Assessing feasibility of using Oral Fluid assay as Alternative method in the Detection of Rubella Virus-Specific IgM Antibodies in routine disease surveillance Programme in Kenya

Chelangat Rose[1], Kombich Janet[2] and Ongus Juliette R[1]
1. Jomo Kenyatta University of Agriculture and Technology
2. University of Kabianga, Department of Biological Sciences
3. Kenya Medical Research Institute (Centre for Virus Research)

Abstract

Background: The WHO recommends the inclusion of rubella testing in the measles surveillance system. Laboratory diagnosis of measles and rubella virus infection is achieved by serological assay for specific IgM from a sample of blood drawn by vein puncture. This conventional method of sample collection is invasive and less acceptable.

Aim: To assess feasibility of using oral fluid as an alternative method in the detection of rubella-virus specific IgM in routine surveillance of rubella

Settings and Design: A prospective laboratory-based cross-sectional study using matched oral fluid and serum collected from emerging outbreaks of rash-like illnesses across Kenya.

Methods and Material: Matching samples of 176 patients were investigated for IgM specific antibodies using enzyme linked immunosorbent assays.

Statistical analysis used: The kappa (k) statistic was used to measure inter-observer variations.

Results: The prevalence of rubella using serum and oral methods was 26.7% and 23.3% respectively. Sensitivity and specificity for rubella IgM in oral fluid when tested against the gold standard was 86% and 93% respectively. Kappa statistic value was 0.80 suggesting substantial agreement between the two methods.

Conclusion: The study showed that oral fluid method is a promising simple alternative, non-invasive and more acceptable specimen of choice for rubella diagnosis. The alternative method will be more applicable to disease surveillance programmes where clinical settings are varied. The advantage of this method of sample collection is ease and safety with minimum requirement for shipment to laboratory. These findings will support the entire disease surveillance system in Kenya and also can have extended use in conducting epidemiological studies.

Key words: Oral fluid, serum, diagnosis, surveillance, prevalence, diseases, measles and rubella

1. Introduction

Since inception of measles surveillance in Kenya, the assay method of choice has been using blood for IgM detection using a commercially manufactured ELISA kit. As a standard procedure, rubella testing is also performed using the same sample. This is because rubella infection presents with almost similar clinical manifestations as measles.

The WHO recommended rubella testing as a differential diagnosis of measles in routine measles surveillance system of member countries. In support of the initiative, it established a global measles and rubella laboratory network [23]. Data collected over the years showed that rubella is endemic in Kenya and 30% to 50% of suspected measles cases are laboratory confirmed as being caused by rubella virus [23]. This has led to introduction of Measles and Rubella (MR) Vaccine into the National Immunization schedule.

Rubella virus is a teratogenic virus that causes congenital disorders to the children who are born to mothers who had the infection during the first trimester of their pregnancy [4]. Rubella virus affects children, adolescents and the young adults. Around 50% of the rubella infections are subclinical and can only be confirmed through laboratory testing. It may be asymptomatic or mild infection in adults and children [3]. This infection during pregnancy especially during the first trimester can result in miscarriage, stillbirth to congenital defect if the foetus is not miscarried [22]. The main symptoms include inflammation of the lymph nodes and a maculopapular rash that may be preceded by mild catarrhal symptoms. Lyphadenopathy occurs from 5-7 days before the onset of the rash and up to 2 days after [5]. Rubella virus infection occurs via the respiratory route. It infects the nasopharynx and multiplies in the lining of the respiratory tract and in local lymph nodes before getting to viraemic phase that begins within 4-5 days after the infection and spreads to the rest of the body. Rubella virus has an incubation period of 14-18 days [14].

In patients where phlebotomy is not possible, saliva can be collected for salivary rubella-specific IgA testing. Positive contact with other patients known to have rubella adds strong epidemiological evidence to the diagnosis. The contact with any infected person in any way, including semen through sex, saliva, or mucus, can cause infection [19].

In this study oral fluid was used as an alternative method for detection of rubella IgM. This method was explored because of the challenges posed by collection, maintenance and safety of blood sample. In addition published literature has shown that transportation of oral fluid does not require low temperatures during shipment when compared to blood [17].

