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Evaluation of the Abbott ARCHITECT™ cytomegalovirus IgM/IgG, rubella IgM/IgG, and syphilis treponemal antibodies enzyme immunoassays in a mother and child health center population

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ABSTRACT

This study evaluated the concordance of Architect™ chemiluminescent microparticle immunoassays with Captia™ ELISA for cytomegalovirus (CMV) IgM and IgG, with Enzygnost™ and Captia™ ELISA for rubella IgM and IgG and with Trep-Sure™ ELISA for syphilis treponemal antibodies in a mixed pediatric and obstetrical population. Total agreement between assays and Kappa statistic value were 82.5% (95% CI: 75.6–87.7) and 0.65 (95% CI: 0.54–0.77) for CMV IgM, 82.8% (95% CI: 76.7–87.6) and 0.65 (95% CI: 0.55–0.75) for CMV IgG, 89.2% (95% CI: 82.9–93.4) and 0.56 (95% CI: 0.36–0.75) for rubella IgM, 88.6% (95% CI: 82.9–92.6) and 0.74 (95% CI: 0.63–0.84) for rubella IgG, and 97.9% (95% CI: 94.5–99.4) and 0.89 (95% CI: 0.79–1.00) for syphilis treponemal antibodies. This study demonstrates that the Architect™ chemiluminescent microparticle immunoassays correlate well with other FDA-approved ELISA assays in this specific population.

1. Introduction

Cytomegalovirus (CMV), rubella, and syphilis are known for their potential to cause severe congenital infections. These pathogens can lead to miscarriage or permanent abnormalities such as neurodevelopmental retardation, or cardiac or musculoskeletal malformations (Wilson et al., 2016). During pregnancy, acute CMV, rubella, and syphilis infections necessitate specific care and follow-up. Since these infections often present with nonspecific clinical findings, rapid and accurate serology results are essential for appropriate diagnosis and clinical management. Therefore, immunoassays for the detection of CMV IgM and IgG, rubella IgM and IgG, and syphilis treponemal antibodies are routinely used during pregnancy to evaluate the risk of fetal infection. Acquisition of protective titers of rubella IgG (≥10 IU/mL) before pregnancy is known to reduce the risk of fetal infection (Skendzel et al., 1997). For syphilis diagnosis during pregnancy, treponemal antibodies testing is increasingly preferred to nonspecific (anticardiolipins) antibodies testing as a first-step assay (Tsimis and Sheffield, 2017). Indeed, pregnancy is known to be responsible for false-positive rapid plasmin reagin results, whereas past nonsyphilitic treponemal diseases usually account for false-positive treponemal assay reactions (Liu et al., 2014).

Chemiluminescent microparticle immunoassays (CMIAs) for qualitative detection of CMV IgM, rubella IgM, and syphilis treponemal antibodies; for semiquantitative detection of CMV IgG; and for quantitative detection of rubella IgG were performed on the Architect™ platform. This is an automated random-access analyzer with a short turnaround time. Previous studies have shown good correlation between CMIA and other assays such as ELISA for CMV (Lagrou et al., 2009) and rubella (Portella et al., 2010), and ELISA and latex agglutination for syphilis (Zhiyan et al., 2015). None of these studies have evaluated these assays specifically in a pediatric and obstetrical population.

2. Objectives

Using a collection of seronegative, indeterminate, and seropositive sera, this study evaluated the concordance of the Architect™ CMIA with Captia™ ELISA for the detection of CMV IgM, CMV IgG, and rubella IgG; with Enzygnost™ ELISA for rubella IgM; and with Trep-Sure™ ELISA for syphilis treponemal antibodies.

Abbreviations: CMV, cytomegalovirus; CMIA, chemiluminescent microparticle immunoassay; ELISA, enzyme-linked immunosorbent assay.

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Table 1
Demographic distribution of patients.

