Validation of IgG-sandwich and IgM-capture ELISA for the detection of antibody to Rift Valley fever virus in humans

Janusz T. Paweska*, Felicity J. Burt, Robert Swanepoel

Special Pathogens Unit, National Institute for Communicable Diseases, Sandringham 2131, South Africa

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Abstract

Rift Valley fever (RVF) virus is an important zoonotic and a potential bioterrorism agent. This paper describes validation of sandwich and capture enzyme-linked immunosorbent assays (ELISA) based on gamma-irradiated antigens for the detection of RVFV-specific IgG and IgM antibody in humans. Validation data sets derived from testing field-collected sera from Africa (n = 2400) were dichotomised according to the results of a virus neutralisation test. In addition, sera from laboratory workers immunized with inactivated RVF vaccine (n = 93) and serial sera (n = 3) from a single RVF case were used. ELISA data were expressed as percentage of high-positive control serum (PP). Cut-off values at 95% accuracy level were optimised using the misclassification cost term option of the two-graph receiver operating characteristics analysis.

During the routine use of assays there was no evidence for excessive intra- and inter-plate variations within and between runs of assays. At a cut-off of 13.2 PP the sensitivity of the IgG-sandwich ELISA was 100% and specificity 99.95%, while for the IgM-capture ELISA the values were 96.47 and 99.44%, respectively, at a cut-off of 7.1 PP. Compared to the virus neutralisation test, the IgG-sandwich ELISA was more sensitive in detection of immunological responses in vaccines. Following natural infection class-specific antibodies were detected in serum taken 6 days after onset of symptoms. The results demonstrate that both assays will be useful for early diagnosis of infection, epidemiological surveillance and for monitoring of immune response after vaccination. As highly accurate, robust and safe tests, they have the potential to replace traditional diagnostic methods which are unable to distinguish between different classes of immunoglobulins, and pose health risks necessitating their use being restricted to high containment facilities outside RVF endemic areas.

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1. Introduction

Rift Valley fever (RVF) is a mosquito-borne virus disease that poses a significant threat to domestic ruminants and human health in Africa where it occurs at irregular intervals when heavy rains facilitate breeding of the mosquito vectors (Swanepeol and Coetzer, 1994). The occurrence of the first confirmed outbreaks of RVF among humans and livestock outside Africa, in the Arabian Peninsula (Jupp et al., 2002, Shoemaker et al., 2002), carries the implication of further spread of infection into non-endemic RVF areas since the causative agent, RVF virus (RVFV), is capable of utilizing a wide range of mosquito vectors (Turrel et al., 1998). The virus is also a potential bioweapons agent (Peters, 2003). Delay in diagnosis associated with traditional virus isolation and identification techniques may represent a significant problem for regulatory healthcare authorities faced with an epidemic of RVF, especially outside its traditional geographical confines. Hence, considerable efforts have been made recently to develop nucleic acid techniques for rapid detection and identification of RVFV (Ibrahim et al., 1997; Garcia et al., 2001; Drosten et al., 2002). However, traditional and molecular procedures for diagnosis of RVF may be beyond the expertise and capabilities of many laboratories, particularly in developing countries.

Accurate diagnosis of RVF can be achieved when serological tests are used in combination with clinical observation and...
2. Materials and methods

2.1. ELISA serum controls and internal quality control (IQC)

Freeze-dried, gamma-irradiated serum controls were used. Selection, inactivation, and characterization of high, low, and negative controls were carried out as described previously (Paweska et al., 2003a). High-positive control serum was prepared by pooling serum from 10 individuals infected with RVFV in domestic and wild animals; have been recently developed and validated (Paweska et al., 2003a, 2003b). ELISA based on β-propiolactone inactivated antigen for the detection of IgG and IgM antibodies to RVFV in humans have been reported (Niklasson et al., 1984). However, methods used for the expression of ELISA absorbance readings and the selection of cut-off values is now obsolete. Also, due to the limited number of field-collected sera tested, diagnostic performance of these assays could not be estimated accurately.

Reasons for test validation include the need for reliable estimates of sensitivity and specificity with respect to clinical diagnosis, risk assessment and risk-factor studies. There is an increasing international demand for capabilities in diagnosis and surveillance of RVF. This paper describes validation of a sandwich ELISA for the detection of IgG and a capture ELISA for the detection of IgM antibody to RVFV in human sera with special emphasis on test performance characteristics.

