ORIGINAL ARTICLE

DIAGNOSTIC PERFORMANCE OF SD BIOLINE IMMUNOCHROMATOGRAPHY TEST FOR THE DETECTION OF RUBELLA SPECIFIC IGM AND IGG ANTIBODIES IN RESOURCE LIMITED SETTINGS

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ABSTRACT

Background: *The diagnosis of rubella and congenital rubella syndrome (CRS) cases by only clinical parameters is unreliable. They are mostly confirmed by enzyme linked immunosorbent assay (ELISA). However, currently, there is a need of more rapid, cost effective and less complicated assays.*

Objective: *The main aim of this study was to evaluate the diagnostic performance of commercially available and newly developed Standard Diagnostic Bioline [SD Bioline] immunochromatographic (ICT) assay with the conventional ELISA method for the diagnosis of rubella IgM and IgG antibodies.*

Method: *A comparative cross-sectional study was conducted in Dessie, Felege-Hiwot and University of Gondar Referral Hospitals, from December 2015 to February 2017. After obtaining written informed consent, blood sample was collected from each pregnant woman for the laboratory analysis of rubella antibodies using both ELISA and SD Bioline methods.*

Result: *A total of 600 serum samples were analyzed by using ELISA and SD Bioline methods. For rubella IgM antibody determination, the sensitivity and specificity of SD Bioline against the ELISA method were 66.7% (95% CI: 52.9%-78.6%) and 98.9% (95% CI: 97.6%-99.6%), respectively (kappa=0.730, 95% CI: 0.62-0.83). Similarly, sensitivity and specificity of the SD Bioline to diagnose rubella IgG antibody against the ELISA method were 82.1% (95% CI: 78.6%-85.3%) and 96.0% (95% CI: 88.6%-99.2%) (Kappa= 0.511, 95% CI: 0.43-0.59). These results indicated that the SD Bioline had a substantial and moderate agreement with conventional ELISA for the diagnosis of rubella IgM and IgG antibodies, respectively.*

Conclusion: *The SD Bioline might be an alternative approach for the diagnosis of both rubella and CRS cases in the areas where ELISA or other advanced laboratory techniques are impractical.*

Key words*: Diagnostic performance, SD Bioline, rubella, resource limited setting, ELISA*

BACKGROUND

The clinical diagnosis of rubella virus (RV) infection alone is unreliable as it mimics other diseases like measles [1]. Furthermore, 50–75% of rubella cases are subclinical and the infected individuals may be difficult to be diagnosed clinically[2]. In addition, laboratory diagnosis is also mandatory for the confirmation of congenital rubella syndrome (CRS) cases [3].Furthermore, laboratory diagnosis can also play an important role for the surveillance of rubella and CRS cases [4].

Like other viral infections, rubella virus infection can be diagnosed by using serological testing, culturing or molecular analysis [5]. It can be detected by culturing the virus in Vero/SLAM/RK13 cells and incubated at 35°C for 3-5 days [5, 6]. However, this method is extremely labor-intensive and time-consuming [7] and can take 1 to 3 weeks to get a positive result [8]. Furthermore, as a rubella virus

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does not cause a cytopathic effect in cell cultures, culturing technique is not usually recommended[9]. Recently, the diagnosis of rubella and CRS cases is mainly using two important techniques [serological and molecular techniques] [10-13].However, in developing countries, molecular techniques may not be practical [14].Rather**,** due to the laboratory capabilities and cost issues, detection of rubella and CRS cases serologically are more likely to be available in developing countries [15].

For many years, serological testing is universally used to determine immune status and to diagnose rubella/CRS cases [16, 17]. Earlier, the assessment of rubella immunity and diagnosis of recent/acute rubella infections have been carried out mainly by the hemagglutination inhibition **(**HAI) tests [18]. But later, the HAI assay has been entirely replaced by more sensitive, specific and technically less demanding enzyme-linked immunosorbent assay (ELISA) [19].

Screening for rubella antibodies using ELISA as part of pre-conceptual or antenatal care represents an additional tool for the prevention and control of CRS. It is also useful to identify unprotected/non-immunized women to offer an active rubella vaccination [20]. In addition, in many countries, clinically recognized maternal rubella during the first 8 weeks of gestation is an indication for therapeutic abortion due to the high incidence of congenital rubella defects [8]. Therefore rubella screening in pregnancy helps to identify women at risk so as to offer the rubella vaccination during postpartumin order to protect future pregnancies [21, 22].Therefore, ELISA continued as a gold standard assay for detection of immunity against rubella. However, it is burdensome to perform, requires trained technical personnel and reports have long turnaround time as compared with rapid diagnostic techniques like SD Bioline kits and hence there is no routine antenatal screening in a public health facilities of many countries [23, 24].