2. Materials and methods
Ethical consideration
Ethical clearance was sought from Ethical Review Committee and also from Jomo Kenyatta University of Agriculture and Technology. Authority was also sought from the Ministry of Health since the samples were collected as part of routine surveillance of measles and rubella. The research was conducted in accordance with KEMRI guidelines on human sample use and care and the internationally accepted principles for laboratory procedures using standard operating procedures [26].

Study site and design
The study site was determined by outbreaks of rash-like illness in the country. On notification of emerging rash-like outbreaks, samples were collected using an established disease surveillance system. The samples were collected from different districts in Kenya and transported to the Kenya Medical Research Institute (KEMRI) laboratory for processing. The districts were; Fafi, Garissa, Kaloleni, Kamukunji, Lagdera, Nairobi North, Nairobi West, Nakuru North, Tana River, Taveta, Turkana North, Wajir East, Wajir South, Wajir West and Kakuma (Sudanese living in Kenya).This was a prospective laboratory-based cross-sectional study design over a period of six months from June- December 2010.
Study population
Samples were collected using the WHO standard [25] case definition for based on the clinical presentation under routine surveillance system.

Sample size
A hundred and eighty (180) matched samples were collected based on the Ministry of Health (MoH) surveillance system 2008-2009 requirement for laboratory confirmation of suspected measles outbreaks.

Specimens collection
Samples of oral fluid and blood were collected from each study participant within 28 days of rash onset. Venous blood (3mm) was collected in a vacutainer tube technique described by WHO [25]. Whole blood specimen was allowed to fully clot for 15-30 minutes at room temperature before harvesting of serum. Serum was transported using cool boxes with frozen ice packs and stored at 4^oC waiting in the laboratory until analysis. The period of storage within the laboratory was less than 3 days.
Oral fluid was collected using an ORACOL test kit (Malvern Medical Developments, Worcester, UK). The device has a sterile swab, with an absorbent material that was placed into the patient's mouth between the lower cheek and gum and left in until adequately moistened. The pad was removed and inserted in the bottom of a vial containing preservative. Consistency in labelling for the two samples from a patient was ensured. The same identification number was entered in the case-based request form. The oral fluid in the swabs was extracted as soon as it arrived in the laboratory by adding 1ml of the virus transport medium to the tube containing the oral fluid swab. The swab was then agitated by vortexing to ensure foaming of the transport medium. The swab was removed from the tube by a twisting motion; centrifugation at 2000 rpm for 5 minutes to ensure that much liquid was recovered from the swab. Extracted fluid was stored at 4^oC until analysis was done.

Diagnosis of Rubella Virus IgM using Serum method
The Enzygnost®Anti-Rubella-Virus/IgM immunoassay (Siemens Healthcare Diagnostics Products, Marburg, Germany) was used to analyse the serum specimens to detect Rubella-Virus IgM. Enzyme Immunoassay is for both qualitative and quantitative determination of IgM antibodies to rubella virus in human serum and plasma. The test was developed for testing individual samples, not for pooled samples. The method is ELISA-based and uses a commercially available kit therefore manufacturer instructions were used. Results were interpreted qualitatively using optical densities (OD). The reading of optical density was done at 450/650 nm using spectrophotometric plate reader.

Diagnosis of Rubella Virus IgM using Oral fluid method.
The Microimmune Rubella IgM capture Enzyme Immunoassay kit (Microimmune Ltd, Middlesex, UK) was used to detect Rubella IgM in oral fluid. Samples and test reagents were brought to room temperature (18-24 ^oC) prior to testing. Manufacturer instructions were followed in the testing using the kit. The reading of optical density was done at 450/650 nm using spectrophotometric plate reader.

Oral fluid diagnostic test interpretation Data analysis and presentation

In each test, the relative sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of oral-fluid results were calculated relative to serum being the gold standard. Concordance between the oral fluid-based and corresponding serum-based assay results was evaluated by considering all sample pairs in the study.

Descriptive findings were presented in form of tables, graphs and charts to show the comparison of the results. The kappa (k) statistic was used to measure inter-observer variability.

