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Evaluation of the time resolved fluorescence immunoassay (TRFIA) for the detection of varicella zoster virus (VZV) antibodies following vaccination of healthcare workers

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Abstract

Determination of varicella zoster virus (VZV) immunity in healthcare workers without a history of chickenpox is important for identifying those in need of vOka vaccination. Post immunisation, healthcare workers in the UK who work with high risk patients are tested for seroconversion. To assess the performance of the time-resolved fluorescence immunoassay (TRFIA) for the detection of antibody in vaccinated as well as unvaccinated individuals, a cut-off was first calculated. VZV-IgG specific avidity and titres six weeks after the first dose of vaccine were used to identify subjects with pre-existing immunity among a cohort of 110 healthcare workers.

Those with high avidity (≥60%) were considered to have previous immunity to VZV and those with low or equivocal avidity (<60%) were considered naive. The former had antibody levels 400mIU/mL and latter had levels <400 mIU/mL. Comparison of the baseline values of the naive and immune groups allowed the estimation of a TRFIA cut-off value of >130 mIU/mL which best discriminated between the two groups and this was confirmed by ROC analysis. Using this value, the sensitivity and specificity of TRFIA cut-off were 90% (95% CI 79–96), and 78% (95% CI 61–90) respectively in this population. A subset of samples tested by the gold standard Fluorescence Antibody to Membrane Antigen (FAMA) test showed 84% (54/64) agreement with TRFIA.

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Keywords

Varicella zoster virus; Time-resolved fluorescence immunoassay; vOka vaccine; Fluorescent Antibody to Membrane Antigen Antibody

1. Introduction

Universal vaccination of children against varicella zoster virus was introduced in the USA in 1995 and more recently in other countries including Germany, Canada, Uruguay and Australia (Robert Koch Institute, 2001; National Advisory Committee on Immunization, 2004; National Health and Medical Research Council, 2003). In the UK, concerns that interrupting transmission of varicella might reduce exogenous boosting and lead to increased rates of shingles, has limited the use of vaccine to healthcare workers or family members of immunocompromised subjects (Department of Health, 2006). To accomplish targeted vaccination healthcare workers are screened for susceptibility to chickenpox by antibody testing of those who have no history of chickenpox. Antibody testing is also used to identify those at risk of serious infection who are eligible for post-exposure prophylaxis, including varicella-zoster immunoglobulin (VZIG). There is considerable variation in the sensitivity of commercially available assays (Maple et al., 2006, 2009b,c). Low assay sensitivity results in over use of post exposure prophylaxis and the unnecessary immunisation of immune individuals. Moreover, many commercial assays are poor at detecting vaccine induced antibodies.

Although most healthcare workers who have been immunised with the vOka vaccine are protected against chickenpox, up to 10% have been shown to develop breakthrough infections over the five years following vaccine (Saiman et al., 2001). Such individuals pose a potential risk to vulnerable patients who are seronegative for VZV. To identify those at risk of breakthrough infection, the UK currently recommends that healthcare workers caring for vulnerable patients should be tested post immunisation for VZV antibodies (Department of Health, 2006). The presence of either vaccine or natural antibody as measured by the Fluorescent Antibody to Membrane Antigen (FAMA), which detects antibodies against VZV glycoproteins (Williams et al., 1974) has been shown to correlate with protection against clinical varicella infection (Gershon et al., 1988, 1994; Grose et al., 1979; Iltis et al., 1982; Michialik et al., 2008; Saiman et al., 2001; Williams et al., 1974). The Merck in-house gpELISA is also sensitive for vaccine antibody but is not generally available (Wasmuth and Miller, 1990), and may be overly sensitive for determining protection (Michialik et al., 2008). The time resolved fluorescent immunoassay (TRFIA) which is more sensitive and specific than most commercial tests in unvaccinated populations has already been described (Maple et al., 2006). In this paper the performance of the TRFIA as a reference test was analysed for the detection of vaccine antibody following immunisation of healthcare workers with the Oka strain vaccine. Using the antibody titre and avidity following the first dose of vaccine, the TRFIA cut-off appears to be nearer to 130 mIU/mL (Maple et al., 2009a). Using this cut-off there is a good correlation between TRFIA and the gold standard FAMA test. Furthermore, the use of avidity and antibody titres following antigenic challenge to define immune status has allowed us to analyse discrepant results between TRFIA and FAMA.

