

Dried venous blood samples for the detection and quantification of measles IgG using a commercial enzyme immunoassay

Michaela A. Riddell,¹ Graham B. Byrnes,² Jennie A. Leydon,³ & Heath A. Kelly⁴

Objectives To determine whether samples of dried venous blood (DVB) were an acceptable alternative to serum for detecting measles-specific IgG in a commercial enzyme immunoassay.

Methods Paired samples of serum and DVB were collected from 98 suspected cases of measles and 1153 schoolchildren in Victoria, Australia. All samples were tested using the Dade Behring Enzygnost® Anti-Measles-Virus/IgG immunoassay. DVB samples were eluted using either the sample buffer provided with the kit or 5% dry milk powder in phosphate-buffered saline–Tween 20.

Findings DVB samples eluted by sample buffer showed significantly better linear correlation to the serum samples than did DVB samples eluted in 5% dry milk in phosphate-buffered saline–Tween 20. To improve the comparability of serum and DVB samples an adjustment factor of 1.28 was applied to the optical density (OD) values of DVB. This adjustment also enabled quantification of the titre of measles IgG in mIU/ml directly from the OD value using the alpha calculation as specified by the kit protocol. For DVB samples stored for less than six months at 4 °C, the assay showed an overall sensitivity of 98.4% and a specificity of 97.2% compared with the results of serum testing.

Conclusion These results illustrate the potential for DVB samples to be widely used with the Dade Behring enzyme immunoassay system for determining the immunity of the individual and the population to the measles virus.

Keywords Measles/immunology/diagnosis; Blood specimen collection/methods; Immunoenzyme techniques; Immunoglobulin G; Sensitivity and specificity; Seroepidemiologic studies (*source: MeSH, NLM*).

Mots clés Rougeole/immunologie/diagnostic; Prélèvement sang/méthodes; Méthode immunoenzymatique; Immunoglobuline G; Sensibilité et spécificité (Epidémiologie); Etude séroépidémiologique (*source: MeSH, INSERM*).

Palabras clave Sarampión/inmunología/diagnóstico; Recolección de muestras de sangre/métodos; Técnicas para inmunoenzima; Inmunoglobulina G; Sensibilidad y especificidad; Estudios seroepidemiológicos (*fuentes: DeCS, BIREME*).

الكلمات المفتاحية: الحصبة، مناعيات الحصبة، تشخيص الحصبة؛ جمع عينات الدم، طرق جمع عينات الدم؛ أساليب وطرق المقايسة المناعية؛ الغلوبولين المناعي جي؛ الحساسية والنوعية؛ الدراسات الوبائية المصلية. (المصدر: رؤوس الموضوعات الطبية – منظمة الصحة العالمية – إقليم شرق المتوسط)

Bulletin of the World Health Organization 2003;81:701-707

Voir page 705 le résumé en français. En la página 706 figura un resumen en español.

يمكن الاطلاع على الملخص بالعربية في صفحة ٧٠٦

Introduction

WHO has identified an ongoing need to develop sensitive and specific tests for both diagnosis and seroepidemiological surveys of measles using specimens other than serum (1). The use of alternative specimen types such as dried blood would enable the problems associated with transportation and storage to be overcome. In addition, the adaptation of a commercial assay to alternative samples would enable testing to be completed in any adequately equipped laboratory.

Dried blood samples have previously been used for detecting measles haemagglutination inhibition (HI) antibodies (2–4). Other recent studies have described the use of dried

blood samples with enzyme immunoassay (EIA) methods in the investigation of rash illnesses and assessment of children following vaccination for measles, mumps, and rubella (MMR) (5–7). However, these studies used “in-house” EIAs, restricting the investigations to research and reference laboratories.

Helfand et al. used an indirect measles-specific IgG “in-house” immunoassay to show excellent concordance between serum and dried blood spot samples when testing for measles IgM, but somewhat reduced concordance between the two specimen types when testing for measles-specific IgG (7). To date, only one group has reported a study showing good correlation between dried filter-paper samples and serum using a commercial enzyme immunoassay for detecting measles-specific IgG (8).

¹ Scientist/PhD Scholar, Victorian Infectious Diseases Reference Laboratory (VIDRL)/WHO Western Pacific Measles Regional Reference Laboratory, 10 Wreckyn Street, North Melbourne, 3051 Victoria, Australia; and Department of Public Health, School of Population Health, University of Melbourne, Australia. (email: michaela.riddell@mh.org.au). Correspondence should be sent to this author at the former address.

