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Short communication

# Measles immunity testing: Comparison of two measles IgG ELISAs with plaque reduction neutralisation assay

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**Abstract**

Two commercial IgG ELISAs, one based on recombinant nucleocapsid antigen and one based on cell culture grown native virus antigens, were evaluated for measles immunity testing by comparison with plaque reduction neutralisation test (PRNT). Qualitative results of the two ELISAs showed 92% agreement with those of PRNT. The sensitivity of the two ELISAs was 89.6%. False negative ELISA results were obtained in 10% of sera, mainly sera containing low levels of neutralising antibody. The specificity of both ELISAs was 100%. Measles IgG ELISAs perform adequately for immunity testing, correctly identifying seronegative individuals for vaccination.


*Keywords:* Measles; Immunity; Neutralisation; ELISA

Measles is a leading cause of vaccine preventable death in infants and children globally (World Health Organisation, 2005a). While mortality rates are highest in developing countries, industrialised countries with sub-optimal vaccination programmes are also at risk of outbreaks and resulting fatalities (Cronin and O'Connell, 2000). In the context of increased efforts by World Health Organisation and other agencies to achieve a reduction in mortality due to measles (World Health Organisation, 2005b), there is an on-going need for reliable laboratory assays to assess measles immunity.

Plaque reduction neutralisation test (PRNT, Albrecht et al., 1981) is regarded as the gold standard method for measles immunity because it measures functional neutralising antibody. It is a quantitative assay and protective levels of antibody measured by PRNT have been determined. A PRNT titre (serum dilution that reduces the number of plaques by 50%) of >1:120 is considered to be a level of antibody protective against measles disease (Chen et al., 1990).

PRNT is technically demanding and takes up to seven days to complete. It is a biological assay that is difficult to standardise and does not lend itself readily to large-scale surveys. Measles IgG ELISA, on the other hand, is easy and rapid to perform and can be automated. It does not measure specifically neutralising antibodies and its performance characteristics for immunity testing need to be determined. New commercial ELISAs based on recombinant measles nucleocapsid antigen expressed in *Saccharomyces cerevisiae* have become available recently and an evaluation of measles IgM ELISA with the new antigen has been reported (Samuel et al., 2003). In this study, the performance of measles IgG ELISA based on the recombinant antigen and that of an ELISA based on cell culture grown native virus antigen were compared to that of PRNT.

One hundred serum samples submitted for immunity testing to the measles laboratory at the Health Protection Agency Centre for Infections during 2003 were included in the study. The indications for immunity testing as stated on laboratory request forms are shown in Table 1.

All sera were tested by PRNT (Albrecht et al., 1981) and by two measles IgG ELISAs, one based on recombinant nucleoprotein antigen and IgG capture (Microimmune Ltd., Brentford, UK) and one based on cell culture grown native virus antigens and an indirect assay format (Dade Behring, UK, Milton Keynes, UK). PRNT was performed with the following modifications. Serum samples, diluted four-fold from 1:8 to 1:8192, were mixed with an equal volume of challenge virus and incubated at room temperature (~22 °C) for 90 min. The challenge virus used was

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Table 1
Indications for measles immunity testing and results with three assays

| Indication for testing | No. sera | PRNT | | Microimmune ELISA | | | Dade Behring ELISA | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Equivocal | Negative | Positive | Equivocal | Negative |
| Assessment of vaccination response | | | | | | | | | |
| Single antigen measles vaccination | 29 | 16 | 13 | 14 | 2 | 13 | 13 | 0 | 16 |
| Pre-MMR booster | 9 | 9 | 0 | 7 | 0 | 2 | 8 | 1 | 0 |
| Problem with primary MMR | 3 | 3 | 0 | 3 | 0 | 0 | 3 | 0 | 0 |
| Sub-total | 41 | 28 | 13 | 24 | 2 | 15 | 24 | 1 | 16 |
| Occupational requirement (health care workers) | 12 | 12 | 0 | 11 | 0 | 1 | 11 | 0 | 1 |
| Pregnant, in contact with measles | 6 | 6 | 0 | 6 | 0 | 0 | 5 | 0 | 1 |
| Immunocompromised patient | 5 | 3 | 2 | 2 | 0 | 3 | 2 | 0 | 3 |
| Visa requirement | 5 | 5 | 0 | 5 | 0 | 0 | 5 | 0 | 0 |
| No information provided | 31 | 23 | 8 | 19 | 1 | 11 | 20 | 1 | 10 |