2. Methods

2.1. Sample collection

Ethical permission for the study was granted by the East London and the City Health Authority Local Research Ethics Committee 3 (05\Q0605\1). Healthcare workers who were eligible for varicella vaccination were screened for VZV antibodies by the diagnostic virology laboratory using a commercial assay (Diamedix®). One hundred and ten healthcare workers with negative or repeatedly equivocal readings were consented for inclusion in the study in accordance with the ethical approval obtained. Healthcare workers were bled at baseline and the first dose of Oka vaccine was administered subcutaneously. Six weeks later a second sample was taken and the second dose of vaccine was administered. A third sample was taken six weeks after the second dose. In 75 patients a final sample was taken 12–18 months after the second dose of vaccine. Samples were spun, separated and stored at –80°C prior to analysis.

2.2. VZV IgG avidity (EUROIMMUN) assay

Samples were tested by the EUROIMMUN assay according to the manufacturer's instructions. Only samples with antibody levels in the PBS test control with VZV specific antibody i.e. >TRFIA 100 mIU/mL (Maple et al., 2006), were tested for avidity.

2.3. VZV IgG antibody assays

TRFIA assays were carried out on serum samples as described previously (Maple et al., 2006). The Diamedix® assay was carried out according to the manufacturer's instructions.

2.4. Fluorescent Antibody to Membrane Antigen (FAMA)

FAMA assays were carried out on 64 samples from 16 subjects, as described previously (Williams et al., 1974).

2.5. Statistical analysis

2.5.1. ROC analysis—ROC analysis was performed by hand and using Minitab Inc. (2006, PA, USA) and SPSS Inc.. The 'gold standard' was taken as avidity at six weeks after the first dose of vaccine (avidities of <60% were considered low and ≥60% high). ROC analysis was carried out using 98 corresponding baseline samples. Cut-offs were applied in log₁₀0.02 increments from a value of 1.18–4.8, covering the entire range of the data. Confidence intervals for sensitivity and specificity were calculated using the exact method in Minitab.

All *p*-values were generated by independent 2 tailed *t*-tests unless otherwise stated. Standard deviations were calculated in Excel (Microsoft 2003, Reading, UK).

3. Results

Table 1 summarises the demographic characteristics of study participants and their baseline Diamedix® test results.

Antibody titres measured by TRFIA at 6 weeks following the first dose of vaccine were plotted against avidity measured on the same samples (Fig. 1a). Eight subjects were excluded as their antibody levels at six weeks were below 100 mIU/mL by TRFIA and therefore could not be tested for avidity. There was a good correlation ($R = 0.822$ between the EUROIMMUN OD values in the control well and TRFIA antibody, confirming that the antibodies being tested in both assays were similar. As shown in Fig. 1a, the results were clearly dichotomously distributed. Sixty one subjects (61%) had low or equivocal avidity antibody (<60%) suggesting that they had made a primary response to vaccine while thirty five subjects (35%) had high avidity antibodies (>60%) which was consistent with prior immunity and a secondary antibody response or boost to vaccine antigen (Fig. 1a). The correlation between TRFIA antibody levels following one dose of vaccine and avidity was high ($R 0.93$) with TRFIA antibody levels <400 mIU/mL in the primary responders and >400 mIU/mL among those with a secondary response (Fig. 1a). The differences in mean log antibody levels between the two avidity groups were highly significant (independent 2 tailed test; $p < 0.0001$). Even at 12 weeks, following the second dose of vaccine, the two groups remained distinct with antibody avidities significantly higher in those assumed to have previous immunity to VZV ($p < 0.0001$) (Fig. 1b). Two subjects (2%) did not group with the primary or secondary responders after one dose of vaccine (labelled 1 and 2 in Fig. 1). However, after two doses, it was clear that both individuals had had a secondary antibody response (Fig. 1b).

To determine a TRFIA cut-off, the baseline values for the 63 primary and 35 secondary vaccine responders were plotted separately (Fig. 2). The 61 VZV naive individuals had significantly lower antibody titres at baseline (GMT 45 ± 2 mIU/mL) than the 35 secondary responders (GMT 229 ± 3 mIU/mL) (independent 2 tailed t -test; $p < 0.0001$) (Fig. 2). From the intercept of the two population curves, a cut-off of >130 mIU/mL which discriminated best between primary and secondary responders was identified (Fig. 2). Using this cut-off, the eight subjects whose low antibody levels had precluded testing for antibody avidity at 6 weeks, were, as expected, negative at baseline.