Recently, there is an urgent need for more rapid, cost effective and less complicated assays for the diagnosis of rubella and CRS cases. Terada et al [25] have used immunochromatography technique (ICT) as a new rapid tool for rubella IgM and IgG antibodies detection with fairly good success. This technique is inexpensive, simple to perform and can give same day result. This might have great contribution for the prevention and control of rubella and/or CRS cases especially in resource limited countries. Currently, there is only one ICT technique Standard Diagnostic Bioline [SD Bioline] [SD-Bioline, Republic of Korea] that could be used for the diagnosis of rubella specific IgM and IgG antibodies in Ethiopian market. However, there were no data on the performance of this rapid rubella IgM and IgG diagnostic test kit in the country. Therefore, the aim of this study was to evaluate the diagnostic performance of this commercially available and newly developed SD-Bioline with the conventional ELISA method for the diagnosis of rubella specific IgM and IgG antibodies.

METHOD

Study design, area and period: A comparative cross-sectional study was conducted in three Amhara Regional State Referral Hospitals, namely Dessie, Felege-Hiwot and University of Gondar Referral Hospitals, from December/2015- May/2017. Dessie referral hospital is found in Dessie Town, which is located in South Wolo Administrative Zone, Northeast Ethiopia and it is 388km far from the capital city, Addis Ababa. Felege-Hiwot referral hospital is found in Bahir-Dar Town which is located in the Northwest part of Ethiopia and it is the capital city of the Amhara Regional State. It is approximately 578 km far from Addis Ababa. University of Gondar Hospital is found in Gondar Town which is 747 km far from the capital city of the country, Addis Ababa

and is found in Northwest Ethiopia. The respective referral hospitals have specializations in internal medicine, pediatrics, gynecology, surgery, ophthalmology and other health related specializations. Furthermore, these referral hospitals also act as teaching hospital or clinical attachment sites for different health professionals.

Study participants, sample size and sampling technique: The study participants were all pregnant women who visited the respective referral hospitals' antenatal care (ANC) clinics during the study period and gave informed consent and required amount of blood sample for laboratory analysis. The study participants were selected using simple random sampling technique and the sample size was calculated using single population proportion formula by considering 95% confidence interval, 4% margin of error and 50% proportion. The sample size was proportionally allocated for the selected referral hospitals based on the previous flow of the pregnant women to visit the ANC clinics of the respective referral hospitals.

Blood collection, handling and transportation: After obtaining informed written consent, 5ml venous blood was collected aseptically from each pregnant woman by medical laboratory professionals. Then blood sample was allowed to clot for 1 hour at room temperature, centrifuged at 3500 rpm for 5 minutes and then serum was separated and collected into sterile vials and stored at-20^{o}C in the respective referral laboratories until transported into the School of Biomedical and Laboratory Sciences, University of Gondar to be stored at -70^{o}C until the laboratory analysis.

Laboratory analysis and result interpretations: The collected serum samples were tested for rubella virus specific antibodies (IgM and IgG) by using ELISA (Linear Chemicals SL, Spain) and rubella virus rapid diagnostic test (SD-Bioline, Republic of Korea) in parallel in the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar.

The rubella IgM ELISA test is a solid phase enzyme immunoassay based on immunocapture principle for the qualitative detection of IgM antibodies to rubella in human serum or plasma. The microwell plate is coated with anti-human IgM antibodies. During testing, the specimen diluent and the specimens are added to the antibody coated microwell plate and then incubated. If the specimens contain IgM antibodies to rubella, it will bind to the antibodies coated on the microwell plate to form immobilized anti-human IgM antibody-rubella IgM antibody complexes. However, if the specimens do not contain IgM antibodies to rubella, the complexes will not be formed. As to the rubella IgG ELISA test, it is a solid phase enzyme immunoassay based on indirect principle for the qualitative and quantitative detection of IgG antibodies to rubella in human serum or plasma. The microwell plate is coated with rubella antigens. During testing, the specimen diluent and the specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain IgG antibodies to rubella, it will bind to the antigens coated on the microwell plate to form immobilized antigen-rubella IgG antibody complexes. If the specimens do not contain IgG antibodies to rubella, the complexes will not be formed.

