The differences in short- and long-term varicella-zoster virus (VZV) immunoglobulin G levels following varicella vaccination of healthcare workers measured by VZV fluorescent-antibody-to-membrane-antigen assay (FAMA), VZV time-resolved fluorescence immunoassay and a VZV purified glycoprotein enzyme immunoassay

P. A. C. MAPLE[1,2]*, J. HAEDICKE[3], M. QUINLIVAN[3], S. P. STEINBERG[4],
A. A. GERSHON[4], K. E. BROWN[1] AND J. BREUER[3]

[1] *Virus Reference Department, Public Health England, Reference Microbiology Services, Colindale, London, UK*
[2] *East Yorkshire Microbiology, York Science Park, Heslington, York, UK*
[3] *Department of Infection, The Cruciform Building, University College London, London, UK*
[4] *Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, USA*

SUMMARY

Healthcare workers (HCWs) reporting no history of varicella frequently receive varicella vaccination (vOka) if they test varicella-zoster virus (VZV) immunoglobulin G (IgG) negative. In this study, the utilities of VZV-IgG time-resolved fluorescence immunoassay (VZV-TRFIA) and a commercial VZV-IgG purified glycoprotein enzyme immunoassay (gpEIA) currently used in England for confirming VZV immunity have been compared to the fluorescent-antibody-to-membrane-antigen assay (FAMA). A total of 110 HCWs received two doses of vOka vaccine spaced 6 weeks apart and sera collected pre-vaccination ($n = 100$), at 6 weeks post-completion of vaccination ($n = 86$) and at 12–18 months follow-up ($n = 73$) were analysed. Pre-vaccination, by FAMA, 61·0% sera were VZV IgG negative, and compared to FAMA the sensitivities of VZV-TRFIA and gpEIA were 74·4% [95% confidence interval (CI) 57·9–87·0] and 46·2% (95% CI 30·1–62·8), respectively. Post-completion of vaccination the seroconversion rate by FAMA was 93·7% compared to rates of 95·8% and 70·8% determined by VZV-TRFIA and gpEIA, respectively. At 12–18 months follow-up seropositivity rates by FAMA, VZV-TRFIA and gpEIA were 78·1%, 74·0% and 47·9%, respectively. Compared to FAMA the sensitivities of VZV-TRFIA and gpEIA for measuring VZV IgG following vaccination were 96·4% (95% CI 91·7–98·8) and 74·6% (95% CI 66·5–81·6), respectively. Using both FAMA and VZV-TRFIA to identify healthy adult VZV susceptibles and measure seroconversion showed that vOka vaccination of HCWs is highly immunogenic.

Key words: Fluorescent-antibody-to-membrane-antigen-assay, healthcare workers, vOka vaccine, VZV glycoprotein EIA, VZV immunoglobulin G, VZV time-resolved fluorescence immunoassay.

INTRODUCTION

Primary infection with varicella zoster virus (VZV) manifests as varicella (chickenpox) and reactivation

later in life produces herpes zoster (shingles). In the UK, historically, most cases of varicella occurred in the 5–14 years age group; however, in recent years there has been a sharp increase in the prevalence of varicella in the 1–4 years age group so that most cases are now reported in children aged 0–5 years [1, 2]. Primary varicella infection in healthy children is generally a mild, self-limiting disease which typically

* Author for correspondence: Dr P. A. C. Maple, East Yorkshire Microbiology, Innovation Centre, York Science Park, York YO10 5DG, UK.
(Email: eastyorksmicrobiol@gmail.com)

presents as a cropping vesicular rash; however, in immunocompromised individuals and susceptible adults, particularly pregnant women, the infection can be more serious and even life threatening [3–5].