Results

Rubella seropositivity using serum and oral fluid were 26.7% and 23.3% respectively (Table 1) Samples took an average of three days to arrive in the laboratory.

Table 1: Comparison of oral fluid and serum prevalence

Out come	Rubella	
	Serum	*Oral fluid*
Positive	47(26.7)	41(23.3)
Negative	113 (64.2)	117 (66.5)

➤ ***Rubella seropositivity using serum and oral fluid were 26.7% and 23.3% respectively.***
➤ ***Samples took an average of three days to arrive in the laboratory***

Sensitivity and specificty for rubella

Serum test for rubella showed that 64.2% of the participants tested negative while 26.7% tested positive. Oral fluid on the other hand revealed that 63.8% tested negative while 36.2% tested positive.

In the rubella test, concordance was confirmed in 176 of samples resulting in sensitivity rate of 86%.and specificity rate of 93%

Table 2: Sensitivity and specificity for Rubella virus

Rubella test	Sample size	Sensitivity (%)	95% CI	Specificty (%)	95% CI	NPV	PPV
Serum	*176*						
Oral fluid	*176*	*86*	*0.05*	*93*	*0.05*	*93*	*86*

➤ ***Serum test for rubella showed that 64.2% of the participants tested negative while 26.7% tested positive.***
➤ ***Oral fluid on the other hand revealed that 63.8% tested negative while 36.2% tested positive.***
➤ ***In the rubella test, concordance was confirmed in 176 of samples resulting in sensitivity rate of 86%.and specificity rate of 93%***

Rubella test

The calculation based on the difference between how much agreements were actually present ("observed" agreement) compared to how much agreement would be expected to be present by chance alone ("expected" agreement). The statistical analysis was necessary to determine whether the oral fluid assays were effective in detecting the ribonucleic acid paramyxovirus in the same manner as invasive serum assays. Since the hypothesis of this study was to examine whether Rubella oral fluid IgM assays can be used as an alternative to Rubella serum IgM assays, the study checked the relationship between the two tests using this tool, the calculation based on the difference between how much agreements were actually present ("observed" agreement) compared to how much agreement would be expected to be present by chance alone ("expected" agreement) is determined. Calculations based on results for rubella tests are 0.56

Therefore, observed agreement is simply the percentage of all tests for which the two test evaluations agree, 0.91 Kappa, K is 0.80.

Discussion

The findings in this study confirmed that an oral fluid sample can be used as an alternative to blood in the surveillance of rubella in Kenya. This proposed and tested method will be useful especially post introduction of measles and rubella (MR) vaccination in Kenya (ref). As a follow of the introduction of MR vaccine in routine immunization, active surveillance of rubella cases in assessing the impact of the intervention and also serosurveys will benefit greatly from these results. The samples used were collected from geographically diverse regions in Kenya confirming versatility of the new method. Failure to perform laboratory confirmation of suspected outbreaks can lead to missed opportunities to prevent transmission. Laboratory confirmation has

become important to both measles and rubella control programs world over [7]. Existing laboratory methods rely largely on the detection of significant rises in rubella antibody titer or the detection of rubella IgM antibody. Collection of blood sample is often considered invasive requiring specially trained persons which can limit usability. A non-invasive method of sample collection can have an important role especially in areas with limited resources[12].

The findings in this study demonstrated high level of concordance between serum and oral fluid IgM results which was demonstrated by the high specificity and sensitivity rates and an acceptable agreement using Kappa statistic. Other investigators have also confirmed versatility of oral fluid for rubella IgM detection. The results of Talat et al., yr?[24] showed indeed that whole saliva contains IgM antibody at concentrations high enough to be diagnostically useful. Its applicability has been tested widely as demonstrated by Author? Where antibody-capture radio immunoassay, showed that virus specific IgM was detected in 100% of rubella saliva samples collected between 1 and 5 weeks after onset of disease [21]. Also in another community-based study in England and Wales found out that the sensitivity of saliva rubella IgM testing was 81% when compared to blood. Another study done in Ethiopia by Nokes et al.,[15] showed that the overall sensitivity and specificity were 79% and 90% for rubella in a study done in Ethiopia.. The, timing of salivary specimen collection is another important factor in determining whether viral specific IgM will be detected. Although this study did not focus on duration after rash onset for sample collection several studies have confirmed that rubella IgM can be detected well shortly after rash onset but within 14 days of rash onset [7]