For rubella specific IgMand IgG determination using ELISA method, results were read by a micro well reader at 450nm compared in a parallel manner with calibrators and controls. The qualitative result IgM result was interpreted as positive if the rubella IgM index was > 1.1, negative when the index was < 0.9 and equivocal when the index was $\geq$ 0.9 and $\leq$1.1. For the quantitative determination of rubella specific IgG antibody, the IgG result was expressed in inter-

national units per milliliter (IU/ml). According to the manufacturer's instruction, the IgG result was interpreted as positive when the IgG index-value was >10 IU/ml, equivocal when the index-value was 5-10 IU/ml and negative when the index-value was <5 IU/ml.

As to the SD Bioline rubella IgG/IgM kit, it is a solid phase immunochromatographic assay for the rapid, qualitative and differential tests for the detection of rubella IgG/IgM antibodies in human serum or plasma. SD Bioline rubella IgG/IgM test device has three pre-coated line: "G" (rubella IgG test line), "M" (rubella IgM test line) and "C" (Control line) on the surface of the device. All these three lines in result window are not visible before applying any samples. As to the result interpretation, the control line is used for procedural control and it should always appear if the test procedure is performed properly and the test reagents of control line are working. If a purple color is visible on the control line (C) and IgG line (G) only on the test device, the result is positive for rubella specific IgG. If a purple color is visible on the control line (C) and IgM line (M) only on the test device, the result is positive for rubella specific IgM. If a purple color is visible on the control line (C), IgG line (G) and IgM line (M) on the result window of the test device, the result is positive for both rubella specific IgG and IgM. If the control line (C) fails to appear, the result is invalid and the test was repeated using new test device. According to the manufacturer, the SD Bioline has sensitivity of 98.33% and 99.14% and specificity of 97.64% and 91.55% to diagnose rubella IgM and IgG antibodies, respectively[26].

Quality assurance mechanisms: The rubella test kits (IgM and IgG ELISA kits) have their own quality control materials that can be run in parallel with patient samples. The SD Bioline test kits have also internal quality control mechanisms. All test procedures of ELISA and SD Bioline were done strictly following the manufacturer's instructions. In addition, standard operational procedures were strictly followed.

Data analysis procedure: Data were entered and analyzed using SPSS version 20 statistical package. Data was summarized using graphs and frequency tables. The sensitivity, specificity, positive and negative predictive values of SD Bioline test against the ELISA result was calculated using MedCalc statistical software. The kappa coefficient (kappa tests) was performed to see the agreement between SD Bioline and ELISA methods. The kappa coefficient can be interpreted as poor and fair agreements when the calculated kappa value was<0.20 and 0.21-0.40, respectively. If the kappa value is between 0.41-0.60, 0.61-0.80 and 0.81-1.00, it can be also interpreted as moderate, good or substantial and very good or perfect agreement, respectively[27].

Ethical approval and consent to participate: The study was conducted after obtaining institutional ethical clearance from University of Gondar Ethical Review Board (UOG-IRB). Letter of agreement and cooperation from each referral hospital clinical director/chief executive officer (CEO) was obtained. The purpose and importance of the study was explained to the study participants prior to their participation. Informed written consent was also obtained from each study participant as per the *National Research Ethics Review Guideline.*

RESULT

Of the total 600 serum samples analyzed by ELISA, 57 (9.5%) of them were positive and the remaining and 543 (90.5%) of them were negative for rubella specific IgM antibody. However, using SD Bioline ICT methods,44 (7.3%) of the serum samples were positive and 556 (92.7%) of them were negative for rubella specific IgM antibody (Figure 1). As to the determination of rubella specific IgG, of the total

serum samples, 526 (87.7%) of them were ELISA positive and 74 (12.3%) of them were ELISA negative. However, using SD Bioline, 435 (72.5%) samples were positive for rubella specific IgG antibody and the remaining 165 (27.5%) of them were rubella IgG negative (Figure 2).

Sensitivity and specificity of SD Bioline to detect rubella specific IgM antibody against the conventional ELISA method were 66.7% (95% CI: 52.9%-78.6%) and 98.9% (95% CI: 97.6%-99.6%), respectively. Similarly, the sensitivity and specificity of SD -Bioline to detect rubella specific IgG antibody against ELISA method were 82.1% (95% CI: 78.6%-99.2%) and 96.0% (95% CI: 88.6%-99.2%), respectively.