To validate the cut-off a receiver operating curve (ROC) was plotted using the baseline TRFIA values. Baseline TRFIA values were classified as positive or negative based on the results of the TRFIA and avidity at six weeks. Log₁₀ baseline antibody titres were ordered and successive cut-offs (increasing by log₁₀0.02) were applied to the data, for which the sensitivity and specificity were calculated. The ROC curve was produced by plotting the true and false positive rates for different cut-offs (Fig. 3). The cut-offs which gave the highest sensitivity and specificity were log₁₀2.12 (132 mIU/mL) and log₁₀2.14 (138 mIU/mL), and therefore similar to the conclusion from Fig. 2 of >log₁₀2.11 (>130 mIU/mL).

From the combined results a cut-off of >130 mIU/mL was established. The sensitivity and specificity of the TRFIA, at baseline i.e. in unvaccinated individuals, using response to a dose of vaccine as the gold standard, were 90% (95% CI 79–96), and 78% (95% CI 61–90) respectively. The majority of results classified as equivocal by Diamedix® 83% (10/12) and 24% (23/96) of results classified as negative by Diamedix® were positive by TRFIA.

Comparison between seropositive status determined using TRFIA and the gold standard reference assay; FAMA. To compare TRFIA against an assay which is known to detect protective antibody, 64 serology samples (16 subjects, 4 visits each) were tested by FAMA (Fig. 4). Borderline results (≥ 2) and results which were indistinct were repeated. Taking a FAMA cut-off of ≥ 4 as positive (Michialik et al., 2008) and from our studies, a TRFIA of >130 , agreement between the FAMA and TRFIA was 84.4% (54/64).

Just over 80% (82.6%) (38/46) of samples with a FAMA reading of ≥ 4 were positive (>130 mIU/mL) by TRFIA while 87.5% (14/16) samples with a FAMA of ≥ 2 were negative by TRFIA. TRFIA antibody titres were significantly higher in the former compared with the latter ((mean $\log_{10} 2.793$), 621 ± 537.515 mIU/mL versus (mean $\log_{10} 1.878$) 75.5 ± 54.558 mIU/mL; independent 2 tailed t -test, $p < 0.0001$) (Fig. 4). Overall 10 results occurring in seven patients were discordant between the assays (Table 2). Four individuals (1–4) differed at baseline (Table 2). Subjects 1 and 2 had a positive FAMA and a negative TRFIA, at baseline. The discrepancies between the two tests continued following vaccination, with TRFIA negative and FAMA positive in three of four samples for both subjects. In both subjects antibody avidity was low ($<40\%$) and failed to mature. Subject 3 was also negative for TRFIA and positive for FAMA at baseline, but made a high (80%) avidity antibody response to vaccine suggesting prior immunity and a false negative TRFIA (Fig. 1 and Table 2). Subject 4 was positive for TRFIA but negative for FAMA (≥ 2) at baseline. This person had high levels of highly avid antibody following vaccination suggesting a false negative FAMA at baseline. Subjects 5–7 seroconverted to vaccine but in all three antibody avidity, where measurable, remained low ($<40\%$). In comparison to FAMA, TRFIA had a sensitivity of 82.6% (38/46) and a specificity of 87.5% (14/16). The positive and negative predictive values were 87.5% (35/40) and 79.2% (19/24) respectively.

4. Discussion

The detection of antibodies to VZV has been shown to correlate well with clinical protection against chickenpox. However, most commercial antibody tests lack sensitivity and do not detect low levels of antibody, such as occur after vaccine (Maple et al., 2009b). The time resolved fluorescent immunoassay, or TRFIA was developed to provide a high throughput reference test for a targeted healthcare worker vaccination programme in the UK (Maple et al., 2006). Like the Merck gpELISA, TRFIA has a wide dynamic linear read out allowing good discrimination between low levels of antibody (Li et al., 2002; Maple et al., 2006). Post-vaccination antibody titres have been estimated to be a log lower than antibody from VZV infection. To determine whether the reference TRFIA test was suitable for the detection of vaccine antibody, the antibody response following a single dose of Oka vaccine was used as the “gold standard” for defining VZV immune status at baseline. Exposure of immune individuals to previously encountered antigen results in a rapid rise in IgG antibodies which bind strongly with high avidity to immunodominant epitopes. This is known as a secondary antibody response. In contrast the primary humoral response results in low levels of IgG antibodies with mixed affinity binding to epitopes and overall low avidity. Clonal selection for B cells excreting higher affinity antibodies occurs over a period of weeks.