		CMV IgM	CMV IgG	Rubella IgM	Rubella IgG	Syphilis IgM/IgG
		$n = 154$	$n = 186$	$n = 139$	$n = 175$	$n = 188$
M:F		50:104	56:130	17:122	16:159	39:149
Children:adults		90:64	98:88	22:117	27:148	13:175
Children	Age range	0–18	0–18	0–17	0–18	0–18
	Median age	5.3	8.4	2.1	5.7	14.7
	Interquartile range	1.9–10.7	2.5–14.3	1.2–6.9	2.5–11.7	11.1–16.5
Adult	Age range	19–93	19–90	19–64	19–43	19–65
	Median age	32.5	33.0	30.8	31.5	32.8
	Interquartile range	24.5–37.5	27.8–39.8	26.1–36.0	26.6–34.8	27.6–36.9

3. Material and methods

3.1. Serum specimens

Correlations between CMV IgM, CMV IgG, rubella IgM, rubella IgG, and syphilis treponemal antibodies were, respectively, performed on 154, 186, 139, 175, and 188 samples. These sera were randomly selected among all clinical specimens submitted for routine serology testing to the Sainte-Justine University Hospital Center clinical laboratory during the study period. Most included specimens were those of patients attending obstetric (73.0%) or pediatric rheumatology and oncology clinics (27.0%) within our institution. These specific study populations were chosen to be highly represented in this study since they regroup patients at higher risk of having non-specific circulating antibodies, autoantibodies, and/or low levels of total immunoglobulins compared to the general population. Sera testing negative, intermediate, and positive on each comparator ELISA were randomly selected for testing on CMIA platform. Those samples were stored at −20 °C immediately after separation and were thawed once for batched testing on CMIA. All specimens were handled and tested by trained laboratory personnel at Sainte-Justine University Hospital Center, Montreal, Canada. Table 1 details the demographic distribution of the sera included.

3.2. Chemiluminescence microparticle immunoassays

CMIAs were performed using the Architect™ CMV IgM, rubella IgM, and syphilis TP (qualitative); CMV IgG (semiquantitative); and rubella IgG (quantitative) kits (Abbott, Germany). Specific antigens include a lysate of the CMV strain AD169; the whole rubella viral strain HPV 77; or the recombinant *Treponema pallidum* antigens TpN15, TpN17, and TpN47. After hybridization and a washing step, acridinium-labeled anti-human IgM and/or IgG antibody conjugate is added, and chemiluminescent signal is measured as a ratio of sample relative light units (RLU) over calibrator established cutoff RLU (S/CO) (Abbott, 2008). For semiquantitative (units/mL) CMV IgG and quantitative (IU/mL) rubella IgG assays, multiple calibrators RLU are used to generate a calibration curve. All sera were tested simultaneously after adequate system calibration and successful quality controls.

Table 2
Results of Abbott Architect™ CMIA and TrinityBiotech Captia™ ELISA for detection of CMV IgM and CMV IgG.

		Architect™ CMIA				
		CMV IgM ($n = 154$)			CMV IgG ($n = 186$)	
		Negative	indeterminate	Positive	Negative	Positive
Captia™ ELISA	Negative	43 (28)	3 (2)	2 (1)	55 (30)	18 (10)
	Indeterminate	11 (7)	3 (2)	3 (2)	1 (0)	14 (8)
	Positive	16 (10)	6 (4)	67 (44)	0 (0)	98 (52)

3.3. Microtitration ELISA assays

Standard microtitration ELISA assays were performed using the Captia™ CMV IgM and IgG and Rubella IgG (TrinityBiotech, Ireland), Enzygnost™ Rubella IgM (Siemens, Germany), and TrepSure Syphilis TP (Phoenix Biotech, United States) assays on a Triturus™ automated immunoassay analyzer (Grifols, Spain). To minimize occurrence of false-positive reactions, rubella IgM samples were pretreated with a rheumatoid factor absorbent-containing solution. In all ELISAs, serum samples were incubated on antigen-coated polystyrene solid phase medium. Goat anti-human IgM or IgG conjugated with a peroxidase was then added to bind antigen–antibody complexes, if present. Subsequent addition of a chromogenic substrate produced a colorimetric signal, which was quantified by a microplate spectrophotometer (Biotek, United States). An optical density (OD) ratio was calculated using a control calibrator as denominator.

3.4. Statistical analysis

Kappa statistic values and positive, negative, and total agreements between CMIA and ELISA assays were calculated. Kappa values are frequently used to assess interrater agreement between categorical variables. Since kappa values account for potential agreement due to chance, they are more robust than agreement percentages. By definition, Kappa values above 0.75 indicate excellent agreement, values between 0.40 and 0.75 indicate fair to good agreement, and values below 0.40 represent poor agreement beyond chance (Fleiss, 1981). Total agreement between assays was the percentage of paired tests with identical results. ELISA assays were considered the reference standard in the positive and negative agreements calculation. The modified Wald method was used to calculate 95% confidence intervals around binomial distribution. The strength of the linear association between ELISA measured OD levels and Architect RLU (S/CO) was evaluated using the R^2 value. All statistical analyses were performed with GraphPad Prism™ statistics software version 6.0.

