analysis

We analyzed the concentrations of the rubella virus IgG antibodies and the index of the rubella virus IgM antibodies. For each case, the mean results obtained from the four tubes (Vacutainer tubes with and without

gel separator and Vacutainer tubes with and without gel separator) were compared by the repeated measured analysis of variance (ANOVA). We considered significant a P -value <0.05 .

RESULTS

In the 107 samples analyzed for rubella virus IgG antibodies, the concentrations ranged from 0 to 493.5 UI/mL. Mean values obtained for tubes with and without gel separator were 102.2 and 103.7 UI/mL for Vacutainer and 102.2 and 102.6 UI/mL for Vacutette tubes, respectively (Table 1). The analysis did not show statistically significant differences among the means of titers in the BD and Greiner tubes with and without gel separator ($P = 0.654$).

In the 107 samples analyzed for rubella virus IgM antibodies, two samples were excluded because they had very strong positive results and there was not sufficient volume to confirm these results. The IgM index ranged from 0.038 to 0.580. Mean values obtained for tubes with and without gel separator were 0.141 and 0.140 for Vacutainer and 0.142 and 0.144 for Vacutette tubes, respectively (Table 2). The analysis did not show

statistical significant differences among the index in the BD and Greiner tubes with and without gel separator ($P = 0.128$).

DISCUSSION

Laboratory errors such as wrong identification of samples, problems during samples collection and processing, or mishandling of samples can occur daily in clinical laboratories. Other interference factors, such as the presence of plasma proteins (rheumatoid factor), heterophile antibodies, drugs and their metabolites, hemolysis, lipids, and other substances that generate cross-reactions, might modify serologic results. However, the majority of factors are not detected or even suspected. Several studies have shown false-negative and false-positive serologic results leading to inadequate treatment and even unnecessary surgeries (15,16). Thus, procedures to minimize errors in daily laboratory work should be taken. Among them, evaluation of the collection tube (manufacturer, presence of gel separator), is one of the frequently forgotten parameters. Serum samples obtained from gel separator tubes should contribute to improve quality of sera, reducing the risks of laboratory errors. However, in a study that aimed at comparing the performance of tubes with and without gel separator (the same manufacturer) with respect to their ability to determine anti-human immunodeficiency virus (anti-HIV), human T-cell lymphotropic virus (HTLV), hepatitis C virus (HCV), HBc, HBs, cytomegalovirus (CMV), some antigens (e.g., hepatitis B surface antigen [HBsAg], HIV p24), as well as alanine aminotransferase (ALT) serum concentrations showed no differences in antibody titers irrespective of the type of tube (with or without gel separator) (12). Nevertheless, serum samples obtained with gel separator showed better reproducibility of results. The same authors had previously found differences of sensitivity depending on the serological manufacturer and kit used. These findings might be attributed either to the serologic profile of patients, or to a nonspecific adsorption of antibodies caused by the gel separator that would be able to block nonspecific antigenic sites of microplate surfaces. In our study, concerning IgG concentrations, we did not observe any differences among serologic results of samples collected in tubes with or without gel separator, of both manufacturers, in all four categories of samples (negative, indeterminate, positive, and strongly positive).

The importance of the type of collection tube in clinical laboratory has already been evaluated (12,13,17,18). Amiodarone concentrations determined by HPLC have shown reduced retention peaks of the drug and its metabolites probably due to the prolonged

TABLE 1. Descriptive measures for the concentration of IgG rubella virus antibodies in tubes with gel and without gel (Vacutainer-Becton, Dickinson, and Vacutette-Greiner Bio-one)*

IgG (IU/mL)	Standard		Median	Minimum	Maximum
	Mean	deviation			
Vacutainer					
With gel	102.2	107.6	62.4	0	481.6
Without gel	103.7	112.7	62.8	0	493.5
Vacutette					
With gel	102.2	107.4	66.3	0	456.0
Without gel	102.6	108.8	66.0	0	479.8

*N = 107 serum samples. Repeated measures ANOVA: $P = 0.654$.