An effective vaccine (vOka) against varicella has been available for a number of years and universal childhood vaccination [6, 7] is undertaken in a number of countries, e.g. Germany, Japan and the USA. The UK does not undertake universal varicella immunization and current UK policy [8] advocates vOka vaccination of significant contacts of immunocompromised individuals and healthcare workers (HCWs). Screening of HCWs through recall of history of chickenpox and laboratory testing to determine VZV IgG status in those with uncertain or negative histories followed by vOka vaccination of specific antibody-negative individuals is widely practised [9]. The use of highly sensitive assays to determine VZV IgG status is recommended [10].

The accepted gold standard assays for the determination of immunity/protection against VZV include the fluorescent-antibody-to-membrane-antigen assay (FAMA) [11, 12], and complement-enhanced VZV neutralization assay [13]. The FAMA and VZV neutralization assays are technically complex, subjective and unsuitable for testing large numbers of sera. Alternative, indirect immunofluorescence-based assays [14, 15] which are less technically demanding due to the use of glutaraldehyde or acetone-fixed infected cells are also available. Following the introduction of vOka vaccination [16] there has been a need for VZV IgG detection assays of high sensitivity because less VZV IgG is produced following vaccination compared to natural infection [17]. The Merck glycoprotein enzyme immunoassay (gpEIA; Merck EIA) was developed for this reason [18, 19] and was extensively evaluated, particularly in children [20]; however, the assay has extremely limited availability and is oversensitive in indicating immunity to varicella [21]. Commercial EIAs with purified VZV glycoproteins as antigen (gpEIAs) which differ methodologically from the Merck EIA are available; but, there are limited data comparing their performance in comparison with FAMA for measuring VZV IgG following vOka vaccination [22]. An 'in house' VZV IgG time-resolved fluorescence immunoassay (VZV-TRFIA) has also been developed using VZV-infected whole-cell lysate [23] and offers high analytical sensitivity and a large linear dynamic range due to the use of lanthanide chelates, which when coordinated with specific ligands and excited by ultraviolet absorption emit fluorescence

for several hundreds of microseconds. Specific fluorescence is emitted over a much longer time period than background fluorescence and time-resolved measurement of fluorescence decay enables non-specific fluorescence which lasts a matter of nanoseconds to be excluded from the measured signal enabling highly sensitive and specific detection.

The immunization of adolescents or adults with vOka in a population where wild-type VZV freely circulates presents its own set of challenges; for instance, choosing appropriate assays to reliably identify VZV susceptibles in need of vaccination and then adequately measuring production of VZV IgG. In this study 110 HCWs with no reported history of chickenpox and testing VZV IgG negative, or equivocal, by a commercial non-glycoprotein-based EIA received two doses of vOka and their levels of VZV IgG at 6 weeks and 12–18 months post-second dose of vOka were measured. The utilities of VZV IgG TRFIA and a commercial gpEIA, which is recommended by UK Public Health England for testing VZV susceptibility, have been compared to FAMA.

METHODS

Study population

The serum samples ($n = 259$) used in this study were obtained from HCWs who were eligible for varicella vaccination as described previously [24]. The East London and the City Health Authority Local Research Ethics Committee (05/Q0605/1 granted ethical permission for the study. In brief, 110 HCWs who tested VZV IgG negative, or equivocal, by a commercial assay (Diamedix®, Diamedix Corporation, USA) received two doses of vOka vaccine (Sanofi Pasteur, Merck Sharp & Dohme, France) 6 weeks apart. Serum samples available for this study were collected pre-vaccination ($n = 100$), 6 weeks following the second vaccine dose ($n = 86$) and 12–18 months following the second dose ($n = 73$). Sera were stored at $\leqslant -20$ °C prior to testing.