² Statistician, Department of Mathematics and Statistics, University of Melbourne, Melbourne Australia.

³ Senior Scientist, VIDRL/WHO Western Pacific Measles Regional Reference Laboratory, Victoria, Australia.

⁴ Head, Epidemiology Division, VIDRL/WHO Western Pacific Measles Regional Reference Laboratory, Victoria, Australia.

Ref. No. 02-0426

The Victorian Infectious Diseases Reference Laboratory (VIDRL), a WHO regional measles reference laboratory, currently uses the Dade Behring Enzygnost® Anti-Measles-Virus commercial EIA for investigating measles-specific antibodies. We have previously reported the diagnosis of measles infection using dried venous blood (DVB) samples with the Dade Behring anti-measles/IgM EIA (9) and now report the detection of measles-specific IgG antibodies using the Dade Behring anti-measles/IgG EIA.

Materials and methods

Patients and samples

VIDRL provides laboratory support for the enhanced measles surveillance programme in the state of Victoria, Australia (10). As part of this programme, 98 paired samples of serum and DVB (subsequently referred to as diagnostic samples) were collected from 97 patients suspected of having measles during April to October 2001. In addition, paired samples of serum and DVB were collected from 1153 Victorian schoolchildren during August to November 1999 to evaluate the 1998 national measles "catch-up" immunization campaign in the state of Victoria (subsequently referred to as seroprevalence samples) (11). Written informed consent was obtained from all participants (or legal guardian if the participant was less than 18 years of age) who provided samples.

The methods for collecting and preparing diagnostic samples have been described elsewhere (9). In brief, approximately 100 µl of whole blood was applied to each of three 13 mm diameter circles on Schleicher & Schuell #903 filter-paper at the time the blood sample was drawn from the patient. The serum and DVB samples were transported to VIDRL, where the DVB sample was dried before storing at 4 °C in a sealed plastic bag until testing. The whole blood was separated by centrifugation and the serum tested for measles-specific IgG and IgM (Dade Behring Enzygnost, Marburg, Germany).

The seroprevalence samples from the 1153 schoolchildren were prepared in a similar manner, at the time the whole blood was drawn. The DVB samples were dried then stored at 4 °C and the serum was tested for measles-specific IgG (Dade Behring Enzygnost, Marburg, Germany). The diagnostic DVB samples were tested within six months of collection, whereas the seroprevalence DVB samples were stored for 15–23 months before testing.

Control samples and validation

Spiked DVB samples for elution and assay optimization were prepared as previously described (9). Ethylenediaminetetraacetic acid-anticoagulated blood was collected from two healthy volunteers with known negative and positive antibody reactivity to measles. DVB samples were prepared and included on each test plate as DVB sample measles IgG-negative and IgG-positive controls. In addition, the kit positive control was tested on each plate to facilitate correction of the optical density (OD) values and to ensure that each plate met the kit batch validity requirements as specified by the manufacturer.

Optimization of elution and EIA conditions

Different elution and testing dilutions were assessed, with the assumption that a 6 mm disk of DVB on filter-paper was equivalent to approximately 5 µl of serum (12). Sample buffer

(Dade Behring Enzygnost sample buffer supplied with the kit) and dry milk powder (blotting grade, non-fat dry milk; Biorad, Hercules, CA, USA) diluted to 1%, 2%, or 5% in phosphate-buffered saline–Tween 20 (PBST) (0.5%) were compared as elution buffers. Disks of filter-paper were cut with a metal paper hole-punch and placed into wells on a microtitre plate. After addition of the eluant the microtitre plate was agitated for 30 minutes at room temperature to ensure thorough soaking of the disks. The plate was covered with tape, placed in a sealed moistened box, and incubated at 4 °C overnight. After overnight elution with either sample buffer or dry milk in PBST (0.5%), the microtitre plate was agitated at room temperature for a further 30 minutes before centrifugation of the plate (15 minutes, 2200 g).

The diagnostic DVB samples ($n = 98$) were eluted concurrently in both sample buffer and 5% dry milk in PBST (0.5%). Seroprevalence DVB samples were eluted in either sample buffer ($n = 499$) or 5% dry milk in PBST (0.5%) ($n = 654$).