The positive and negative predictive value of the SD-Bioline to detect rubella specific IgM antibody were 86.4% (95% CI: 73.7%-93.5%) and 96.6% (95% CI: 95.1%-97.6%), respectively (kappa=0.730, 95% CI: 0.62-0.83). The positive and negative predictive values of the SD-Bioline to detect rubella specific IgG antibody were 99.3% (95% CI: 97.9%-99.8%) and 43.0% (95% CI: 38.5%-47.7%), respectively (Kappa= 0.511, 95% CI: 0.43-0.59) compared with ELISA method (Table 1).

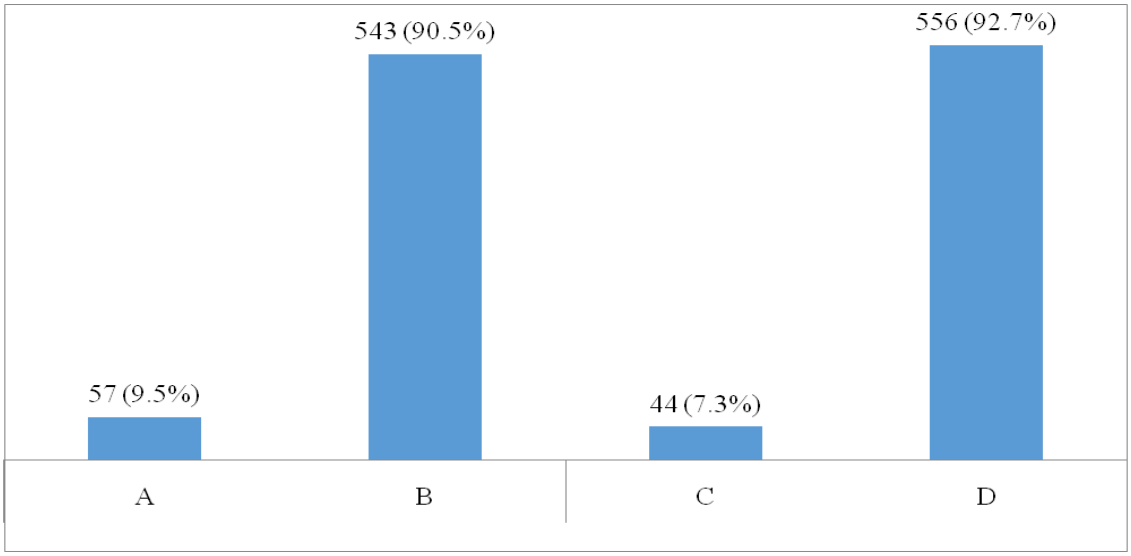

***Figure 1**: The positivity rates of rubella specific IgM antibody using ELISA and SD-Bioline methods. The letter "**A**" indicates ELISA IgM positive samples, **B**: ELISA IgM negative samples, **C**: SD-Bioline IgM positive samples, **D**: SD-Bioline IgM negative samples.*

