NOTES

Dried Blood Spots versus Sera for Detection of Rubella Virus-Specific Immunoglobulin M (IgM) and IgG in Samples Collected during a Rubella Outbreak in Peru

Rita F. Helfand, 1* Cesar Cabezas, 2 Emily Abernathy, 1 Carlos Castillo-Solorzano, 3 Ana Cecilia Ortiz, 2 Hong Sun, 1 Fernando Osores, 2 Lucia Oliveira, 3 Alvaro Whittinebury, 4 Myrna Charles, 1 Jon Andrus, 3 and Joe Icenogle 1

Centers for Disease Control and Prevention (CDC), Atlanta, Georgia 1; Instituto Nacional de Peru (INS), Ministerio de Salud, Lima, Peru 2; Pan American Health Organization, Washington, D.C. 3; and Dirección General de Epidemiología, Ministerio de Salud, Lima, Peru 4

Received 23 March 2007/Returned for modification 4 June 2007/Accepted 10 September 2007

Most persons with rubella virus-specific immunoglobulin M (IgM)- or IgG-positive sera tested positive (98% [n = 178] and 99% [n = 221], respectively) using paired filter paper dried blood spot (DBS) samples, provided that DBS indeterminate results were called positive. For persons with IgM- or IgG-negative sera, 97% and 98%, respectively, were negative using DBS.

Simplification of specimen collection, storage, transport, and processing in the field would be a great advantage to rubella surveillance. Recent studies have suggested that filter paper dried blood spots (DBS) are suitable for laboratory detection of measles-specific immunoglobulin M (IgM) (2, 3, 6–8). In this study, we compared the detection of rubella virus-specific IgM and IgG in DBS to their detection in serum samples collected from health care provider-diagnosed rubella patients.

The presence of rubella virus-specific IgM in serum according to enzyme immunoassay is diagnostic for rubella, and thus, results from sera were used as the standard. However, because most specimens were collected in the first week after rash onset, a time period when serum IgM and IgG enzyme immunoassays do not detect many rubella cases, we do not refer to serum samples as a “gold standard” (1, 9).

Health care workers at the local health care centers in five Regional Health Directorates in Peru enrolled persons 8 months or more in age seen within 28 days of rash and fever onset (clinically suspected rubella). Persons who were vacci-

| Antibody | Result for serum sample | No. of DBS samples from: | Persons with suspected rubella | Healthy blood donors |
|----------|-------------------------|-------------------------|-------------------------------|---------------------|
|          | Positive                | Indeterminate           | Negative                      | Total               |
| IgM      | 146                     | 28                      | 4                             | 178                 |
|          | 2                       | 17                      | 17                            | 36                  |
|          | 1                       | 3                       | 55                            | 59                  |
|          | Total                   | 149                     | 48                            | 76                  | 273                 | 0     | 1 | 102 | 103 |
| IgG      | 105                     | 22                      | 2                             | 129                 |
|          | 3                       | 14                      | 23                            | 40                  |
|          | 1                       | 0                       | 103                           | 104                 |
|          | Total                   | 109                     | 36                            | 128                 | 273                 | 91    | 1 | 11 | 103 |

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, 1600 Clifton Rd., NE, Mailstop C-12, Atlanta, GA 30333. Phone: (404) 639-3788. Fax: (404) 639-3039. E-mail: rzh7@cdc.gov.

* Published ahead of print on 19 September 2007.
nated within 8 weeks of presentation, were pregnant, or had chronic illnesses were excluded. Healthy adult blood donors from a single blood donation center in Lima, Peru, were enrolled as controls.

Ethics approval was obtained at the Ministry of Health in Peru and at the Centers for Disease Control and Prevention (CDC), and informed consent was obtained. The health care workers collected demographic information, serum samples via venipuncture using universal precautions, and two to three DBS via fingerstick (onto Schleicher & Schuell, Inc., grade 903 filter paper; Whatman Inc., Florham Park, NJ). Study personnel collected sera by venipuncture and DBS via fingerstick from the controls.