FAMA

VZV FAMA was performed using previously described methodology [11, 12]. MRC-5 cells were grown in Eagle's Minimum Essential Medium containing L-glutamine (Sigma, UK), supplemented with 10% fetal calf serum (FCS; Sigma, UK), 100 × non-essential amino acids (Life Technologies, UK), 500 × gentamicin and amphotericin B (Life

Technologies, UK), and 1 ml vancomycin at 5 mg/ml (Sigma, UK). Cells were infected at 90% confluence with an in-house wild-type VZV strain (THA). The multiplicity of infection was 0·5. Infected cells were harvested at 80–90% cytopathic effect by careful trypsinization, gently pelleted (400 g) and resuspended in an appropriate volume of phosphate buffered saline (PBS) for use fresh in setting up FAMA.

To set up the FAMA, 25 μl PBS was added to control and sample wells of a round-bottom 96-well suspension culture plate (Grenier Bio-one, Germany). Control sera included VZV positive, intermediate positive, and negative sera diluted 1:2, 1:4 and 1:8 in consecutive wells. Serum negative, antibody negative and antibody/serum negative controls were employed in each run by replacing the respective components with 25 μl PBS, except for the antibody-negative control to which a 1:8 dilution of the British standard VZV IgG antibody (90/690 NIBSC, UK) was added. A volume of 25 μl of infected cell suspension was added to all wells and the plate incubated at room temperature for 30 min. The cells were then washed three times with 100 μl PBS with centrifugation at 400 g for 5 min. A volume of 25 μl of 1:50 dilution of polyclonal rabbit anti-human IgG FITC conjugate (Dako, UK) was added to all wells except the antibody-negative and antibody/serum-negative controls, and the plate was incubated at room temperature for 30 mins in the dark. Unbound antibody was removed by washing, as described previously and 10 μl of 50% glycerol was added to each well. Volumes of 2 μl from each well were transferred to a slide and visualized by confocal microscopy using a Zeiss AxioPlan 2 Microscope System (Campbell, USA). A bright fluorescent ring around the surface of infected cells indicated a sample positive for the presence of anti-VZV IgG. On average, between 10 and 20 infected cells were visualized per field and 10–20 fields examined depending upon the degree of fluorescence observed.

Results were reported as the lowest dilution at which distinct membrane fluorescence was visible. No membrane fluorescence at 1:2 dilution was graded as FAMA negative (susceptibility to VZV), and fluorescence at 1:2, 1:4 or 1:8 was graded as FAMA positive (VZV protective). Typical fluorescence is shown in Figure 1.

VZV-TRFIA

DELFIA microtitre plates (PerkinElmer, UK) were coated with VZV ELISA grade antigen (Serion Immundiagnostica GmbH, Germany) at concentrations of 1·0–2·0 μg/ml (depending on batch) prepared in 0·05 m carbonate/bicarbonate buffer (pH 9·6). The antigen was a sucrose-density gradient-purified extract of VZV strain Ellen grown in human embryo lung culture. The coating concentration of antigen selected was that which gave a Europium count of 400 000– 600 000 with British Standard VZV antibody (NIBSC, UK) at a concentration of 50 mIU/ml. The plates were stored overnight at 4 °C and washed four times with DELFIA wash buffer (PerkinElmer) using a DELFIA plate washer (PerkinElmer). Sera for testing were diluted 1:50 in DELFIA assay buffer (Perkin Elmer) and 100 μl loaded into appropriate wells. A standard curve was run on each plate, prepared from British Standard VZV antibody diluted in DELFIA assay buffer at concentrations ranging from 50 mIU/ ml to 0·39 mIU/ml. The plates were sealed and incubated in a humid chamber for 2 h at 37 °C and then washed four times, as before. Europium-labelled anti-human IgG conjugate (PerkinElmer) diluted 1:500 in DELFIA assay buffer was added at 100 μl per well using a multichannel pipette. The plates were then incubated for 1 h at 37 °C, washed four times, as before, and 150 μl DELFIA enhancement solution (PerkinElmer) added to all wells. Following 10 min rotating incubation at room temperature, in the dark, the plates were read using a DELFIA 1234 reader (PerkinElmer) and data analysed using Multicalc software, v. 2000 (Wallac Oy, Finland). Interpolated antibody concentrations were expressed as mIU/ml.