The validity of this approach is supported by the clearly dichotomous response to a single dose of vaccine, with clustering of primary and secondary responders (Fig. 2) and significant differences in avidity and antibody titres between the two groups. As expected, antibody avidity also increased significantly after the second vaccination in the naive group ($p < 0.0001$) but not in those with prior immunity. By extrapolating back to the values at baseline it was possible to set a cut-off of >130 mIU/mL, which discriminated between the naive and immune groups. This cut-off was supported by ROC analysis. Using the 130 mIU/mL the sensitivity of the TRFIA in this population of unvaccinated adults who are negative by the commercial Diamedix[®] assay is calculated as 90% (95% CI 79–96) and specificity as 78% (95% CI 61–90). The correlation with Diamedix[®] was 73% ($r = 0.728$).

In order to measure the clinical protection afforded by antibody follow-up of antibody positive and negative individuals exposed to varicella zoster virus is necessary. However, this has only been successfully achieved with FAMA and then largely when performed in one laboratory (Gershon et al., 1994). Even then, while most patients are FAMA positive immediately post vaccination, up to 30% of immunised individuals lose FAMA antibody over time and this is associated with increased risk of breakthrough infection (Ampofo et al., 2002). A FAMA result of ≥ 4 has been shown to correlate well with protection against natural and vaccine breakthrough infection (Michialik et al., 2008). In the study reported here, limited comparison of TRIFIA showed 84.4% agreement with FAMA and for a FAMA of ≥ 4 there was similar agreement with a TRFIA > 130 mIU/mL (82.6%). Similarly the agreement for negative FAMA (≤ 2) with TRFIA ≤ 130 mIU/mL was also high i.e. 87.5%. Of the seven subjects with discordant FAMA and TRFIA results, most occurred in subjects (1, 2, 5, 6 and 7) who failed to produce a highly avid antibody result even 12–18 months after vaccination (Table 2). In these subjects FAMA was positive while TRFIA was negative. FAMA results have been shown to correlate with neutralising antibody probably directed against surface glycoproteins (Grose et al., 1979) and the results therefore suggest that TRFIA fails to detect these well in low avidity samples. Subjects 5–7 who seroconverted to vaccine, took longer to become positive by TRFIA which might reflect poorer detection of IgM or certain IgG isotypes. All three lost antibody by TRFIA at 12 months post vaccination while remaining FAMA positive. Further studies to examine the immunological basis for this result are underway. Of the remaining two patients who had discrepant results at baseline, the data suggest a false negative TRFIA result for subject 3, and a false positive FAMA result for subject 4. FAMA, unlike TRFIA, has been validated extensively in follow-up clinical studies, but even so, a positive antibody postvaccination may not always predict long term protection (Saiman et al., 2001). This analysis of maturation of antibody avidity and the pattern of antibody boosting following Oka vaccine has provided a unique insight into the performance of both tests. The results suggest that both FAMA and TRFIA misclassified one patient each at baseline. However, FAMA and TRFIA results correlated 100% for antibody measured after the second vaccination, suggesting that clinical advice based on TRFIA levels at this stage is compatible with the data from longitudinal studies of healthcare workers tested by FAMA.

The data also show that antibody titres from the in-house TRFIA assay combined with avidity values following one dose of vaccine reflect baseline immunity to VZV. The approach taken in this paper has allowed confirmation of the usefulness of the TRFIA assay

and has established cut-off of >130 mIU/mL for both vaccine and natural antibody, although given the variability of the assay (Maple et al., 2009b) it maybe necessary to operate a wider equivocal range, but this remains to be confirmed. The increased sensitivity improved the performance of this test for the detection of vaccine induced antibody. The correlation between TRFIA titres and FAMA antibody scores, particularly following two doses of vaccine, provides reassurance that the specificity of TRFIA is retained notwithstanding its greater sensitivity compared with commercial enzyme immunoassays.