Serum samples were processed and validated with an automated ELISA processor according to the manufacturer's instructions (for diagnostic samples, ETI-LAB, DiaSorin, Saluggia, Italy; and for seroprevalence samples, ROSYS, Dade Behring Diagnostics, Lane Cove, NSW, Australia). All equivocal serum and DVB samples were retested and the repeat result was recorded. DVB samples were manually tested independently of the serum samples. OD values of the DVB samples were compared with the corresponding serum OD values. Conditions investigated for the adaptation of the assay to test DVB samples included sample and conjugate incubation time (1 or 1.5 hours), final sample dilution (1:100, 1:200, 1:220, 1:440, 1:880), plate incubation (still or shaking), and wash conditions (3–6 washes with PBST (0.05%)). OD values were determined at $\lambda = 450$ nm ($\lambda = 620$ nm reference wavelength, Labsystems Multiskan Ascent plate reader).

Data analysis

To improve reproducibility, OD values for all samples of serum and DVB were corrected using the average OD of the kit positive control and the batch-specific nominal value for each plate and kit batch number as described by the manufacturer. Stata statistical software (version 6.0, 1999; Stata Corporation, College Station, TX, USA) was used for analysing these corrected data.

Initial analysis showed a systematic proportional difference between DVB and serum OD (data not shown). To improve quantitative agreement of the methods, the DVB OD was multiplied by an adjustment factor derived such that the correlation between the difference and mean of the OD pairs would be zero. Bland–Altman regression analysis (difference on mean) was performed with both adjusted and unadjusted DVB OD values to assess the suitability of DVB to replace serum as the sample type used in this commercial EIA (13).

Samples (serum or adjusted DVB) registering an OD ≥ 0.2 were considered to indicate protection against measles, whereas samples that repeatedly tested OD ≤ 0.2 were considered to indicate susceptibility to infection with measles virus.

Measles antibody titres were calculated using the alpha method as specified by the manufacturer, and converted to geometric mean titres (GMTs). Specimens for which the OD values were < 0.2 were arbitrarily assigned a titre of half the detection limit of the assay (i.e. 75 mIU/ml) to provide an unbiased calculation of GMTs (14).

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated by comparing the DVB sample OD values to the serum OD values. The cut-off and validity requirements for each plate were as recommended in the kit protocol. In addition to a known positive DVB sample the kit IgG positive control was used to calculate coefficients of variation (% CV) to assess interassay variation.

Agreement of both categorical (susceptible to measles) and quantitative (OD) results was assessed using kappa and R^2 statistics. Exact 95% confidence intervals for categorical agreement were calculated using the binomial distribution.

Results

Elution and EIA conditions

The sample buffer, which was provided with the kit, and the 5% dry milk in PBST (0.5%) were initially found to be equally effective as eluants; however, each required different test conditions for optimal use. Optimal elution occurred when one 6 mm disk was incubated overnight at 4 °C with 250 μ l of either sample buffer or 5% dry milk in PBST (0.5%). This represented a 1:50 dilution of the DVB sample.

Optimal assay conditions were achieved after adding 50 μ l of eluate (either sample buffer or 5% dry milk in PBST (0.05%)) to 170 μ l of sample buffer in each test and control well. This equated to a final specimen dilution of 1:220.

The sample incubation time was 1 hour at 37 °C and 1.5 hours at 37 °C for DVB samples eluted in sample buffer and for