In this study based on previous work [25] a cut-off VZV IgG level of <100 mIU/ml has been used to indicate VZV susceptibility in non-vaccinated (pre-vaccination) adults and levels of VZV IgG ⩾100 mIU/ml were considered protective. In adults vaccinated with vOka previous work [24] has shown that VZV IgG levels >130 mIU/ml can be considered protective although relevant clinical studies are needed to confirm this assertion. Therefore the VZV-TRFIA cut-offs used in this study were 100 mIU/ml for HCWs pre-vaccination and 130 mIU/ml for HCWs post-vaccination.

Commercial VZV gpEIA

The commercial gpEIA used in this study was the VaccZyme™ VZV glycoprotein IgG Low Level EIA kit supplied by The Binding Site (Birmingham, UK). The methodology for this assay has been described elsewhere [26] and the cut-off criteria used for the
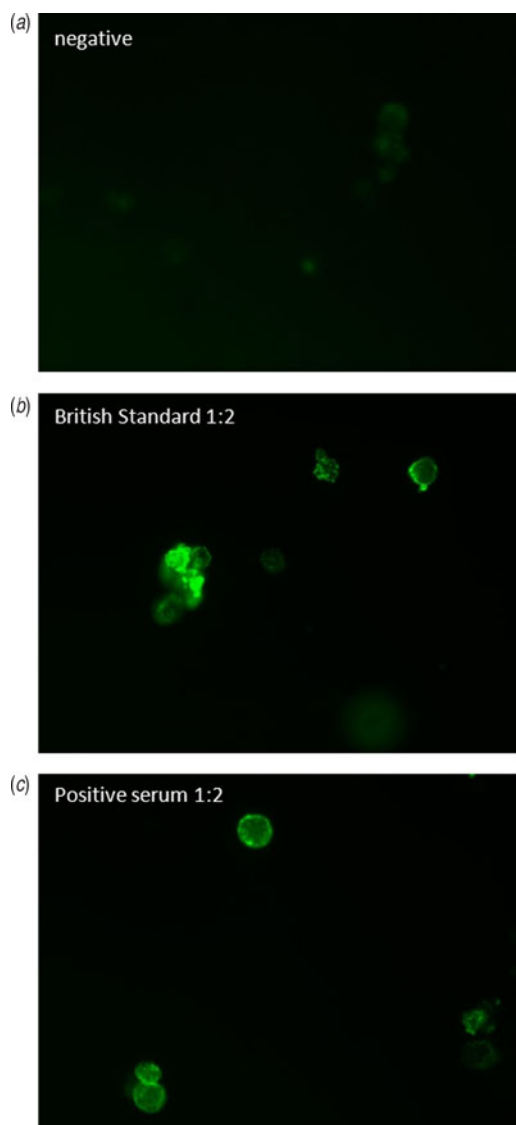
Fig. 1. Confocal microscopy (20× objective, green fluorescent filter) of VZV-infected MRC-5 cells. (*a*) Exposed to VZV negative serum, (*b*) exposed to British Standard 90/690 VZV IgG and (*c*) exposed to serum from vOka-vaccinated individual. Note typical ring fluorescence in VZV IgG-positive sera.

purposes of this study were the same as those described above for the VZV-TRFIA. This commercial gpEIA has been used for a number of years by the Virus Reference Department, Colindale for confirmatory testing of VZV immunity status, particularly in pregnant women but is not recommended for assessing vaccine response [26].