2.2. Serum specimens

2.2.1. Field sera

A total of 2404 individual sera were used. Sera were collected in Kenya (n = 817), South Africa (n = 1253), Tanzania (n = 221) and Uganda (n = 109). South African sera represented specimens collected during the 1974 outbreak of West Nile in the country (n = 953), and the routine 1999–2003 diagnostic submissions (n = 300) to the Special Pathogens Unit, National Institute for Communicable Diseases. East African sera were specifically taken to monitor the 1997–1998 outbreak of RVFV in the region (Woods et al., 2002). In addition, three serial serum samples from an individual infected with RVFV in Namibia in 2004 were tested.

Field-collected sera found to be negative in virus neutralisation tests were regarded as reference panels from non-infected, and sera that tested positive as reference panels from individuals infected with RVFV. ELISA results obtained on the field-collected sera were used for the selection of cut-off values and determination of diagnostic accuracy of IgG-sandwich and IgM-capture ELISA.

2.2.2. Sera from individuals vaccinated with inactivated RVF vaccine

A total of 93 individual sera from South African medical and veterinary laboratory workers immunized with formalin-inactivated RVF vaccine (Randall et al., 1962, 1964) were used. Sera were taken 4–6 weeks after primary (n = 17) or booster vaccination (n = 76).

Post-vaccination sera were used to compare the analytical sensitivity of the IgG-sandwich ELISA with that of virus neutralisation test under control conditions.

2.3. Serum neutralisation test

Duplicates of serial two-fold dilutions of sera inactivated at 56°C for 30 min were tested using a microneutralisation inhibition test. Low-positive serum control was prepared by diluting high-positive serum control in negative control.

The internal quality control (IQC) data were generated as described previously (Paweska et al., 2003b). Means and standard deviations (S.D.) of ELISA net optical density readings (OD) and percentage positivity (PP) of high-positive control serum (see Section 2.5) were calculated from replicates of all internal controls in each plate and each run of the assay to assess intra- and inter-plate variation. Additionally, coefficients of variation (C.V. = standard deviation of replicates/mean of replicates × 100) were calculated for positive controls. Data obtained from this analysis were used to estimate the assay repeatability and to establish the upper and lower control limits for each of the internal controls. Upper and lower control limits together with C.V. values (<10%) were applied as IQC rules for further analysis. During routine runs of the ELISA each plate had four replicates of high-positive, two of low-positive and two of negative serum controls.
procedure as previously described (Swanepeel et al., 1986a), except for using the AR 20368 isolate of RVFV recovered in 1981 from Culex zombaensis in South Africa. Titres were expressed as the reciprocal of the serum dilution that inhibited \(\geq 75\%\) of viral cytopathic effect. A serum sample was considered positive when it had a titer of \(\geq 100\) or equivalent to a serum dilution of 1:10.

### 2.4. ELISA reagents

#### 2.4.1. Antigens and antisera

Production, inactivation, preservation, and safety testing of RVFV antigen, mock antigen, and hyperimmune mouse anti-virus antibody followed methods described previously (Paweska et al., 2003a, 2003b).

#### 2.4.2. Optimisation of reagents and protocols

Optimal reagent concentrations/dilutions and protocol parameters were determined through standard checkerboard titration procedures (Crowther, 1995).

### 2.5. Procedure for IgG-sandwich ELISA

A 100 \(\mu\)l well of polyclonal mouse anti-RVFV capture antibody diluted 1:10000 in phosphate buffered saline (PBS) was passively adsorbed onto ELISA plates (Maxisorp, NuncTM, Denmark) overnight in a humidity chamber at +4 °C. After incubation, plates were washed three times with 0.1% Tween-20 in PBS; the same washing procedure followed each subsequent stage of the assay. Plates were blocked with 200 \(\mu\)l well of 10% skim milk (Merek) in PBS and incubated at 37 °C for 1 h. After washing, a volume of 100 \(\mu\)l well of virus antigen and mock antigen, diluted 1:1000 in 2% skim milk in PBS (diluting buffer), was added to rows of the top half of the plate (rows A-D:1–12) and to the bottom half of the plate (rows E–G:1–12), respectively. After incubation at 37 °C for 3 h, plates were washed and duplicate volumes of 100 \(\mu\)l of each test and control serum diluted 1:400 in dilution buffer were added to wells in rows A–D:1–12 and to corresponding wells in rows E–G:1–12, and plates were incubated at 37 °C for 1 h. After washing, 100 \(\mu\)l/well of antigens diluted 1:800 were added; virus antigen was added to rows A–D:1–12, and mock antigen to rows E–G:1–12 of the plate. After incubation at 37 °C for 60 min and washing, a 100 \(\mu\)l/well of mouse anti-virus antibody diluted 1:5000 was added to each well, and plates were incubated again at 37 °C for 1 h. After washing, a 100 \(\mu\)l/well of goat anti-mouse IgG (H+L chain) HRPO-conjugate (Zymed Laboratories) diluted 1:6000 was added to each well and plates were incubated at 37 °C for 30 min. Plates were washed and a 100 \(\mu\)l ABTS was added to each well and the plate incubated in the dark for 30 min at room temperature (22–25 °C). Stopping of color development, measurement of OD, and converting of net OD readings into PP values were the same as described for the IgG-sandwich ELISA.