the wild type strain, 'Loss' (Sinitsyna et al., 1990) which was used at a dilution that gave 20–30 plaques per well. Serum/virus mixtures were transferred to wells of TC 24 cell culture plates and Vero cell suspension ($5 \times 10^5$ cells per well) added and the plates incubated at 37 °C in a humidified $CO_2$ incubator for 2–3 h. Cell culture supernatant fluid was then removed and replaced with an overlay consisting of 0.8% carboxymethyl cellulose in Dulbecco's minimal essential medium containing 1.5% fetal bovine serum and gentamicin (0.5 mg/mL). The plates were incubated for 5–7 days and then the overlay was removed, the monolayer washed with phosphate buffered saline (PBS), fixed with 5% formalin in PBS and stained with crystal violet. The numbers of plaques were counted and the 50% neutralising dose ($ND_{50}$) of each serum calculated using the Kärber formula. The measles international antibody standard (66/202, National Institute of Biological Standards and Control, South Mimms, UK), containing 5000 milli international units per mL (mIU/mL), was included with each batch of tests, which enabled quantitative results to be transformed into mIU/mL as described below:

$$\text{mIU/mL} = \text{ND}_{50} \times \text{unitage constant}$$

where

$$\text{unitage constant} = \frac{\text{unitage of International Standard}}{\text{ND}_{50} \text{ of International Standard}}.$$

A 'test limit' in terms of mIU/mL was determined for each assay run as follows:

$$\text{test limit} = \text{unitage constant} \times 8$$

where 8 is the lowest reciprocal dilution of each test serum.

Test sera with reactivity greater than the test limit were considered PRNT positive.

A three-way comparison of qualitative PRNT, Microimmune and Dade Behring ELISA results with one another is shown in Table 2. Treating equivocal results as positive, Microimmune ELISA and PRNT results showed 92/100 (92%; 95%CI 84.8%–96.5%) agreement; Dade Behring ELISA and PRNT results also showed 92/100 (92%) agreement. Compared to PRNT, the sensitivity of Microimmune ELISA was 69/77 (89.6%; 95%CI 80.6–95.4%) and specificity was 23/23 (100%; 95%CI 85.2%–100%); the sensitivity of Dade Behring ELISA was also 69/77 (89.6%) and specificity 23/23 (100%). A similar pattern of serostatus determined by PRNT and ELISA was reported in a recent study of measles vaccination in young infants (Carson et al., 2005).

When optical density (OD) signals in the two ELISAs for each of the serum samples were compared with the PRNT titre (expressed in mIU/mL), the rank correlation with Microimmune was 72% and with Dade Behring was 83%. The absence of a high correlation between Microimmune ELISA and PRNT is not surprising since the ELISA detects antibodies solely to the nucleocapsid protein whereas PRNT detects neutralising antibodies to the haemagglutinin and fusion proteins (Griffin, 2001). Although Dade Behring ELISA is based on cell culture grown measles virus antigen, which presumably contains haemagglutinin and fusion protein, the relatively low correlation with PRNT suggests that the ELISA is predominantly detecting antibodies to the NP antigen which is the most abundant protein found in measles infected cells. It should be noted that, while antibody to nucleocapsid does not contribute directly to neutralisation, it is the most abundant antibody formed in response to infection or immunisation, so its absence is considered a reliable indicator of seronegativity (Griffin, 2001). In the present study OD values obtained in ELISA were not transformed to quantitative values in terms of mIU/mL since the primary aim was to determine whether

Table 2
Comparison of qualitative PRNT, Microimmune and Dade Behring ELISA results

| PRNT | Microimmune ELISA | Dade Behring ELISA | | |
|---|---|---|---|---|
| | | Negative | Equivocal | Positive |
| Negative | Negative | 23 | 0 | 0 |
| | Equivocal | 0 | 0 | 0 |
| | Positive | 0 | 0 | 0 |
| Positive | Negative | 3[a] | 1[a] | 4[a] |
| | Equivocal | 0[a] | 0 | 3[b] |
| | Positive | 5[a] | 1[b] | 60 |

[a] Sera giving discordant results: 13 sera were negative in at least one ELISA and positive by PRNT.
[b] Sera giving discordant results: four sera were equivocal in at least one ELISA and positive by PRNT.