DBS were dried at room temperature for a minimum of 4 h, placed in a zip-closure bag with a desiccant pack and humidity indicator card, and transferred on wet ice to the district office and then to the Ministry of Health’s National Reference Laboratory (NRL) for measles/rubella, where they were frozen at

FIG. 1. (a) Comparison of the rubella virus-specific IgM ODs for serum/DBS sample pairs. The space between the horizontal and vertical dashed lines represents the indeterminate range. The diagonal dashed line represents a perfect correlation of 1.00, and the solid line is the trend line. The Pearson correlation coefficient is 0.91. (b) Comparison of the rubella virus-specific IgG ODs for serum/DBS sample pairs. The space between the horizontal and vertical dashed lines represents the indeterminate range. The diagonal dashed line represents a perfect correlation of 1.00, and the solid line is the trend line. The Pearson correlation coefficient is 0.94.
−20°C. Blood samples were sent to the NRL on wet ice and centrifuged, and aliquots of the resulting sera were either refrigerated (4°C) or frozen (−20°C) for 1 to 4 months. Sera and DBS were shipped on dry ice to the CDC, where they were stored at −70°C. The elution of antibodies from DBS was performed by using protocol number 6 described by Mercader and colleagues (5). Sera and DBS eluates were tested for rubella virus-specific IgM and IgG using Dade Behring Enzynost anti-rubella virus IgM and IgG enzyme immunoassays (Marburg, Germany) per the manufacturer’s instructions, including definitions of positive, indeterminate, and negative results. There were insufficient DBS samples from some patients with indeterminate results to allow retesting as recommended by the manufacturer. Thus, DBS/serum comparisons used the initial results.

Most calculations were performed using SAS9.10 and EpiInfo 2000 software. Correlation coefficients were calculated using Excel software.

IgM. The serum and DBS results for IgM are compared in Table 1 and Fig. 1a. The serum/DBS sample pairs that were analyzed were from 273 persons with suspected rubella enrolled between June 2004 and February 2005 from >70 health centers and from 103 healthy adult blood donors enrolled in April and May 2005. The IgM positivity rates for serum samples from persons with suspected rubella were 34% (13/38), 64% (51/80), 74% (55/74), 72% (46/64), and 86% (12/14), while the indeterminate rates were 26%, 19%, 8%, 5%, and 7%, from days 1, 2, 3, 4 to 7, and 8 to 28, respectively, after rash onset (data not shown) (for the indeterminate rates in the first week after rash onset, the chi square test result for the trend was <0.001). For serum and DBS sample pairs from persons with suspected rubella, the concordance for IgM increased from 76% (146/192) in the first 3 days after rash onset to 88% (69/78) from days 4 to 28 after rash onset (P, 0.02; Mantel-Haenszel chi square test), when more persons would be expected to have IgM levels which had risen out of the indeterminate range.

There are many ways to adjust for the slightly lower optical density (OD) values from DBS relative to those of sera; the simplest that has been used is to include DBS indeterminate results with positive results (4). Doing this resulted in an increase in the ability to detect the presence of rubella virus-specific IgM in DBS compared with the ability to detect it in serum (relative sensitivity) from 82% (146/178) to 98% (174/178; P of <0.001 by Mantel-Haenszel chi square test) and an unchanged ability to detect the absence of rubella virus-specific IgM in DBS compared with the ability to detect the absence of it in serum (relative specificity) (155/159; 97%).

IgG. The serum and DBS results for IgG are compared in Table 1 and Fig. 1b. The IgG positivity rates of serum samples from persons with suspected rubella were 26% (10/38), 28% (22/80), 49% (36/74), 73% (47/64), and 86% (12/14), while the indeterminate rates were 18%, 26%, 11%, 5%, and 0%, on days 1, 2, 3, 4 to 7, and 8 to 28, respectively, after rash onset (data not shown) (for the indeterminate rates in the first week after rash onset, the chi square test result for the trend was <0.01). The concordance for IgG increased from 78% (149/192) in the first 3 days after rash onset to 90% (70/78) from days 4 to 28 after rash onset (P, 0.02; Mantel-Haenszel chi square test). For the 221 persons with IgG-positive serum results (persons with suspected rubella and healthy blood donors), 196 (89%) of their corresponding DBS samples were IgG positive. Similar to the IgM results, the ability to detect the presence of rubella virus-specific IgG in DBS increased from 89% (196/221) to 99% (218/221; P of <0.001 by Mantel-Haenszel chi square test) when indeterminate and positive results from DBS were combined, while the ability to detect the absence of rubella virus-specific IgG in DBS compared with the ability to detect the absence of it in serum remained unchanged at 98% (112/114).