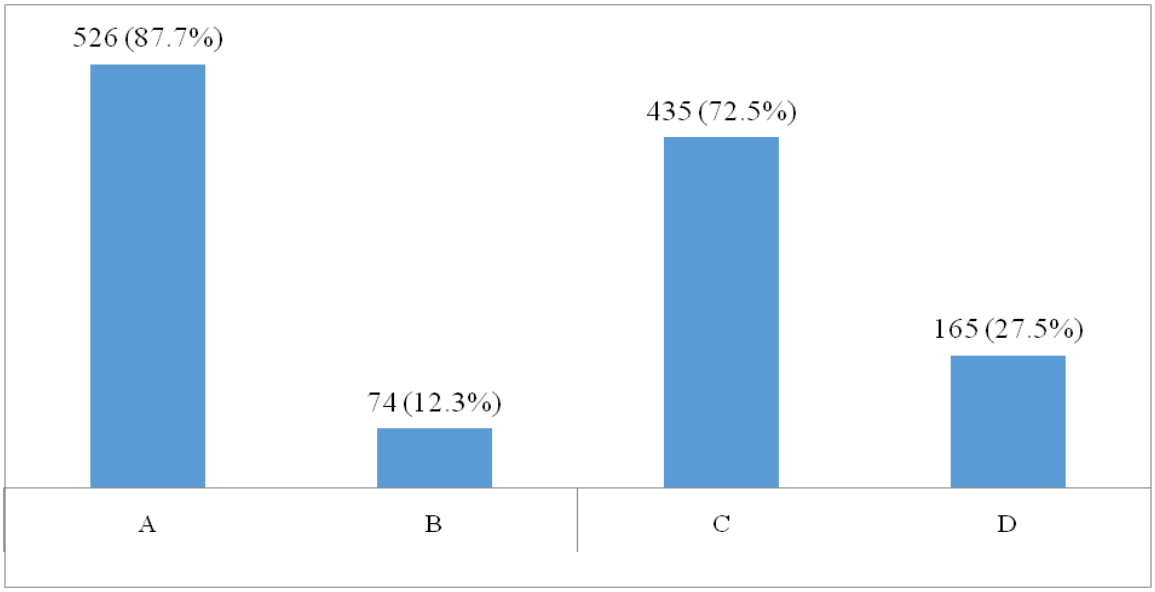

***Figure 2**: The positivity rates of rubella specific IgG antibody using ELISA and SD-Bioline methods. The letter "**A**" indicates ELISA IgG positive samples, **B**: ELISA IgG negative samples, **C**: SD-Bioline IgG positive samples, **D**: SD-Bioline IgG negative samples.*

Table 1: The sensitivity, specificity, positive and negative predictive values of SD-Bioline against the conventional ELISA method

Rubella specific antibodies	SD-Bioline				
	Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Kappa-value (95% CI)**
Rubella IgM	66.7% (52.9-78.6)	98.9% (97.6-99.6)	86.4% (73.7-93.5)	96.6% (95.1-97.6)	0.730 (0.62-0.83)
Rubella IgG	82.1% (78.6-85.3)	96.0% (88.6-99.2)	99.3% (97.9-99.8)	43.0% (38.5-47.7)	0.511 (0.43-0.59)

***Key:** PPV: Positive predictive value, NPV: Negative predictive value*

DISCUSSION

Prevention of rubella associated morbidity and mortality depends on the prevention of infection in childbearing women and early recognition of maternal infection [28]. The detection of rubella specific antibodies serologically has a great importance for the determinations of the immune status of childbearing women and to diagnose acute rubella infection [5]. Although rubella and CRS cases can be diagnosed by different techniques, the most commonly used and the conventional diagnostic method is ELISA[29]. However, for the prenatal counseling and laboratory assessments, inexpensive, simple to perform and assays that can give same day result is mandatory especially in resource limited settings like Ethiopia. The performance of a diagnostic test might be depend on factors like the intrinsic ability of the diagnostic test, the particular characteristics of each individual and the environment in which the diagnostic tests is going to be applied[30]. In this study, we have assessed the performance of an ICT (SD Bioline) against the conventional and commercially

available ELISA method for the diagnosis of rubella specific IgM and IgG antibodies.

According to the present study, 9.5%and 7.3%of serum samples were IgM positive by ELISA and SD Bioline methods, respectively. To see the performance of a given test against the conventional gold standard method [31], it is mostly measured by its sensitivity and specificity. In the present study, the sensitivity and specificity of the SD Bioline to diagnose rubella specific IgM against the ELISA method were 66.7% and 98.9%, respectively. These implied that 33.3% of the ELISA IgM positive samples were falsely reported as IgM negative by SD Bioline. However, only 1.1% of ELISA IgM negative samples were falsely reported as IgM positive. This can be explained that in practical situation, the sensitivity and specificity might be inversely proportional (as one increases the other decreases and vice versa) [32].In the present study, the low false positivity of the SD Bioline to diagnose the rubella specific IgM antibody indicates that the SD Bioline specificity was greater than its sensitivity.

In the present study, the probability of SD Bioline to identify those rubella ELISA IgM positive and negative samples were 86.4% and 96.6%, respectively. These results indicate that 13.6%and 4.3% of ELISA IgM positive and negative samples have the probability to be reported falsely as IgM negative and positive, respectively. However, as these predictive values might be affected by the prevalence of a given disease [33], the calculated kappa coefficient might be more practical to see the level of agreement between two laboratory assays [34]. In the present study, the calculated kappa value was 0.730. This indicates that the SD Bioline had a substantial (good) agreement with that of the ELISA method for the diagnosis of rubella specific IgM antibody among individuals with acute or recent rubella and congenital rubella infections. In another study, even a better agreement between ICT and ELISA has been also reported [35]. Hence, this ICT assay might be useful for the diagnosis of recent/acute postnatal or congenital rubella infections (CRI) in areas where ELISA or other advanced laboratory assays might not be available.