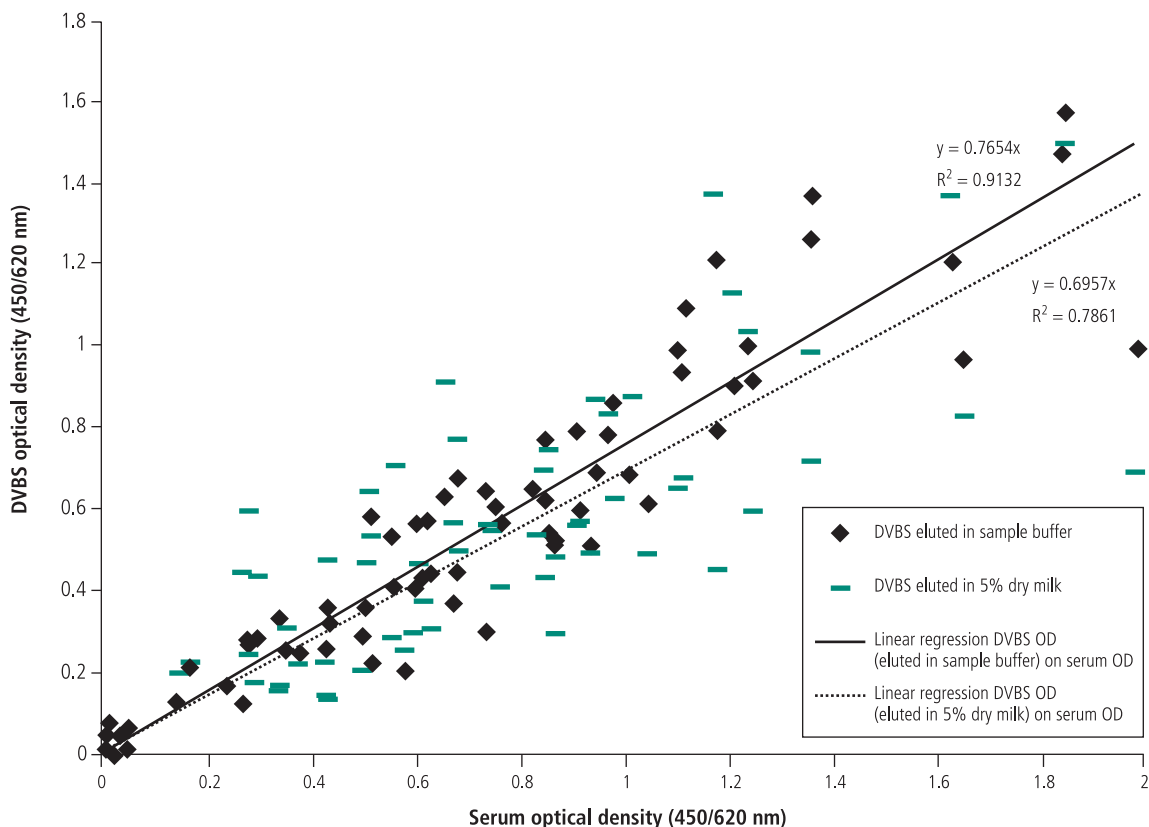
DVB samples eluted in 5% dry milk in PBST (0.5%), respectively. Apart from the initial sample incubation differing in both methods and the plate wash step comprising five instead of four washes (Wellwash Ascent plate washer; Labsystems) in PBST (0.05%), the assay was completed in accordance with the manufacturer's protocol, using reagents provided with the kit. The % CV for the positive control and for the known positive DVB samples were 14% and 18.9%, respectively, for DVB samples eluted with sample buffer and 14.5% and 11.3%, respectively, for DVB samples eluted with 5% dry milk in PBST (0.5%), indicating acceptable interassay variability for both elution and the testing methods.

Fig. 1 shows the linear relation and regression coefficients between the corrected OD values of paired serum and the DVB diagnostic samples. The correlation between the serum and DVB sample OD values was significantly better for DVB samples eluted in sample buffer than with 5% dry milk in PBST (0.5%) (difference between slopes = 0.07 (95% confidence interval (CI) 0.01 to 0.13, $P = 0.02$). The remaining results presented here are restricted to DVB eluted in sample buffer because it gives a better correlation than with the dry milk in PBST and because this assay method using sample buffer from the kit is much simpler.

Adjustment factor

The adjustment factor was generated from the square root of the ratio of the regression coefficient of serum OD on DVB sample OD and the regression coefficient of DVB sample OD on

Fig. 1. Corrected optical density (OD) values ($\lambda=450$ nm) of serum and corresponding dried venous blood diagnostic samples eluted using either sample buffer or 5% dry milk in phosphate-buffered saline–Tween 20 (0.5%) and tested in the Dade Behring Enzygnost® Anti-Measles-Virus/IgG enzyme immunoassay. Serum samples were generally tested within three days and dried venous blood (DVB) samples within six months of collection after storage at 4 °C. Regression lines were constrained to pass through the origin



WHO 03.174

Table 1. Comparison of positive (OD ≥ 0.2)^a and negative (OD < 0.2) assay results for diagnostic samples eluted in sample buffer, after serum correction and DVB^b sample OD correction and adjustment^c

DVB sample ($\times 1.28$)	No. serum samples		Total
	+	-	
+	62	1 ^d	63
-	1 ^e	34	35
Total	63	35	98
Assay parameters			
% Sensitivity	98.4 (91.4–99.9) ^f		
% Specificity	97.1 (85.1–99.9)		
% Positive predictive value	98.4 (91.5–99.9)%		
Negative predictive value	97.1 (85.1–99.9)		
Kappa value	0.96; 98% ^g		

^a OD = optical density.

^b DVB = dried venous blood.