3.5. Ethical approval

Institutional board review and ethical approval were not necessary for this study. All samples were anonymized, and the study did not include clinical interventions.

4. Results

4.1. CMV IgM and IgG assays

Paired results and agreement percentages for CMV antibody assays are respectively presented in Table 2 and Table 5. For CMV IgM, total agreement was 82.5% (95% CI: 75.6–87.7), and kappa value was 0.65 (95% CI: 0.54–0.77). Architect™ CMIA produced less indeterminate results than Captia™ ELISA (7.8% vs. 11.0% $P = 0.33$). For CMV IgG,

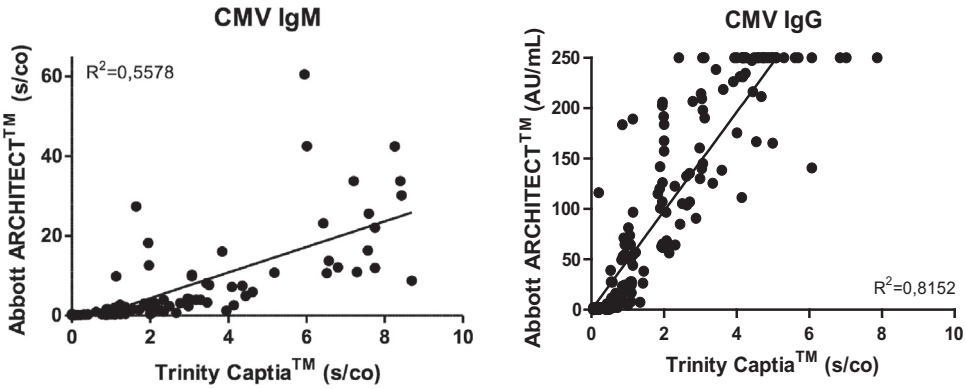

Fig. 1. Correlation between Captia™ OD and Architect™ S/CO or AU/mL for CMV IgM and CMV IgG; *n* (%).

total agreement was 82.8% (95% CI: 76.7–87.6), and kappa value was 0.65 (95% CI: 0.55–0.75). Captia™ ELISA assay reported 15/186 (8.1%) samples as indeterminate, whereas the Architect™ CMIA does not provide an indeterminate S/CO range. Focusing on CMV IgG discordant results, Architect™ CMIA reported 32/186 (17.2%) samples as positive, while they were reported as indeterminate/negative by Captia™ ELISA. All positive samples by Captia™ ELISA were also positive by Architect™ CMIA. Architect™ CMIA reported 130/186 (69.9%) samples as having titers of CMV IgG ≥ 6 AU/mL, while the qualitative Captia™ ELISA found 98/186 (52.7%) samples with OD in the positive range. The majority of patients with indeterminate results on Captia™ ELISA were under the age of 18 years old (78.6%). Fig. 1 shows the integral raw data and the imperfect linear correlation between the OD ratio on Captia™ ELISA and the S/CO or AU/mL on Architect™ CMIAs for CMV IgM and CMV IgG, respectively. The R^2 value was higher for CMV IgG (0.82) than for CMV IgM (0.56).

4.2. Rubella IgM and IgG assays

Paired results of rubella antibody assays are presented in Table 3, and agreements are shown in Table 5. For rubella IgM, total agreement was 89.2% (95% CI: 82.9–93.4), and kappa value was 0.56 (95% CI: 0.36–0.75). The Architect™ CMIA produced less indeterminate results than the Enzygnost™ ELISA (2.9% vs. 23.7% $P < 0.001$). For rubella IgG, total agreement was 88.6% (95% CI: 82.9–92.6), and kappa value was 0.74 (95% CI: 0.63–0.84). Architect™ CMIA reported 8/175 (4.6%) samples as positive and 12/175 (6.9%) samples as indeterminate/negative, while these specimens were, respectively, reported as indeterminate/negative or positive by Captia™ ELISA. Architect™ CMIA reported 53/175 (30.3%) patients as having ≥10 IU/mL rubella IgG, while Enzygnost ELISA reported 57/175 (32.6%) patients as positive. Among women of childbearing age, Architect™ CMIA yielded more indeterminate results than did the Captia™ ELISA assay (50.0% vs. 18.2% $P < 0.001$). Fig. 2 shows the integral raw data and the imperfect linear correlation observed between the OD ratio on Enzygnost™ or Captia™ ELISA and the S/CO or IU/mL on Architect™ CMIAs for RUBELLA IgM and rubella IgG, respectively. The R^2 value was higher for Rubella IgM (0.70) than for Rubella IgG (0.54).