In this study, we have also determined rubella specific IgG antibody by using both ELISA and SD Bioline methods. Of the total samples, 87.7% and 72.5% were IgG positive by using ELISA and SD Bioline, respectively. When we compared the diagnostic performance of SD Bioline against ELISA for the detection of rubella specific IgG, SD Bioline had sensitivity and specificity of 82.1% and 95.9%, respectively. Similar to our findings, the ICT assay had high sensitivity (99.4%) and specificity (100%) for the diagnosis of rubella specific IgG antibody in the previous study [36]. But in another study[24], the SD Bioline had lower sensitivity (36.6%)and specificity (22.5%) to diagnose rubella IgG antibody. These variations on the sensitivity and specificity of ICT against the rubella IgG ELISA methods in different studies might be associated with manufacturing defects, storage condition, transportation problem and end user performance differences.

According to the present study, 17.9% of the rubella ELISA IgG positive samples were falsely reported as IgG negative by SD Bioline. In addition, 4.1% of the rubella ELISA IgG negative samples were also falsely reported as IgG positive. This low false positivity indicates that the SD Bioline specificity is also greater than its sensitivity for the diagnosis of rubella specific IgG antibody as discussed earlier. In the present study, the probability of SD Bioline to identify those ELISA IgG positive samples was 99.3%. However, the probability of this SD Bioline to identify those ELISA IgG negative samples was only 43.0%. This low negative predictive value might be due to the low prevalence of IgG negative samples. When

we see the agreement of the SD Bioline with that of the ELISA, the calculated kappa value in the present study was 0.511. Despite its low NPV, this kappa value indicates that the SD Bioline has moderate agreement with ELISA for the diagnosis of IgG antibody or to determine the immune status of an individual and categorized as immuned or susceptible. As disused earlier, this ICT assay might be also important to determine the immune status of a childbearing women before getting pregnancy to prevent congenital rubella infection or rubella associated anomalies of the new born in areas where ELISA assays might not be practical.

In general, when large volumes of samples are to be tested, a test kit that do not require high technology, simple and fast to perform, cost effective, easily interpreted, reliable and same day results is required [37]. Therefore, despite its sensitivity, specificity and kappa coefficient variations for the diagnosis of rubella specific IgM and IgG antibodies in different studies, the SD Bioline can add a battery for the available rubella/CRS diagnostic tests especially in resource limited settings. It only requires little technical expertise, takes lesser time (only 20-30minutes), can determine both rubella IgM and IgG antibodies at a time and could be done without elaborate equipment and it can be done in areas where electricity is not avail unlike that of ELISA techniques [24, 25].

Furthermore, the SD Bioline can be easily performed in many public health laboratories unlike advanced molecular assays like Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)[38]. Hence, this SD Bioline would be useful in clinical laboratories especially in resource limited settings when immediate laboratory results are required for the management of patients [24]. In addition, this would be increasingly important and can guide prenatal management as well as identify the need for long-term follow up in the case of CRS in resource limited settings like Ethiopia.

Limitation of the study: Due to the lack of molecular reagents and other rapid kits for rubella diagnosis in Ethiopian market during the study period, the performance of SD Bioline was evaluated only against ELISA method.

CONCLUSION

According to the present study, the SD Bioline had a substantial agreement with the ELISA assay for the diagnosis of rubella specific IgM antibody. This ICT had also moderate agreement with commercially available ELISA method to diagnose rubella specific IgG antibody. Furthermore, it could be also an alternative approach for the diagnosis of both rubella/ CRS cases especially in resource limited settings like Ethiopia.

Competing interests: The authors have declared that no competing of interest with respect to the authorship and/or publication of this research paper.

Availability of data and materials: All data generated and analyzed during this study are included in this manuscript. All the findings reported in this research can be freely available for any researchers or scientists throughout the globe to use them for none commercial purposes.

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Authors' contributions: YW: Participated in conceived, designed and proposed the research idea, data collection, data clearance, entry, analysis and interpretation of the findings and drafting the manuscript and write up. **MT**: Participated in the conception, design and proposed the research idea, data analysis and interpretations of the findings. **GF:** Participated

in design of the study, data analysis and interpretations of the findings. **BT**: Participated in the conception, design and proposed the research idea, data analysis and interpretations of the findings. All authors reviewed and approved the final manuscript.

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