^c See text for explanation of correction and adjustment. Serum samples and DVBS were generally tested within three days and six months of collection, respectively, after storage at 4 °C. Sensitivities, specificities, positive predictive values, and negative predictive values of the Dade Behring Enzygnost® Anti-Measles-Virus/IgG immunoassay for DVB samples relative to serum samples, and 95% exact binomial confidence intervals, are shown.

^d DVB sample OD = 0.28, paired serum OD for measles IgG = 0.16 (sample was positive for measles IgM and measles virus RNA by polymerase chain reaction).

^e DVB sample OD = 0.17 (equivocal), paired serum OD = 0.27.

^f Figures in parentheses indicate 95% confidence intervals.

^g Figure in italics indicates % agreement.

serum OD, with the regression lines constrained to pass through the origin. Adjustment of the DVB sample OD using the Bland–Altman method comparison analysis (13) resulted in OD values that were not significantly different from the serum OD values (data not shown). The adjustment factor of 1.28 was applied to all corrected DVB sample OD values.

Comparison of serum and DVB samples

Table 1 shows the assay parameters (sensitivities, specificities, PPV, NPV) and level of agreement (kappa statistic) of the diagnostic samples comparing adjusted DVB sample OD values to serum OD values. Adjustment of the DVB sample OD values for samples stored up to six months did not alter the sensitivity or specificity relative to serum OD values (data not shown).

However, Table 2 shows that the sensitivity of the assay, for samples stored at 4 °C for 15–17 months, was significantly improved after adjusting the DVB sample OD (difference between unadjusted and adjusted DVB sample OD assay sensitivity 5.5% (95% CI, 2.4–8.6%; $P = 0.007$). These results suggest that antibody reactivity may be degraded if samples are stored for periods of longer than six months at 4 °C.

In the assessment of population immunity in Victorian schoolchildren, adjusted OD values of DVB sample eluted by sample buffer produced an estimate of population immunity not significantly different to that obtained from the paired serum samples (94.9% vs 91.8%, difference 3.1% (95% CI, 0–6.2%; $P = 0.05$)) (11).

There was no significant difference of GMTs from serum or adjusted DVB samples for primary- and secondary-school children (Table 3).

Discussion

We have previously shown the feasibility of using DVB in a commercial EIA for diagnosing measles (9). We now report the adaptation of a commercial assay to use DVB samples for detecting measles-specific IgG. We have derived an adjustment factor and established that, after adjustment, OD values for the DVB samples were not significantly different to the OD values obtained from the paired serum sample. Furthermore, we have shown that population estimates of measles immunity and measles antibody GMTs derived from adjusted DVB samples and from serum were not significantly different, indicating that this method of sampling and testing could be successfully used in large-scale population seroprevalence studies.

We found that DVB samples could be stored for up to six months at 4 °C without apparent loss of detectable antibody activity, but not for longer than 15–17 months. This phenomenon has been observed in other studies (9, 15).

Several groups (5, 8) have shown a good correlation between serum and dried peripheral blood samples in the investigation of measles antibody activity. However, the correlation between dried samples prepared from peripheral and venous blood for the quantification of measles IgG antibody activity remains uncertain.

The Bland–Altman analysis enabled us to quantify the agreement between serum and DVB sample types and showed that the latter samples could replace serum in this immunoassay. Independent validation of the method and adjustment factor reported here will be necessary before adopting this method for routine surveillance of immunity to measles. Validation of the

Table 2. Comparison of sensitivity, specificity, positive predictive value, negative predictive value, and kappa values relative to serum samples for the seroprevalence of DVB^a samples eluted by sample buffer after 15–17 months storage at 4 °C before and after adjustment

Assay parameter	DVB sample OD without adjustment ($n = 499$)	DVB sample OD adjusted ($\times 1.28$) ($n = 499$)
% Sensitivity	90.7 (87.7–93.2) ^b	96.2 (94.1–97.7)
% Specificity	100 (86.3–100)	92 (73.9–99.0)
% Positive predictive value	100 (99.1–100)	99.6 (98.4–99.9)
% Negative predictive value	36.2 (24.9–48.7)	56.1 (39.8–71.5)
Kappa value	0.49; 91% ^c	0.68; 96%

^a DVB = dried venous blood.

^b Figures in parentheses are 95% confidence intervals.

