Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A review of testing used in seroprevalence studies on measles and rubella

Wayne Dimech [a,*], Mick N. Mulders [b]

[a] NRL, 4th Floor Healy Building, 41 Victoria Parade, Fitzroy 3065, Victoria, Australia
[b] World Health Organization, Global VPD Laboratory Networks, 20, Avenue Appia, CH-1211 Geneva 27, Switzerland

ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form 15 May 2016
Accepted 1 June 2016
Available online xxxx

Keywords:
Seroprevalence
Measles
Rubella

ABSTRACT

Seroprevalence studies are an essential tool to monitor the efficacy of vaccination programmes, to understand population immunity and to identify populations at higher risk of infection. An overarching review of all aspects of seroprevalence studies for measles and rubella published between 1998 and June 2014 was undertaken and the findings reported elsewhere. This paper details the considerable variation in the testing formats identified in the review. Apart from serum/plasma samples, testing of oral fluid, breast milk, dry blood spots and capillary whole blood were reported. Numerous different commercial assays were employed, including microtitre plate assays, automated immunoassays and classical haemagglutination inhibition and neutralisation assays. A total of 29 of the 68 (43%) measles and 14 of the 58 (24%) rubella studies reported qualitative test results. Very little information on the testing environment, including quality assurance mechanisms used, was provided. Due to the large numbers of testing systems, the diversity of sample types used and the difficulties in accurate quantification of antibody levels, the results reported in individual studies were not necessarily comparable. Further efforts to standardise seroprevalence studies may overcome this deficiency.

1. Introduction

Seroprevalence studies are conducted to evaluate the level of protective immunity of a country or discrete communities, to identify gaps in immunity and to monitor the efficacy of vaccination programmes. Seroprevalence studies underpin the World Health Organization's (WHO) Global Measles and Rubella Strategic Plan 2012–2020 [1] and the Global Vaccination Action Plan 2011–2020 [2], however these studies require extensive planning and are resource intensive. To evaluate population immunity, individuals from the selected population are tested for the presence of specific antibodies. Recently, a comprehensive 16-year literature review of published seroprevalence studies for measles and rubella was undertaken [3]. The review identified considerable variation in the testing used to assess protective immunity and highlights the need to standardise the approach to conducting seroprevalence studies.

2. Materials and method

A Medline search was conducted using the National Library of Medicine's PubMed online search engine; the starting year selected was 1998; the end date was June 2014. Keywords included 'rubella' and 'measles' combined with 'serosurvey', 'seroprevalence', 'immunity' and 'population immunity'. An article was included in the review if it reported seroprevalence results and contained a description of study design, study population, age group(s) tested, and laboratory method used to determine antibody status. One article was not retrievable on-line [4]. As no language priority was chosen, one article was in Spanish [5]. The method of testing and the sample type used to determine immunity in each of the selected studies were reviewed and compared.

3. Results

The literature search for the 16-year period identified a total of 97 articles fulfilling the criteria, of which 68 described serosurveys for measles and 58 described serosurveys for rubella. Thirty of the articles addressed both measles and rubella.

* Corresponding author.
E-mail addresses: wayne@nrl.gov.au (W. Dimech), muldersm@who.int (M.N. Mulders).

http://dx.doi.org/10.1016/j.vaccine.2016.06.006
0264-410X/

3.1. Specimen type

Where storage conditions were specified, all but one study reported storing serum specimens frozen at −20 °C or below prior to testing. A single study [6] tested fresh, not yet frozen specimens on the same day of collection. A total of 58 of the 68 (85%) measles studies used only serum samples to determine seroprevalence. Of the other 10 studies, several other specimen types were used. For instance, two measles studies reported using oral fluid exclusively [7,8]. Other sample types included breast milk [9], oral fluid [10,11] and blood derived from the umbilical cord, earlobe or finger prick [12,13]. Of the 58 rubella studies, 54 (93%) reported using serum only. One rubella survey reported using plasma and umbilical cord serum [14] and one combined measles and rubella study used plasma [15].

Five measles studies used fresh, non-serum/plasma specimens. One measles study tested human breast milk [9] using an in-house enzyme immunoassay. Four studies from Bangladesh [10], Ethiopia [8], Kenya [7] and Norway [11] used oral fluid samples tested in the Measles IgG capture EIA (Microimmune Ltd., Middlesesex, UK). Two [10,11] of these four studies also tested serum specimens in the Enzygnost EIA as a comparison. Although the Microimmune EIA has been licenced for oral fluid testing, one study reported poor correlation between the serum-based assays, with only 62% agreement [16]. The other study using oral fluid and serum [11] reported a high concordance between both specimen types (95% correlation for sero-positive and 96% for sero-negative specimens).

3.2. Testing format

Detection of measles and rubella antibodies was achieved through a range of testing systems. While neutralisation assays, particularly some form of plaque neutralisation test (PRNt) for measles or fluorescent focus reduction test for rubella remains the gold standard [17,18], few studies used these time-consuming and labour-intensive neutralisation tests as the primary laboratory method. The most popular assay type used was some form of microtitre-plate enzyme-immunoassay (MTP EIA) (Table 1). Since 1999, a range of automated, commercial immunoassays (IA) have been released to the market and were utilised in more recent studies, especially for rubella testing. Several other test types were used for measles studies including haemagglutination inhibition assays (HAI) and the bioplex tests. Interestingly, HAI was not used for any rubella studies even though it has previously been considered a gold standard, especially prior to 1980s.