4.3. Syphilis antibody assays

Paired results of Syphilis treponemal antibody assays are presented in Table 4, and agreement values are in Table 5. Total agreement was 97.9% (95% CI: 94.5–99.4), and kappa value was 0.89 (95% CI: 0.79–1.00), indicating excellent agreement. All 4/188 (2.1%) discordant

Table 3
Results of Abbott Architect™ CMIAs and TrinityBiotech Captia™ or Siemens Enzygnost™ ELISA for detection of rubella IgM and rubella IgG.

		Architect™ CMIA					
		Rubella IgM ($n = 139$)			Rubella IgG ($n = 175$)		
		Negative	Indeterminate	Positive	Negative	Indeterminate	Positive
Captia™ or Enzygnost™ ELISA	Negative	80 (58)	0 (0)	1 (1)	39 (22)	48 (28)	2 (1)
	Indeterminate	30 (21)	2 (1)	1 (1)	2 (1)	21 (12)	6 (3)
	Positive	11 (8)	2 (1)	12 (9)	1 (1)	11 (6)	45 (26)

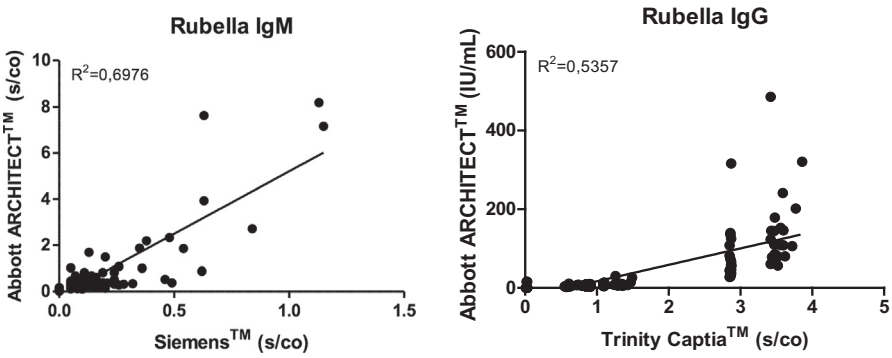

Fig. 2. Correlation between Architect™ (IU/mL or S/CO) and Captia™ or Enzygnost™ OD for Rubella IgM and Rubella IgG detection; *n* (%).

Table 4
Results of Abbott Architect™ CMIA and Phoenix Biotech Trep-Sure™ ELISA for detection of syphilis treponemal antibodies.

		Architect™ CMIA	
		Syphilis antibodies (n = 188)	
		Negative	Positive
Trep-Sure™ ELISA	Negative	165 (88)	0 (0)
	Positive	4 (22)	19 (10)

results were positive on Trep-Sure™ ELISA and negative on Architect™ CMIA. These specimens were subsequently confirmed as being negative with *Treponema pallidum* particle assay. Fig. 3 shows the integral raw data and the imperfect linear correlation between the OD ratio on Phoenix Biotech Trep-Sure™ and the S/CO on Architect™ CMIA for syphilis treponemal antibodies. The R^2 value was 0.81.

5. Discussion

In this study, we evaluated the concordance of Architect™ CMIAs with commercially available FDA-approved ELISA-based techniques for the detection of CMV IgM and IgG, rubella IgM and IgG, and syphilis treponemal antibodies. We used sera collected from patients attending obstetrical and pediatric specialized clinics and therefore at higher risk for circulating nonspecific antibodies, autoantibodies, and/or low levels of total immunoglobulins compared to the general population.

Specimen selection was based on results previously obtained by the comparator assays. To minimize the impact of this potential selection bias, a wide range of specimens testing negative, intermediate, and positive on these comparator assays were randomly included in the study. This selection method yielded sera with discordant results between the evaluated assays. Kappa statistic values concordance analyses revealed fair to excellent agreement for all paired assays (Fleiss, 1981). Agreement was the lowest between both CMV IgG detection assays, with a total agreement value of 82.8% (95% CI: 76.7–87.6). Agreement was particularly high between both syphilis treponemal antibodies detection assays, with total agreement values of 97.9% (95% CI: 94.5–99.4). Despite showing good agreement percentage, rubella IgM assays had the lowest kappa statistic value. This is partly explained by the relatively few number of included rubella IgM–positive samples, a limitation of our study. Agreement values between Architect™ CMIAs and other ELISAs are globally lower than those reported in the literature (Lagrou et al., 2009; Portella et al., 2010). Using sera from the general population, Lagrou et al. found Architect™ and other commercial platforms including Enzygnost™ to have a correlation between 93% and 97% for CMV IgM and between 98% and 99% for CMV IgG. Similarly, Portella et al. found Architect™ to have a correlation between 97.5% and 97.9% for rubella IgM and between 95.0% and 97.3% for rubella IgG. These higher correlation percentages could be explained by the previously mentioned specificities of our population. Especially in the context of our smaller sample size, higher numbers of discrepant samples can negatively impact concordance.