The mildness of the majority of rubella cases makes parents and medical practitioners reluctant to take blood for diagnosis. Moreover, in developing countries rubella outbreaks can occur with no clinical recognition, even in a community in which health is being monitored. The use of non-invasive specimens for diagnosis offers several advantages over blood such as: acceptability to patients, applicability to children and reuse of disposable equipment can be ruled out. In addition occupational risk from needle stick injures is eliminated. The present work and other studies indicate that oral fluid is a viable alternative to serum for monitoring the impact of vaccination programmes and disease surveillances in the future.

Data has shown that approximately 30 to 50% of suspected measles cases are often confirmed as being caused by rubella virus (, [23]. Diagnosis of rubella using oral fluid samples instead of serum samples offers many advantages that can support disease surveillance programmes in any country [12]. Among the many advantages are ease of specimen collection, non-invasiveness and also comparative acceptability in many communities. .

The results demonstrated that oral fluid specimens are a convenient alternative to serum for diagnosis of recent rubella infection. The widespread acceptability of oral fluid collection should facilitate the investigation of rubella outbreaks and have an important role in controlling the disease in regional and national public health laboratories worldwide. Additionally, refusal in collecting blood samples due to cultural or religious traditions and vein puncture related problems may increase the difficulty in obtaining specimens for testing [8].
Oral fluid is a valid alternative to serum for IgM detection of rubella antibodies that could play a major role in surveillance of rubella and also for use in rubella serosurveys. This study therefore confirms the potential of incorporating enzyme linked immunosorbent assay using oral fluid as a sample of choice in detection of rubella IgM. For routine surveillance and epidemiological surveys nationwide and also for other regions with similar settings. Moreover, the turnaround time for results in this method does not deviate from that of the gold standard yet it offers additional advantages comparatively.

5. Conclusion
The study showed that oral fluid method is a promising simple alternative, non-invasive and more acceptable specimen of choice for rubella diagnosis. The alternative method will be more applicable to disease surveillance programmes where clinical settings are varied. The advantage of this method of sample collection is ease and safety with minimum requirement for shipment to laboratory. These findings will support the entire disease surveillance system in Kenya and also can have extended use in conducting epidemiological studies.

References

1. Amado C, Murray C, Lopez A, Mathers C, Stein C. The global burden of disease. Project: aims, methods and data sources [discussion paper no.36]. Geneva: *World Health Organization/Global Programme on Evidence for Health Policy*, 2006; 1–57.
2. Brown DG, Ramsay MB, Richards AF and Miller E. Salivary Diagnosis of Measles: A Study of Notified Cases in the United Kingdom, 1991-3. *BMJ*, 1994;308: 1015-7,
3. Cradoc-Watson J E. Laboratory diagnosis of rubella :past, present and future. *Epidemiol. I fect,* 1990; 107: 1-15.
4. Cutts FT, Robertson SE, Diaz- Ortega JL, and Samwel R. Control of rubella and syndrome (CRS) in

developing countries, Part 1: the burden of disease from CRS. *Bulletin of World Health Organization,* 1997; 75 (1): 55-68.

5. Feigin RD and Cherry JD. Textbook of pediatric infectious diseases.4th Ed 1997; 2054-2074.

6. George JR, Fitchen JH. Future Application of Oral Fluid Specimen Technology. *Am. J. Med.,* 1997; 102 (Suppl 4A): 21- 25

7. Helfand RF, Kebede S, Alexander JP, Alemu W, Heath JL. Comparative Detection of Measles-Specific IgM in Oral Fluid and Serum from Children by an Anti-body-Capture IgM EIA. *J. Infect. Dis*., 1996; 173: 1470-4

8. Holm-Hansen C, Constantine NT, Haukenes G. De- tection of Antibodies to HIV in Homologous Sets of Plasma, Urine and Oral Mucosal Transudate Samples Using Rapid Assays in Tanzania. *Clin. Diagnos. Virol*., 2010; 1: 207-14

9. Kingsbury DW, Bratt MA, Choppin PW, Wunner WH. Paramyxoviridae. Journal *lntervirology*, 2007; 10:137-152,

10. Lenesha W, Rimantas S, Kaw BC, Wondatir N, Kevin E Brown, Kestutis S, Dhanraj S & David B. A point-of-care test for measles diagnosis: detection of measles-specific IgM antibodies and viral nucleic acid. *Bulletin of the World Health Organization* 2011; 89:675-682.