The majority of the measles MTP EIAs used were obtained commercially. There were 52 measles studies that used MTP EIAs and one that used an automated IA. The two most commonly used measles MTP EIA was the Enzygnost® Anti-Measles Virus/IgG (Siemens Health Care Diagnostics GmbH, Marburg, Germany) (40%) and Serion Measles IgG (Institut Virion\Serion GmbH Würzburg, Germany) (17%). However, a range of measles assays were employed, sourced from a total of 19 different commercial sources.

There were 40 rubella studies that used MTP EIAs. The most commonly used rubella MTP EIAs was Enzygnost® Anti-Rubella Virus/IgG (Siemens Health Care Diagnostics GmbH, Marburg, Germany) (35%) and ETI-RUBEK-G Plus (DiaSorin, Saluggia, Italy) (12%). Apart from these two MTP EIAs, assays from 13 different manufacturers were used in the studies. The rubella studies used more automated IAs than the measles studies: 12 rubella studies compared with a single measles study. Of the 12 rubella studies using IAs, five used the AxSYM Rubella IgG assay (Abbott Diagnostics, Abbott Park, Il), four used the VIDAS Rub IgG (bioMerieux, Marcy l'Etoile, France) and a further three used different automated platforms.

Table 1
Laboratory tests used in published measles and rubella seroprevalence studies.

Laboratory test	Measles publications	Rubella publications
Neutralization assay	**7** *(10%)*	**1** *(2%)*
MTP EIAs	**52** *(76%)*	**40** *(69%)*
Siemens Enzygnost	**21 (31%)**	**14 (24%)**
Virion/Serion	**9 (13%)**	**2 (3.5%)**
Other commercial MTP EIAs	**3 (4%)**	**22 (38%)**
MTP EIA not identified	**19 (28%)**	**2 (3.5%)**
Automated immunoassay	**1** *(1%)*	**12** *(21%)*
Abbott AxSYM		**5 (9%)**
BioMerieux VIDAS	**1 (1%)**	**4 (7%)**
Other commercial automated immunoassays		**3 (5%)**
Bioplex assay	**1** *(1%)*	**1** *(2%)*
Haemagglutination inhibition assay	**4** *(6%)*	**0**
MTP EIA and neutralisation	**2** *(3%)*	**0**
Assay not described	**1** *(1%)*	**3** *(5%)*
MTP EIA and gel haemolysis	**0**	**1** *(2%)*
Totals	**68**	**58**

MTP EIA Microtitre plate enzyme immunoassays.

3.3. Reporting of results

The results of testing were expressed as either quantitative or qualitative values. Five studies did not specify how results were reported and two reported results as titres. Of the measles studies, 39 of 68 (57%) reported quantitative results and 22 (43%) reported qualitative results. Thirteen measles studies and six rubella studies reported having used a WHO international standard to calibrate a non-commercial test system. It should be noted that the current (3rd) WHO international standard for measles is not recommended to be used to calibrate MTP EIAs [19], and the 2nd WHO international standard for measles was only tested against the Enzygnost MTP EIA.

Reporting quantitative results was more common for rubella studies, with 44 of 58 (76%) studies reporting results in IU/mL. This trend was most likely due to the use of commercial assays, almost all having been calibrated against the WHO international standard. Of the six rubella studies reporting qualitative results, all used uncalibrated MTP EIAs.

Almost half of the measles studies (32 of 68; 47%) applied a range for equivocal results. The application of an equivocal range was independent of whether the results were reported qualitatively or quantitatively. Of these 32 measles studies, an equivocal range was reported for 14 studies reporting qualitative results, 17 studies with quantitative results and one study reporting titres. The ranges were often dependent on the testing system used. Six users of the Enzygnost measles IgG EIA used the manufacture's equivocal range of optical density (OD) of 0.1–0.2. However, nine users of the same assay did not report an equivocal range and a further six used a quantitative equivocal range, expressed in mIU/mL.

Of the 58 rubella studies, 22 (38%) including an equivocal range. The equivocal ranges used in the rubella seroprevalence studies varied considerably. Of the studies reporting qualitative results, the most common range was OD 0.1–0.2, associated with the Enzygnost MTP EIA. Quantitative equivocal ranges included 5–10 IU/mL ($N = 5$), 10–15 IU/mL ($N = 4$), 5–15 IU/mL ($N = 2$), but several other quantitative ranges were used. No study used a lower limit of the equivocal range of less than 3 IU/mL or an upper limit of the equivocal range greater than 15 IU/mL. There is some contention regarding the standardization of rubella IgG testing, which has been addressed in detail elsewhere [20].

Studies reported using different cut-offs to differentiate between immune and non-immune individuals. Seven measles

Please cite this article in press as: Dimech W, Mulders MN. A review of testing used in seroprevalence studies on measles and rubella. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.06.006