^c Figures in italics are % agreement

Table 3. Geometric mean titres (GMTs) of Victorian primary- and secondary-school students for paired seroprevalence serum and DVB^a samples eluted using sample buffer^b

Sample type	GMTs of measles-specific IgG	
	Primary-school students ^c (n = 182)	Secondary school students ^d (n = 317)
% Serum OD ^e	1445 mIU/ml (1257–1662) ^f	1175 mIU/ml (1041–1326)
Unadjusted DVB sample OD	1056 mIU/ml (900–1239)	789 mIU/ml (684–911)
Adjusted DVB sample OD	1633 mIU/ml (1394–1912)	1235 mIU/ml (1071–1424)
Ratio in GMTs		
Serum, unadjusted DVB sample	1.4 (1.1–1.7); P = 0.004 ^g	1.5 (1.2–1.8); P < 0.001 ^g
Serum, adjusted DVB sample	0.89 (0.72–1.09); P = 0.254 ^g	0.95 (0.79–1.15); P = 0.300 ^g

^a DVB = dried venous blood.

^b See Materials and methods for details of seroprevalence samples. GMT calculations from DVB sample OD values before and after adjustment (i.e. DVB sample OD × 1.28) were compared with the GMTs calculated from the serum OD values. Serum samples were generally tested within three days and DVB samples within 15–17 months of collection after storage at 4 °C.

^c Aged 5–13 years.

^d Aged 14–17 years.

^e OD = optical density.

^f Figures in parentheses are 95% confidence intervals.

^g P-value for ratio.

specimen collection method, transport, and storage conditions will also be necessary. Preliminary results from field-testing suggest that storage of dried blood spots for more than one week at high ambient temperature and humidity may increase background reactivity of the assay.

Our current investigation, our previous work (9), and the findings reported by Helfand et al. describing the detection of rubella-specific antibodies with commercial assays (7) show the suitability of dried blood samples to facilitate rapid “in country” investigation of measles outbreaks by any laboratory capable of performing an EIA.

Others have noted the advantages of dried filter-paper samples over blood samples — for example, ease of collection, transport, and storage (6–8, 15). This study shows the additional advantage of testing dried blood samples using a commercial kit without requiring further reagents or altered incubation times. Serum and DVB samples could be tested in the same assay strip, albeit with different sample preparation. Furthermore, the same adjustment factor can be applied to the OD of all samples stored up to 17 months and the cut-off and validation values defined by the kit protocol are unchanged. Subject to satisfactory field

testing, any laboratory capable of performing an EIA can therefore carry out measles surveillance or seroprevalence studies using DVB samples. ■

Acknowledgements

We thank Debbie Gercovich for collection of all samples, the Victorian Department of Human Services for collection and clinical details of diagnostic samples, and Chrisni Karunakaran and serology staff at VIDRL for testing of all serum samples. Thanks to Professor Stephen Wesselingh for critical reading of the manuscript.

Funding: MAR received a National Health and Medical Research Council Public Health Research Scholarship. This work was presented in part at the International Congress of Virology, Paris 27 July to 1 August 2002 (abstract # V108). Dade Behring Diagnostics Pty Ltd, (NSW, Australia) provided sponsorship to MAR to attend this conference and provided the IgG kits to VIDRL at a reduced price.

Conflicts of interest: none declared.

Résumé

Recherche qualitative et quantitative des IgG anti-rougeoleuses par la méthode du confetti au moyen d'une méthode immuno-enzymatique du commerce

Objectif Déterminer si la méthode du confetti (prélèvement de sang sur papier filtre, séché et conservé) peut remplacer le prélèvement de sérum quand un test immuno-enzymatique du commerce est utilisé pour la recherche des IgG anti-rougeoleuses spécifiques.

Méthodes Des prélèvements appariés de sérum et des confettis ont été obtenus chez 98 cas présumés de rougeole et 1153 écoliers de la ville de Victoria (Australie). Tous les prélèvements ont été testés avec un produit du commerce, le Dade Behring Enzygnost Anti-Measles-Virus/IgG. Les prélèvements sur papier ont été élués, soit dans le tampon pour échantillon fourni par le fabricant du test, soit dans un

mélange 5 % de lait en poudre – salin tamponné aux phosphates – Tween 20.

Résultats Les prélèvements sur papier élués dans le tampon pour échantillon ont montré une corrélation linéaire meilleure avec les prélèvements de sérum que les prélèvements sur papier élués dans le mélange 5 % lait en poudre – soluté salin tamponné aux phosphates – Tween 20. Pour améliorer la comparabilité des prélèvements de sérum et des prélèvements sur papier, un facteur d'ajustement de 1,28 a été appliqué à la densité optique (DO) obtenue avec les prélèvements sur papier. Cet ajustement a permis de quantifier directement le titre en IgG anti-rougeoleuses en mIU/ml à partir de la DO en calculant alpha selon la méthode

indiquée par le fabricant. Les prélèvements sur papier conservés moins de six mois à 4°C ont montré une sensibilité de 98,4 % et une spécificité de 97,2 % par rapport aux tests utilisant le sérum.