11. Matsubara J, Kamiyama T, Miyoshi T, Ueda H, Saito M, Nakagawa M. Serodiagnosis of Streptococcus pneumoniae Infection in Guinea pigs by Enzyme-linked Immunosorbent Assay. *Lab. Anim*., 1998; 22: 304-308

12. Mortimer PP and Parry JV. The use of saliva for viral diagnosis and screening. *Epidemiol. Infect,* 1998; 101:197-201.

13. Mortimer PP, and Parry JV. Non-invasive virological diagnosis: are saliva and urine specimen adequate substitute for blood? *Rev. Med. Virol,* 1996; 1: 73-78

14. Murray M. The rubella virus microbiology and immunology 3rd Ed; *Oxford University Press pp,* 2006; 429-502.

15. Nokes James D, FikreEnquselassie, WondatirNigatu, Andrew J Vyse, Bernard J Cohen, David WG Brown and Felicity T Cutts. Has oral fluid the potential to replace serum for the evaluation of population immunity levels? A study of measles, rubella and hepatitis B in rural Ethiopia; *Bulletin of the World Health Organization,* 2001; 79 (7).

16. Oliveira SA, Siqueira MM, Brown DWG, Camacho LAB, Faillace T. Salivary Diagnosis of Measles for Surveillance: A Clinic-Based Study in Niteroi, State of Rio-de Janeiro, Brazil. Trans. R. Soc, Trop. *Med. Hyg.,* 1998; 92:636-8,

17. Parry JV, Perry KR, and Mortimer PP. Sensitive assays for viral antibodies in saliva: an alternative to tests on serum. *Lancet II* 2000; 72-75.

18. Perry KR, Brown DWG, Parry JV, Panday S, Pipkin C, and Richards A. Detection of measles, mumps, and rubella antibodies in saliva using antibody capture radio-immunoassay. *Journal of Medical Virology,*1993; 40:235-240.

19. Perry, RobertT, Halsey, Neal A. "The Clinical Significance of Measles: A Review". *The Journal of Infectious Diseases,*2004; 189 (S1): S4–16. doi:10.1086/377712.

20. Piacentini P, Peltola H, Heinonen P, Valle M. The elimination of indigenous measles, mumps and rubella from Finland by a 12-year, two-dose vaccination program. *N Engl J Med*; 1993; 331:1397–1402.

21. Ramsay ME, Brugha R, Brown DWG, Cohen BJ, and Miller E. Salivary diagnosis of rubella: a study of notified cases in the United Kingdom, 1991-4. *Epidemiol. Infect*., 1998; 120:315-319.

22. Reef SE. Rubella and congenital rubella syndrome. *Bulletin of the World Health Organization,* 1998*;* 76:156-157.

23. Robertson SE, Featherstone DA, Gacic-Dobo M and Hersh BS. Rubella and congenital rubella syndrome: *global update. Rev. Panam. SaludPublica,*2003; 14:306-315 (In Spanish.).

24. Talat MA, Anahid E, Parvin YR, Hamkar, Zahra SP, Essalat and Rakhshandeh N. Comparative detection of measles specificigm antibody in serum and Saliva by an antibody-capture igmenzyme immunoassay (EIA) Dept. Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. *Iranian Journal of Allergy, Asthma And Immunology,* 2003; Vol. 2, No. 3, pp154

25. World Health Organization. Manual for the laboratory diagnosis of measles and rubella virus infection 2nd Ed. *World Health Organization, Geneva, Switzerland.*2007.

26. World Health Organization. Nomenclature for describing the genetic characteristics of wild-type measles viruses (update). *WklyEpidemiol Rec*.1996; 76:242–7.