Using the method of Karapanagiotidis and colleagues (4), we were able to obtain good correlation between sera and DBS results using the OD cutoff values established for serum samples with the simple adjustment of including DBS indeterminate results with positive results. This simple adjustment is only one means of dealing with the slightly lower ODs from DBS specimens; others include only using DBS after the first 3 to 5 days of rash onset when positivity rates are higher; adjustment of ODs from DBS; and performing no adjustment at all, since detecting 82% of persons with IgM-positive sera (146/178; Table 1) would be adequate in many circumstances.

The World Health Organization still considers serum samples and virus detection to be the “gold standard” for laboratory confirmation of cases (10). Nevertheless, the present study suggests that the use of DBS, collected in field conditions such as were found here, by properly trained personnel (3; D. Featherstone, personal communication), is an acceptable alternative to detect rubella virus-specific IgM and IgG in settings where it is not feasible to collect and test serum samples.

We thank all of the staff in the DISAS and the clinics in Lima Sur, Lima Ciudad, Lima Este, Taena, and Huánuco for their assistance in this study. In addition, we appreciate the technical input from Susan Relf (CDC) and Rosa Acosta’s role (INS) in performing timely rubella testing as part of routine surveillance. Finally, we acknowledge Edwin Cabezudo from INS and Washington Toledo from the Pan American Health Organization in Peru for their technical and logistical assistance.

The findings and conclusions in this article are those of the individual authors and do not necessarily reflect the views of the Centers for Disease Control and Prevention.

REFERENCES
1. Bellini, W. J., and J. P. Icenogle. 2007. Measles and rubella viruses, p. 1378–1391. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
2. De Swart, R. L., Y. Nur, A. Abdallah, H. Kruining, H. S. El Mubarak, S. A. Ibrahim, B. Van Den Hoogen, J. Groen, and A. D. Osterhaus. 2001. Combination of reverse transcriptase PCR analysis and immunoglobulin M detection on filter paper blood samples allows diagnostic and epidemiological studies of measles. J. Clin. Microbiol. 39:270–273.
3. El Mubarak, H. S., S. Yuksel, O. M. Mustafa, S. A. Ibrahim, A. D. Osterhaus, and R. L. de Swart. 2004. Surveillance of measles in the Sudan using filter paper blood samples. J. Med. Virol. 75:624–630.
4. Karapanagiotidis, T., M. Riddell, and H. Kelly. 2005. Detection of rubella immunoglobulin M from dried venous blood spots using a commercial enzyme immunoassay. Diagn. Microbiol. Infect. Dis. 53:107–111.
5. Mercader, S., D. Featherstone, and W. J. Bellini. 2006. Comparison of available methods to elute serum from dried blood spot samples for measles serology. J. Virol. Methods 137:140–149.
6. Riddell, M. A., G. B. Byrnes, J. A. Leydon, and H. A. Kelly. 2003. Dried venous blood samples for the detection and quantification of measles IgG using a commercial enzyme immunoassay. Bull. W. H. O. 81:701–707.
7. Riddell, M. A., D. Chibo, H. A. Kelly, M. G. Catton, and C. J. Birch. 2001. Investigation of optimal specimen type and sampling time for detection of
measles virus RNA during a measles epidemic. J. Clin. Microbiol. 39:375–376.

8. Riddell, M. A., J. A. Leydon, M. G. Catton, and H. A. Kelly. 2002. Detection of measles virus-specific immunoglobulin M in dried venous blood samples by using a commercial enzyme immunoassay. J. Clin. Microbiol. 40:5–9.

9. Tipples, G. A., R. Hamkar, T. Mohktari-Azad, M. Gray, J. Ball, C. Head, and S. Ratnam. 2004. Evaluation of rubella IgM enzyme immunoassays. J. Clin. Virol. 30:233–238.

10. World Health Organization. 2007. Manual for the laboratory diagnosis of measles and rubella virus infection, 2nd ed. World Health Organization, Geneva, Switzerland. http://www.who.int/immunization_monitoring/LabManualFinal.pdf.