### 2.6. Procedure for IgM-capture ELISA

#### 2.6.1. Antigens and antisera

Production, inactivation, preservation, and safety testing of RVFV antigen, mock antigen, and hyperimmune mouse anti-virus antibody followed methods described previously (Paweska et al., 2003a, 2003b).

#### 2.6.2. Optimisation of reagents and protocols

Optimal reagent concentrations/dilutions and protocol parameters were determined through standard checkerboard titration procedures (Crowther, 1995).

### 2.7. Selection of cut-off values

Cut-off values at 95% accuracy level were optimised using the misclassification cost term option (Greiner, 1996) of the two-graph receiver operating characteristics (TG-ROC) analysis (Greiner, 1995; Greiner et al., 1995). Optimisation of cut-off values was based on the following equation: misclassification cost term option (Greiner, 1996) of the two-graph receiver operating characteristics (TG-ROC) analysis (Greiner, 1995; Greiner et al., 1995). Optimisation of cut-off values was based on the following equation: misclassification cost term option (Greiner, 1996).

### 2.8. Diagnostic accuracy of ELISA

The following statistical approaches (Greiner and Gardner, 2000) were used to estimate: sensitivity \((D – Se) = [Tp/(Tp + Fn)] \times 100\), specificity \((O – Sp) = [Tn/(Tn + Fp)] \times 100\), Youden’s index \((J) = [Sn + (Sp – 1)]\), efficiency \(= ([Se] \times [Sp] = 100\), apparent prevalence \((AP) = ([D – Se] + (O – Sp) \times 100\), and true prevalence \((TP) = ([AP + (Sp – 1)]\times N\) the number of sera tested.

The following statistical approaches (Greiner and Gardner, 2000) were used to estimate:

- Sensitivity: \((D – Se) = [Tp/(Tp + Fn)] \times 100\)
- Specificity: \((O – Sp) = [Tn/(Tn + Fp)] \times 100\)
- Youden’s index: \((J) = [Sn + (Sp – 1)]\)
- Efficiency: \(= ([Se] \times [Sp] = 100\)
- Apparent prevalence: \((AP) = ([D – Se] + (O – Sp) \times 100\)
- True prevalence: \((TP) = ([AP + (Sp – 1)]\times N\) the number of sera tested.
3. Results

3.1. Internal quality control and repeatability

Upper and lower control limits for internal controls used in IgG-sandwich and IgM-capture ELISA and estimates of repeatability of these assays are given in Table 1. During the routine runs of ELISA, IQC data for internal controls were within upper and lower limits and there was no evidence for excessive variations within and between routine runs of the assay (Fig. 1A, B and Fig. 2A, B).

3.2. Safety of ELISA reagents

Mice inoculated with gamma-irradiated reagents were clinically normal for 21 days after inoculation. This confirms that the procedures used in this work yielded safe preparations of reagents for laboratory use.