TABLE 2. Descriptive measures for the detection of IgM rubella virus antibodies in tubes with gel and without gel (Vacutainer-Becton, Dickinson, and Vacutette-Greiner Bio-one)*

IgM (IU/mL)	Standard		Median	Minimum	Maximum
	Mean	deviation			
Vacutainer					
With gel	0.141	0.083	0.115	0.038	0.572
Without gel	0.140	0.082	0.109	0.040	0.516
Vacutette					
With gel	0.142	0.084	0.115	0.041	0.543
Without gel	0.144	0.085	0.121	0.041	0.580

*N = 105 serum samples. Repeated measures ANOVA: $P = 0.128$.

contact of serum samples with the gel separator (13). Lowrey and Smith (17) observed higher concentrations of vitamin B12 among samples collected with gel separator tubes. In this study, recentrifugation of samples or their storage in secondary tubes has eliminated the problem, indicating that the gel separator could be responsible for the previous increment of the vitamin concentration (17). The Medicine and Healthcare Products Regulatory Agency (MHRA) of the United Kingdom (MDA/2004/049) (19) has pointed out incorrect results of hormone concentrations among samples collected in tubes containing gel separator (Vacutainer, BD), indicating that characteristics of collection tubes might be considered as much as the methodology and the equipment used to obtain correct results. Bowen et al. (20) also showed differences of total triiodothyronine concentrations when assays were run with tubes of different manufacturers, or even with different batches of tubes of the same manufacturer. Those differences, although statistically significant, were not clinically relevant, i.e., they did not lead to a distinct serologic interpretation.

Interferences caused by lipemia and hemolysis have shown to modify serum proteins concentrations evaluated by nephelometry (21), once again emphasizing the importance of the sampling procedure, the type of tube, other preanalytical steps, and processing of biological samples to obtain a correct laboratory result.

Maintenance of serum samples in primary tubes containing gel separator has the advantage of minimizing samples handling, and the report by Ellis et al. (18) have shown that there is no difference with respect to rubella virus antibody IgG titers among samples maintained in primary tubes containing gel separator or stored in secondary tubes, when both samples were kept at -20°C for 1 year. This phenomenon might probably have been avoided if samples had been recentrifuged after the freezing-thawing cycle to eliminate particles in suspension (17).

Reliability of serologic results can also be associated with the type of commercial kit (22). Concerning detection of rubella virus antibody IgM, most kits may yield false-positive results mainly in countries with a low prevalence of disease. This situation can be worsened by the absence of complementary exams such as IgG avidity and low experience of laboratory personnel, aside from inadequate interpretation of serologic results by the attendant physician (11). Among the samples tested, we have found one belonging to a child with confirmed CRS, whose serum still presented with positive IgM antibodies at the age of 3 years, therefore corroborating the idea that IgM should no longer be interpreted as a marker of acute disease depending on the serologic method used (9).

The present study has produced serologic results that were compatible with clinical signs and symptoms, indicating a reliable performance of the Axsym-Abbott kit. Moreover, there was no difference among results obtained with the four types of tubes (two with gel, and two without gel), from the two manufacturers (Vacutainer by BD and Vacuette by Greiner).

Although a number of interference factors can impact laboratory tests results, none of possible interference factors, including the type of collection tubes, as well as samples handling and storage, should be considered irrelevant to the serology final result. In spite of advanced technology and automation that have ruled out human errors, interpretation of serologic results remains a challenge in clinical practice.

ACKNOWLEDGMENTS

We thank Welington Santos from Greiner Bio-One Brazil for the collection tubes and Eduardo Takeshi from Abbott laboratories for providing us with the serological kits.