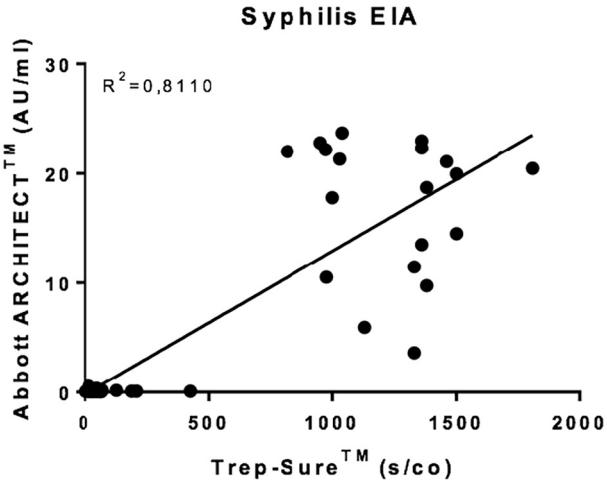

Fig. 3. Correlation between Architect™ S/CO and Phoenix Biotech Trep-Sure™ ELISA for detection of syphilis treponemal antibodies; n (%).

For each paired assay, the linear correlation between S/CO, IU/mL, or AU/mL levels on CMIAs and OD levels ratio on ELISA was imperfect. When looking specifically at sera showing discordant results, CMIA S/CO and ELISA DO varied greatly above the Architect™ and Captia™ or Siemens™ respective cutoffs values. This reinforces the fact that S/CO or OD ratio values cannot be used to facilitate the interpretation of these nonquantitative serology assays' results.

In the absence of an accepted gold standard, no sensitivity and specificity could be calculated. This is a limitation of our study and a frequently encountered problem for serologic assays evaluation. This lack of gold standard is particularly challenging in the case of IgM assays for which, like in our study, more discrepancy between platforms is generally observed. Strategies that have previously been used to overcome this challenge include the use of well-characterized collection sera, but these are limited in number and poorly address the previously mentioned intrinsic complexities of our population. Detailed clinical correlations have also been used to determine expected true serologic profiles. This strategy could not be considered as a gold standard here since acute rubella, CMV, or syphilis clinical manifestations overlap with those of many other pathogens. Lastly, imperfect gold standards integrating multiple assays results and clinical information can be used to determine expected serologic status (CLSI, 2008). Since this study only included 2 different techniques, this strategy could not be used.

Again, in the absence of a gold standard assay, laboratories and clinicians need to discuss reporting and interpretation of indeterminate results and need to decide whether an assay suspected to be more sensitive or more specific is best suited for the clinical context. Among women of childbearing age, Architect™ CMIAs reported fewer patients as immune to rubella virus than did the Enzygnost™ ELISA assay. This type of discordance between serological assays was described in a recent comprehensive study comparing quantitative rubella IgG results reported by different assays (Bouthry et al., 2014). In this case, increased

Table 5
Concordance between results of Abbott Architect™ CMIAs and TrinityBiotech Captia™, Siemens Enzygnost™, and Phoenix Biotech Trep-Sure™ ELISA assays.*[a]

Tested antibody	n	No. (%) of discordant samples[a]		Positive agreement (95% CI)	Negative agreement (95% CI)	Total agreement (95% CI)	Kappa value (95% CI)
		CMIA+ / ELISA−	CMIA− / ELISA +				
CMV IgM	154	5	22	75.3 (65.3–83.1)	92.3 (82.8–97.1)	82.5 (75.6–87.7)	0.65 (0.54–0.77)
CMV IgG	186	32	0	100.0 (95.5–100.0)	63.6 (53.2–72.9)	82.8 (76.7–87.6)	0.65 (0.55–0.75)
Rubella IgM	139	2	13	48.0 (30.0–66.5)	98.3 (93.4–99.9)	89.2 (82.9–93.4)	0.56 (0.36–0.75)
Rubella IgG	175	8	12	79.0 (66.6–87.7)	93.2 (87.0–96.7)	88.6 (82.9–92.6)	0.74 (0.63–0.84)
Syphilis treponemal IgM/IgG	188	0	4	82.6 (62.3–93.6)	100.0 (97.3–100.0)	97.9 (94.5–99.4)	0.89 (0.79–1.00)