Conclusion Il ressort de ces résultats que les prélèvements sur

papier pourraient être largement utilisés avec la méthode immuno-enzymatique Dade Behring pour déterminer le degré d'immunité, individuel ou à l'échelle de la population, vis-à-vis du virus rougeoleux.

Resumen

Muestras de sangre venosa desecada para detectar y cuantificar las IgG antisarampión mediante un inmunoanálisis enzimático comercial

Objetivo Determinar si la sangre venosa desecada (SVD) es una alternativa aceptable al suero para detectar IgG específica contra el sarampión en un inmunoanálisis enzimático comercial.

Métodos Se reunieron muestras emparejadas de suero y SVD de 98 casos sospechosos de sarampión y 1153 escolares en Victoria, Australia. Todas las muestras se analizaron mediante el kit de inmunoanálisis Enzygnost® Anti-Measles-Virus/IgG de Dade Behring. Las muestras de SVD se eluyeron usando ya fuera la solución tampón de muestra proporcionada con el kit o bien leche en polvo al 5% en una solución de tampón fosfato salino - Tween 20.

Resultados La correlación lineal con respecto a las muestras séricas de las muestras de SVD eluidas con el tampón de muestra fue significativamente mejor que la de las muestras de SVD eluidas con la leche en polvo al 5% en tampón fosfato salino -

Tween 20. Para mejorar la comparabilidad de las muestras de suero y de SVD se aplicó un factor de ajuste de 1,28 a los valores de densidad óptica (DO) de la SVD. Este ajuste permitió además cuantificar directamente en mUI/ml el título de las IgG antisarampión a partir del valor de la DO calculando alfa conforme a lo especificado en el protocolo del kit. Aplicado a muestras de SVD almacenadas durante menos de seis meses a 4°C, el ensayo mostró una sensibilidad general del 98,4% y una especificidad del 97,2% en comparación con los resultados del análisis del suero.

Conclusión Estos resultados muestran que, empleando el inmunoanálisis enzimático de Behring Dade, se podría recurrir ampliamente a muestras de SVD para determinar la inmunidad de individuos y poblaciones en lo referente al virus del sarampión.

ملخص

عينات الدم الوريدي الجفّف لكشف مستويات الغلوبولينات المناعية جي للحصبة وتحديد كميتها باستخدام المقايسة المناعية للإنزيم متوافرة تجارياً

٥% في محلول ملحي دارى فوسفاتي (Tween-20) ولتحسين إمكانية الموازنة بين عينات الدم الوريدي الجفّف والمصل تمت إضافة عامل تصحيح مقداره ١,٢٨، لقيم الكثافة البصرية للدم الوريدي الجفّف. وقد أمكن بهذا التصحيح تحديد المقادير الكمية للغلوبولينات المناعية جي للحصبة مقاسة بميلي وحدة دولية لكل ميلي لتر بشكل مباشر باستخدام الحسابات الألفائية، وذلك وفقاً للبروتوكول الذي تنص عليه التعليمات في العبوة المتوافرة تجارياً. وقد أظهرت المقايسة لعينات الدم الوريدي الجفّف المخزون لمدة تقل عن ستة شهور بدرجة حرارة ٤ سيلزيوس حساسية عامة مقدارها ٩٨,٤% ونوعية عامة مقدارها ٩٧,٢% وذلك بالمقارنة بنتائج الاختبارات الجراة على المصل.

الاستنتاج: توضح هذه النتائج إمكانية استخدام عينات الدم الوريدي الجفّف على نطاق واسع في مجموعة المقايسة المناعية للإنزيم (Dade Behring) لتحديد مناعة الأفراد والمجموعات ضد فيروس الحصبة.

الهدف: معرفة فيما إذا كانت عينات الدم الوريدي الجفّف تعتبر بديلاً مقبولاً للمصل في كشف مستويات الغلوبولينات المناعية جي للحصبة وتحديد كميتها باستخدام المقايسة المناعية للإنزيم متوافرة تجارياً.