3.3. Determination of cut-off values

Selection of a cut-off value for IgG-sandwich ELISA was based on field data sets which were dichotomised according to the results of the virus neutralisation test (Table 2). For the selection of a cut-off for IgM-capture ELISA by TG-ROC field data sets were categorised (Table 2) using mean plus three standard deviations of ELISA PP values observed in the RVF-free population (Fig. 3). Optimisation of cut-off values using misclassification cost term option of the TG-ROC analysis was based on the non-parametric programme option (Greiner et al., 1995) due to departure from a normal distribution of data sets analysed. For example, in IgG-sandwich ELISA the correlation coefficients were $r = 0.993$ and $r = 0.991$ for linear correlations of the non-parametric diagnostic sensitivity and specificity versus parametric diagnostic sensitivity and specificity parameters, respectively (Fig. 4A and B). Examples of graphical presentations of the TG-ROC analysis are shown in Fig. 5A and D.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>IgG-sandwich</th>
<th>IgM-capture</th>
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<tbody>
<tr>
<td>IQC</td>
<td>UCL</td>
<td>LCL</td>
</tr>
<tr>
<td>OD C++</td>
<td>1.76</td>
<td>0.84</td>
</tr>
<tr>
<td>PP C++</td>
<td>107.01</td>
<td>92.73</td>
</tr>
<tr>
<td>PP C+</td>
<td>37.72</td>
<td>20.64</td>
</tr>
<tr>
<td>PP C−</td>
<td>1.56</td>
<td>–1.97</td>
</tr>
</tbody>
</table>

Repeatability:

- Intra-plate variation

<table>
<thead>
<tr>
<th></th>
<th>IgG-sandwich</th>
<th>IgM-capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C++</td>
<td>3.21 ± 1.97</td>
<td>5.59 ± 1.05</td>
</tr>
<tr>
<td>C+</td>
<td>4.16 ± 1.45</td>
<td>5.06 ± 1.17</td>
</tr>
</tbody>
</table>

- Inter-plate variation

<table>
<thead>
<tr>
<th></th>
<th>IgG-sandwich</th>
<th>IgM-capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C++</td>
<td>3.38 ± 1.28</td>
<td>5.47 ± 1.08</td>
</tr>
<tr>
<td>C+</td>
<td>6.13 ± 1.75</td>
<td>8.32 ± 1.51</td>
</tr>
</tbody>
</table>

* Upper control limit (UCL) and lower control limit (LCL) for internal quality control (IQC) data were calculated from the mean ± 2 S.D. of 400 replicates of each control over ten runs each including five plates.

* PP: percent positivity.

Mean ± S.D. of replicates/mean C.V. mean of replicates × 100; range of C.V. values.
Fig. 2. Upper (—–) and lower (– ––) IQC limits for PP values of high-positive (■), low-positive (▲), and negative (□/H17033) serum controls in IgG-sandwich (A) and IgM-capture (B) ELISA and the results for these controls (mean ± S.D.) on 24 (A) and 15 (B) routine runs of the assays during a period of 10 weeks. Five plates were used during each run with four replicates of high-positive, and two replicates of low-positive and negative serum controls on each plate.

Table 2

Diagnostic accuracy of Rift Valley fever IgG-sandwich and IgM-capture ELISA

<table>
<thead>
<tr>
<th>Measure</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-off (%)</td>
<td>11.21PP</td>
<td>7.19PP</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>96.47</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>99.95</td>
<td>99.44</td>
</tr>
<tr>
<td>Youden’s index</td>
<td>0.999</td>
<td>0.908</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td>99.95</td>
<td>99.09</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>99.50</td>
<td>96.55</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>100</td>
<td>99.54</td>
</tr>
</tbody>
</table>

* Individuals were dichotomised according to the results of virus neutralisation test (VNT).

Fig. 3. Distribution of IgM capture ELISA PP values in human sera from Africa known to be positive (n=202, dot area) and negative (n=1254, grey area) in the VNT for antibodies to RVFV. All the VNT-positive sera were collected during the 1997–1998 outbreak of RVF in East Africa. Sera ordered according to ELISA PP values. Vertical lines crossing the shaded and dot areas indicate the ELISA cut-off determined as mean plus three standard deviations (5.75 PP) of ELISA PP values observed in the VNT-negative population. At this cut-off results to the left of vertical lines are considered negative and to the right of vertical lines are considered positive.