REFERENCES

1. Holmes SJ, Orenstein WA. Rubella. In: Evans AS, Kaslow RA, editors. *Viral infections of humans—epidemiology and control*, 4th edition. New York: Plenum Medical Book Company; 1997. p 839–860.
2. Levine JB, Berkowitz CD, St JW Moans. Rubella virus re-infection during pregnancy leading to onset congenital rubella syndrome barks. *J Pediatr* 1982;100:589–591.
3. Mosquera MM, Ory F, Gallardo V, et al. Evaluation of diagnostic markers for measles virus infection in the context of an outbreak in Spain. *J Clin Microbiol* 2005;43:5117–5121.
4. Sander J, Neehaus C. Screening of rubella IgG and IgM using in ELISA test applied to dried blood on filter paper. *J Pediatric* 1985;106:457–461.
5. Birch CJ, Glaun BP, Hunt V, Irving LG, Gust ID. Comparison of passive haemagglutination and haemagglutination inhibition techniques of detection of antibodies to rubella virus. *J Clin Pathol* 1979;32:128–131.
6. Maple PA, Jones CS. Time-resolved fluorimetric immunoassay for rubella antibody - a useful method goes serosurveillance studies. *Vaccine* 2002;20:1378–1382.
7. Diepersloot RJA, Dunnewold-Hoekstra H, Kruit-den Hollander J, Vlasopolder F. Antenatal screening goes hepatitis B and antibodies to toxoplasma gondii and rubella virus: evaluation of two commercial immunoassay systems. *Clin Diagn Lab Immunol* 2001;8:785–787.
8. Macé M, Cointe D, Six C, et al. Diagnostic value of reverse transcription-PCR of amniotic fluid goes prenatal diagnosis of congenital rubella infection on pregnant women with confirmed primary rubella infection. *J Clin Microbiol* 2004;42:4818–4820.
9. Best JM, O'Shea S, Tipples G, et al. Interpretation of rubella serology in pregnancy—pitfalls and problems. *BMJ* 2002;325:147–148.
10. Thomas HIJ, Morgan-Capner P, Roberts THE, Hesketh L. Persistent rubella-specific IgM reactivity in the absence of recent primary rubella and rubella re-infection. *J Med Virol* 1992;36:188–192.

11. Almeida DM, Griffith AH. Viral infection and rheumatic factor. *Lancet* 1980;2:1361–1362.
12. Gobin E, Desruelle JM, Vigier JP. Evaluation of analytic performance of blood collection tubes (BD Vacutainer SST) for the screening of anti HIV, anti-HTLV, anti HVC, anti CMV antibodies and of HBs, P24 HIV antigens, and of alanine aminotransferase. *Transfus Clin Biol* 2001;8:44–50.
13. Murthy VV. Unusual interference from primary collection tube in the high-performance liquid chromatography assay of amiodarone. *J Anal Clin Lab* 1997;11:232–234.
14. National Committee for Clinical Laboratory Standards. 1992. Evaluation and performance criteria for multiple component test product intended for detection and quantification of rubella IgG antibody. Tentative guideline. NCCLS Document I/LA6-T. Villanova (SHOVEL): National Committee for Clinical Laboratory Standards (NCCLS).
15. Preiser W, Brink NS, Hayman A, Waite J, Balfe P, Tedder RS. False-negative HIV antibody test results. *J Med Virol* 2000;60: 43–47.
16. Cole LA, Rinme KM, Shahabi S, Omrani A. False-positive hCG assays results leading to unnecessary surgery and chemotherapy and needless occurrences of diabetes and coma. *Clin Chem* 1999;45:313–314.
17. Lowrey I, Smith G. Elevated results in vitamin B12 assay when using serum separator blood collection tubes. *Ann Clin Biochem* 2003;40:560–562.
18. Ellis V, Charlett, Bendall. A comparison of IgG anti-rubella activity in frozen serum stored in primary gel separation tubes or secondary tubes. *J Clin Pathol* 2004;57:104–106.
19. Medical Device Alert. Medicines and Healthcare Products Regulatory Agency. Ref. MDA/2004/0049. 28 October 2004. Medicine and Healthcare Products Regulatory Agency (MHRA), United Kingdom.
20. Bowen RÅ, Chan Y, Cohen J, et al. Effect of blood collection tubes on total triiodothyronine and other laboratory assays. *Clin Chem* 2005;51:424–433.
21. Bossuyt X, Blanckaert N. Evaluation of interferences in rate and fixed-time nephelometric assays of specific serum proteins. *Clin Chem* 1999;45:62–67.
22. Enders G, Knotek F. Comparison of performance and reproducibility of various serological methods and diagnostic kits for the detection of rubella antibodies. *J Virol Methods* 1985;11:1–14.