[a] Indeterminate results were considered negative for agreement percentage and kappa value calculation.

rate of childbearing-age women testing negative with Architect™ CMIAs could lead to either unnecessary immunization recall or appropriate prevention of potential severe neonatal complications in addition to having an impact on postexposure management during pregnancy. These challenges are inherent to serology-based screening algorithms and need to be addressed through a close collaboration between microbiologists and clinicians in a patient-centered approach.

When transitioning from one assay to another, especially when initial validation studies indicate poor correlations like in the case of IgM assays, laboratories have a responsibility to provide guidance to end users. Assays with increased positivity rates can either be more sensitive or less specific, and this needs to be discussed with clinicians so that results are optimally interpreted and appropriate counseling is provided to patients. Different strategies such as repeated testing for high clinical suspicion of acute infection or reflex testing with complementary confirmation assays can be implemented, and medical microbiologists should be involved in the development of such algorithms as part of a laboratory stewardship process.

Even if these elements have not been objectively measured, transferring CMV, rubella, and syphilis antibody assays from microtitration ELISA assays to the Architect™ automated random-access analyzer is expected to reduce technical workload and improve turnaround times.

6. Conclusion

This study demonstrates that Achitect™ CMIAs correlate well with Captia™ ELISA for CMV IgM, CMV IgG, and rubella IgG and with Trep-Sure™ ELISA for syphilis treponemal antibodies in a mixed pediatric and women of childbearing age population. The fair correlation between Achitect™ CMIA and Enzygnost™ ELISA for rubella IgM should be the focus of future studies in pediatric and obstetrical settings.

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Competing interest

None.

Ethical approval

Not required.
See declaration forms for all authors' implications and signatures.

References

Abbott. CMV IgG Package Insert; 2008.
Bouthry E, HD, Revello MG, Furione M, Grangeot-Keros L, Vauloup-Fellous C. An evaluation of nine rubella IgG assays highlights many discrepancies in the interpretation of the results. 24th European Congress of Clinical Microbiology and Infectious Diseases; Barce- lona, Spain; 2014.
Clinical and Laboratory Standards Institute (CLSI). Clinical evaluation of immunoassays, approved guideline. , 28(22). Clinical and Laboratory Standards Institute; 2008.
Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New-York: John Wiley; 1981.
Lagrou K, Bodeus M, Van Ranst M, Goubau P. Evaluation of the new architect cytomegalovirus immunoglobulin M (IgM), IgG, and IgG avidity assays. J Clin Microbiol 2009; 47(6):1695–9.
Liu F, Liu LL, Guo XJ, Xi Y, Lin LR, Zhang HL, et al. Characterization of the classical biological false-positive reaction in the serological test for syphilis in the modern era. Int Immunopharmacol 2014;20(2):331–6.
Portella G, Galli C, Multicenter Italian Group for Hospital to RCe. Multicentric evaluation of two chemiluminescent immunoassays for IgG and IgM antibodies towards rubella virus. J Clin Virol 2010;49(2):105–10.
Skendzel LP, KD, Nakamura MD, Nutter CD, Schaefer LE, Stewart JA. Detection and quantification of rubella IgG antibodies: evaluation and performance criteria for multiple component test products, specimen handling, and use of test products in the clinical laboratory; approved guideline. National Committee for Clinical Laboratory Standards; 1997.
Tsimis ME, Sheffield JS. Update on syphilis and pregnancy. Birth Defects Res 2017;109(5): 347–52.
Wilson Christopher B, Nizet Victor, Maldonado Yvonne, Remington Jack S, Klein Jerome O. Remington and Klein's infectious diseases of the fetus and newborn infant. Elsevier Press; 2016.
Zhiyan L, Meiling W, Ping L, Jinhua D, Zhenlin Y, Zhenru F. Consistency between Treponema pallidum particle agglutination assay and architect chemiluminescent microparticle immunoassay and characterization of inconsistent samples. J Clin Lab Anal 2015;29(4):281–4.