Fig. 4. Linear correlation analysis of the non-parametric (—) diagnostic sensitivity (D-Se) vs. parametric (—–) diagnostic sensitivity (D-Se*) (A), and the non-parametric diagnostic specificity (D-Sp) vs. parametric diagnostic specificity (B).
Fig. 5. Optimisation of cut-offs for Rift Valley fever IgG-sandwich and IgM-capture ELISA in humans using the misclassification cost term (MCT) option of the two-graph receiver operating characteristics analysis. At cut-off value of 13.2 PP for IgG ELISA (A) and of 7.1 PP for IgM ELISA (B), the overall misclassification costs for IgG ELISA (C) and IgM ELISA (D) become minimal (0.002 and 0.013, respectively) under assumption of 50% disease prevalence and equal costs of false-positive and false-negative test results. The two curves represent MCT values based on non-parametric (smooth line) or parametric (dashed line) estimates of sensitivity and specificity derived from data sets in field-collected sera. Optimisation of cut-off values was based on the non-parametric program option due to departure from a normal distribution of data sets analysed.

3.4. Diagnostic accuracy of IgG-sandwich and IgM-capture ELISA

Estimates of sensitivity, specificity, and other estimates of combined measures of diagnostic accuracy (J, Ef, PPV, NPV) of IgG-sandwich and IgM-capture ELISAs derived from the study data sets are given in Table 2.

3.5. Comparison of IgG-sandwich and virus neutralisation in vaccines

Of the total of 93 post-vaccination sera, all were positive in IgG-sandwich ELISA and 90 in the virus neutralisation test. All sera tested positive in virus neutralisation test were also positive in ELISA. The three sera, which tested negative in the virus neutralisation test, were from individuals bled 28 or 36 days after primary vaccination.

3.6. Kinetics of IgG and IgM responses in a natural case of RVF

At the selected cut-offs for IgG and IgM ELISA (Table 2) class-specific antibody to RVFV were easily detectable in sera collected on days 6, 9 and 22 after onset of symptoms in a RVF naturally infected individual (Fig. 6) whose infection status has been confirmed by virus isolation and reverse transcription-PCR (data not shown).

4. Discussion

ELISAs for the detection of virus-specific antibodies have a number of potential advantages compared to traditional
serological tests. In general terms, they are less expensive and time consuming, well suited to the needs of large scale testing and production of kits, and as primary assays they can be well standardised, quality controlled and automated. However, an ELISA can be difficult to develop and validate because of signal amplification of both specific and non-specific components. One of the major problems hindering the diagnostic application of an ELISA can be background absorbance arising from the application of unpurified antigen (Gravel et al., 1977; Forghani and Schmidt, 1979). Production of purified and concentrated stocks of viral antigens for ELISA by classical methods is expensive and laborious, especially when an agent does not multiply to a high titer in tissue culture cells (Chu et al., 1985). In this study we used sucrose–acetone-extracted antigens derived from RVFV-infected and normal mouse livers. These antigen preparations do not readily coat ELISA plates (Meegan et al., 1987). However, the mouse model for the production of RVFV antigen is simple to employ and yields high concentrations of viral antigen in relatively small volumes of infected tissues. Antigen-specific antibodies immobilised on the plate are used to capture unpurified viral antigens. An advantage of the technique is that the antigen is selectively trapped and thus need not be purified (Schrivjer and Kramps, 1998). Capture antibodies can also be used in a non-competitive ELISA for the detection of virus-specific class antibodies, e.g. IgM. In this instance use of class-specific IgM-capture antibody prevents interference by specific antibodies of other classes that are present in excess in post-infection sera (Deshpande, 1996).

Procedures for validation of serological assays for the diagnosis of infectious diseases are complex and subject to many limitations, including availability of standards and representative reference sera (Jacobson, 1998). The ultimate goal of assay validation is to provide test results that identify objects as positive or negative, and by inference accurately predict the infection status of individuals with a predetermined degree of statistical certainty. The determination of the performance characteristics of a diagnostic assay should be based on testing samples from individuals of known infectious status relative to the disease of interest. The accuracy of initial classification of the subjects based on diagnostic discrimination, significantly impacts on the selection of the optimal threshold and subsequently on estimates of diagnostic sensitivity, specificity and other measures of test performance. In the present work we used the virus neutralisation test to dichotomise individuals according to their RVF infection status. Therefore, it is important to note that infection with RVFV induces life long virus neutralising antibody-associated immunity in animals (Barnard, 1979) and humans (Findlay and Howard, 1951), and that there is no evidence of serological subgroups or major antigenic variation between virus isolates of disparate chronologic or geographic origins (Swanepoel and Coetzer, 1979). Antigenic cross-reactivity studies in animals (Davies, 1975; Swanepoel, 1976, 1981; Swanepoel et al., 1980b) failed to provide any evidence that other African phleboviruses could hamper the diagnosis of RVF. The virus neutralisation test is generally regarded as a gold standard for the detection of antibodies to many viruses. However, there are differences in the antigenic specificities of the antibodies being measured by ELISA and the virus neutralisation test (Saunders et al., 1977; Bolton et al., 1981). Thus, it is expected that ELISA, which detects antibodies against all viral components, would be more sensitive than the virus neutralisation test which detects only antibodies to viral neutralizing epitope. This may explain higher sensitivity of IgG-sandwich ELISA compared to the virus neutralisation test in detection of RVFV-specific antibody in vaccines.