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Abbreviations

FAMA	Fluorescent Antibody to Membrane Antigen
TRFIA	time-resolved fluorescence immunoassay
VZIG	varicella zoster immunoglobulin
VZV	varicella zoster virus
ROC	receiver operating curve

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Fig. 1. Scatter plot to show the relationship between TRFIA titres and avidity (relative avidity index) RAI. (A) Six weeks post first vaccination; the dashed horizontal and vertical lines represent the avidity and TRFIA cut-offs (60% and log₁₀ 2.60;400 mIU/mL respectively). Two results which did not cluster within the two populations are circled (1 and 2). (B) Antibody and avidity results six weeks following the second vaccination.

Fig. 2.

Observed and fitted positive and negative distributions of baseline samples classified by avidity readings and TRFIA titres after the first dose of vaccine (six weeks). The arrow indicates the cut-off of $\text{Log}_{10} 2.11$ (130 mIU/mL); the point where the two fitted populations intercept. $N=96$. The horizontal dotted lines highlight the population that were TRFIA negative at baseline.

Fig. 3.
ROC analysis of baseline TRFIA at different cut-offs. The area under the curve is 0.92.

Fig. 4.

Relationship between FAMA scores and log₁₀ TRFIA readings. Whiskers extend to largest or smallest data point within 1.5 times the upper or lower quartile. The mean is indicated by a filled square (■) and the median by the horizontal line within the box. The horizontal dashed line indicates the log₁₀ TRFIA cut-off of 2.11 (130 mIU/mL). On analysis of variance with Tukey's post hoc tests, the difference between the mean of the FAMA ≤ 2 and FAMA ≥ 8 groups was not significant ($p > 0.1$); all other differences were significant at the 0.1% level.

Table 1

Summary of study participants demographics and baseline Diamedix[®] readings.

Total number of study participants	Female (%)	Mean age in years (range: 19–61)	Non-Caucasian (%) ^a	Negative by Diamedix [®] (%) ^b
110	74	33 ± 8	75.5	98 (89%)

^aBlack: 42.8% (African: 25.5%: Caribbean: 17.3%); Indian subcontinent: 21.8%: Filipino: 8.2%: Other: 2.7%.

^bEquivocal by Diamedix[®] *n* = 12(11%).

Table 2

FAMA, TRFIA and avidity readings for study participants with discrepant results.

Study number	Visit	TRFIA (mIU/mL)	TRFIA status	Avidity (%)	FAMA score	FAMA status	History or contacts	Agreement on assay status	Responder status
1015 subject 1	V1	37	N	X	4	P	None	No	Primary
	V2	96	N	26	4	P		No	
	V3	429	P	33	8	P		Yes	
	V4	74	N	X	>8	P		No	
1017 subject 2	V1	64	N	X	4	P	None	No	Primary
	V2	125	N	28	8	P		No	
	V3	374	P	25	>8	P		Yes	
	V4	148	P	29	<2	N		No	
1032 subject 3	V1	94	N	X	4	P	None	No	Secondary
	V2	823	P	55	8	P		Yes	
	V3	464	P	80	>8	P		Yes	
	V4	194	P	81	8	P		Yes	
1013 subject 4	V1	183	P	70	<2	N	None	No	Secondary
	V2	700	P	81	8	P		Yes	
	V3	778	P	85	8	P		Yes	
	V4	463	P	72	4	P		Yes	
1001 subject 5	V1	130	P	28	<2	N	None	Yes	Primary
	V2	207	P	15	8	P		Yes	
	V3	229	P	38	8	P		Yes	
	V4	87	N	X	8	P		No	
1019 subject 6 1019	V1	21	N	X	<2	N	None	Yes	Primary
	V2	64	N	X	8	P		No	
	V3	326	P	30	8	P		Yes	
	V4	15	N	X	<2	N		Yes	
1026 subject 7	V1	41	N	X	<2	N	None	Yes	Primary
	V2	128	N	26	4	P		No	
	V3	481	P	34	>8	P		Yes	
	V4	116	N	28	8	P		No	

Key: V1: Visit 1 (baseline); V2: Visit 2 (six weeks); V3: Visit 3 (12 weeks); V4: Visit 4 (18-month follow-up); P: positive; N: negative. Assay discrepancies are highlighted in bold.