الطريقة: جمعت عينات مزدوجة من كل من الدم الوريدي الجفّف والمصل من ٩٨ حالة حصبة مشتبه بها و١١٥٣ طفلاً من أطفال المدارس في فيكتوريا، أستراليا، وأجريت اختبارات المقايسة المناعية للإنزيم لتحديد كمية الغلوبولينات المناعية جي باستخدام مضاد فيروس الحصبة (Dade Behring Enzygnost) المتوافرة تجارياً. وقد شطفت عينات الدم الوريدي الجفّف إما باستخدام المحلول الداري المتوافر في عبوة الاختبار أو باستخدام محلول الحليب الجفّف بتركيز ٥% في محلول ملحي دارى فوسفاتي (Tween-20).

الموجودات: لقد أظهرت عينات الدم الوريدي الجفّف التي شطفت بالمحلول الداري المتوافر في عبوة الاختبار ارتباطاً خطياً مع عينات المصل أفضل مما أظهرته عينات الدم الوريدي الجفّف التي شطفت في الحليب الجفّف بتركيز

References

1. World Health Organization, Department of Vaccines and Biologicals, Geneva. Research related to measles control and elimination. *Measles Bulletin* 2000;3:2.
2. Wassilak SG, Bernier RH, Herrmann KL, Orenstein WA, Bart KJ, Amler R. Measles seroconfirmation using dried capillary blood specimens in filter paper. *Pediatric Infectious Diseases* 1984;3:117-21.
3. Nakano JH, Miller DL, Foster SO, Brink EW. Microtiter determination of measles hemagglutination inhibition antibody with filter papers. *Journal of Clinical Microbiology* 1983;17:860-3.
4. Rawat D, Chakravarti A, Yadav S. Comparison of two blood collection techniques for measles antibody detection in children. *Indian Journal of Medical Research* 2001;114:117-20.
5. Condorelli F, Scalia G, Stivala A, Gallo R, Marino A, Battaglini CM, et al. Detection of immunoglobulin G to measles virus, rubella virus, and mumps virus in serum samples and in microquantities of whole blood dried on filter paper. *Journal of Virological Methods* 1994;49:25-36.
6. De Swart RL, Nur Y, Abdallah A, Kruining H, El Mubarak HS, Ibrahim SA, et al. Combination of reverse transcriptase PCR analysis and immunoglobulin M detection on filter paper blood samples allows diagnostic and epidemiological studies of measles. *Journal of Clinical Microbiology* 2001;39:270-3.

7. Helfand RF, Keyserling HL, Williams I, Murray A, Mei J, Moscatiello C, et al. Comparative detection of measles and rubella IgM and IgG derived from filter paper blood and serum samples. *Journal of Medical Virology* 2001;65:751-7.
8. Novello F, Ridolfi B, Fiore L, Buttinelli G, Medda E, Favero A, et al. Comparison of capillary blood versus venous blood samples in the assessment of immunity to measles. *Journal of Virological Methods* 1996;61:73-7.
9. Riddell MA, Leydon JA, Catton MG, Kelly HA. Detection of measles virus-specific immunoglobulin M in dried venous blood samples by using a commercial enzyme immunoassay. *Journal of Clinical Microbiology* 2002;40:5-9.
10. Lambert SB, Kelly HA, Andrews RM, Catton MG, Lynch PA, Leydon JA, et al. Enhanced measles surveillance during an interepidemic period in Victoria. *Medical Journal of Australia* 2000;172:114-8.
11. Riddell MA, Leydon JA, Ugoni A, Kelly HA. A serosurvey evaluation of the school-based measles 'catch-up' immunisation campaign in Victorian school-aged children. *Australian and New Zealand Journal of Public Health* 2001;25:529-33.
12. Steger KA, Craven DE, Shea BF, Fitzgerald BR, Schwerzler M, Seage GR. Use of paper-absorbed fingerstick blood samples for studies of antibody to human immunodeficiency virus type 1 in intravenous drug users. *Journal of Infectious Diseases* 1990;162:964-7.
13. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Statistical Methods in Medical Research* 1999;8:135-60.
14. Lee MS, Nokes DJ, Hsu HM, Lu CF. Protective titres of measles neutralising antibody. *Journal of Medical Virology* 2000;62:511-7.
15. Behets F, Kashamuka M, Pappaioanou M, Green TA, Ryder RW, Batter V, et al. Stability of human immunodeficiency virus type 1 antibodies in whole blood dried on filter paper and stored under various tropical conditions in Kinshasa, Zaire. *Journal of Clinical Microbiology* 1992;30:1179-82.