Although the virus neutralisation test used for the verification of infection with RVFV is an appropriate standard for IgG comparative serology, it is not for IgM comparative serology. Due to the transitory nature of IgM antibody, the diagnostic accuracy of any assay designed for its detection will vary over time. In this study the number of cases of known exposure and/or disease history was insufficient to allow precise evaluation of time-dependent changes of the IgM-capture ELISA diagnostic performance in humans. In an experimental animal model a similar assay was shown to be 100% sensitive 5–42 days post infection and 12.5% sensitive 3 weeks later (Paweska et al., 2003a). Though very limited, our results with serial sera from a natural RVF case suggest that the IgM-capture ELISA will detect antibody at least on days 6–22 after onset of RVF symptoms in naturally infected humans. The levels of IgM on day 6 and 22 after onset of clinical disease was about 18–25 times higher than the cut-off derived from population field data sets. This indicates that seroconversion in a case under study most likely took place very early in the course of infection, and that IgM would be detectable for a longer time after onset of clinical disease that determined in the present study. The available data on the case disease history and exposure (the patient was bitten by mosquitoes while camping and become sick 3 days later) suggests that the incubation period was not longer than 1–3 days after infection (Fig. 3). In a sheep model, IgG and IgM antibody to RVFV were detectable as early as 4 days after experimental infection (Paweska et al., 2003b).

Time-dependent changes in the sensitivity of an assay may be of significance for epidemic situations, where the stage of disease may affect the outcome of assay results and interpretation of the data. However, in practice, the impacts and interrelationships of multiple factors, e.g. biological, nutritional, geographic, stage of infection, are mostly unknown and diagnostic sensitivity and specificity estimates are based on average values calculated in non-homogenous populations. Biological variables probably contribute more heavily to false positive than to false negative results (Jacobson, 1998b). Therefore, to account for the probable increased variance that would affect the estimate of diagnostic specificity relatively large numbers of individuals free from Rift Valley fever were analysed in the present study.

The cut-off for a test result is selected at a threshold value and above which findings are interpreted as positive. Although, still widely in use, a cut-off determined as two or three
standard deviations above the mean is not recommended for interpretation of serodiagnostic assays because it assumes normal distribution of test values in populations targeted by the assay. Deviations from normality are often observed in serological data and should be addressed in the selection of cut-off values (Vizard et al., 1990). The two-graph receiver operating characteristics analysis (TG-ROC) provides a simple graphical method to select cut-off of an assay at desired accuracy level (Greiner, 1995), and permits its optimisation to minimise overall misclassification costs (Greiner, 1996). Estimates of diagnostic accuracy vary with disease prevalence (Bremer and Gefeller, 1997). In this study the decision threshold at 95% confidence level was selected under assumption that the cost of a false positive result and the cost of a false negative result were equal and the prevalance of infection was 50%. The prevalence assumed in the study sample may not be representative of the prevalence in the target populations and this should be borne in mind in applying the estimates of diagnostic accuracy reported for the assays in the present work.

The ability of a diagnostic test to produce consistent results within tolerable analytical error limits when specimens are re-tested, is one of prerequisites for any diagnostic device to be accepted for routine applications. While the IgG and IgM ELISA used in this study achieved very high repeatability estimates within the determined IQC limits, the reproducibility (laboratory-to-laboratory) of both assay formats remains to be addressed for more comprehensive evaluation. Antibody-binding levels should be expressed in relative rather than absolute terms. One of the distinct advantages of converting ELISA OD data into PP values relative to a known standard is that this method does not assume uniform background activity, and therefore enables inter-laboratory standardisation (Wright et al., 1993).

Our results demonstrate that the ELISAs reported here are safe, robust and highly accurate, and therefore have the potential to be used in early diagnosis of infection, disease surveillance, and for monitoring of immune response in vaccines.

References