Assay variability

It is widely recognized that FAMA is a subjective and technically demanding assay and to accommodate for this all FAMA work for this study was undertaken by a single author (J.H.). The competency of this worker and the reliability of the FAMA method set up at the Great Ormond Street Hospital, London, UK was checked by testing a proficiency panel of 64 serum samples (comprising pre-vaccination, post-vaccination and 12–18 months follow-up samples) which were then tested also by another author (S.P.S.) at the Columbia University College of Physicians and Surgeons, New York, USA, from where the reference FAMA originates. The agreement (kappa statistic = 0·4) of the classification of the samples as either VZV IgG positive or negative using a cut-off titre of 2 was high with 94% (44/47) of the samples tested giving concordant results. The sensitivity and specificity of the FAMA method used for this study was 93·9% and 92·8% compared to the reference FAMA. Repeat testing of selected samples in separate FAMA runs also assessed the reproducibility of the FAMA methodology used for this study. Using FAMA, the frequency of samples tested with a ⩾fourfold difference in FAMA titres was 1:10–1:11.

The inter-assay variability (percentage coefficient of variation) of VZV-TRFIA in this study for a negative and low-positive sample was 12·5% and 10·9%, respectively. Using the commercial gpEIA the inter-assay variability for a negative and low-positive sample was 4·2% and 6·2%, respectively.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for VZV-TRFIA and the commercial gpEIA together with 95% confidence intervals (CIs) were calculated compared to FAMA overall and by visit. Results for the three assays were plotted against one another on log-scale axes to assess agreement and to calculate Pearson's correlation (*r*). Results for the three assays were also plotted at each visit with geometric means and proportions ⩾2 by FAMA, ⩾0·1 by TRFIA and ⩾0·1 by gpEIA compared using a McNemar's test for paired data.

RESULTS

Pre-vaccination VZV serological status of HCWs

Results for FAMA, VZV-TRFIA and gpEIA were available for 100/110 HCWs, pre-vaccination. By FAMA (Table 1), 61·0% sera had no VZV IgG

detected, 16·0% sera had FAMA titre = 2, 13·0% sera had FAMA titre = 4, 7·0% sera had FAMA titre = 8 and 3·0% sera had FAMA titre >8.

A total of 57/61 (93·5%) FAMA VZV IgG-negative sera tested VZV IgG negative by VZV-TRFIA and four (6·5%) sera tested VZV IgG positive. The mean VZV IgG level by VZV-TRFIA was 46·2 (range 15–183) mIU/ml. Similarly, for the FAMA VZV IgG-negative sera there were 60/61 (98·4%) sera which tested VZV IgG negative by commercial gpEIA and one (1·6%) serum which tested VZV IgG positive. The mean VZV IgG level by commercial gpEIA was 28 (range <10–124) mIU/ml.

For the FAMA VZV IgG-positive sera there were 29/39 (74·4%) sera that tested VZV IgG positive by VZV-TRFIA and 10 (25·6%) that were VZV IgG negative. The mean VZV IgG level by VZV-TRFIA was 356 (range 22–2507) mIU/ml. Similarly, for the FAMA VZV IgG-positive sera there were 18/39 (46·1%) sera which tested VZV IgG positive by commercial gpEIA and 21 (53·8%) sera which were VZV IgG negative. VZV IgG levels for the commercial gpEIA ranged between <10 and >810 mIU/ml.

Compared to FAMA the sensitivity and specificity of VZV-TRFIA at baseline was 74·4% and 93·4%, respectively. Likewise, compared to FAMA the sensitivity and specificity of commercial gpEIA was 46·2% and 100%, respectively.

Post-vaccination VZV serological status of HCWs 6 weeks following the second dose of vOka

Results for FAMA, VZV-TRFIA and gpEIA were available for 86/110 HCWs at 6 weeks post-vaccination. By FAMA (Table 1), 5·8% sera had no VZV IgG detected, 9·3% sera had FAMA titre = 2, 17·4% sera had FAMA titre = 4, 41·9% sera had FAMA titre = 8 and 25·6% sera had FAMA titre >8. The mean VZV IgG level by VZV-TRFIA was 800·9 (range 78–3346) mIU/ml. For the commercial gpEIA a mean VZV IgG value could not be computed as VZV IgG levels >810 mIU/ml were not quantitated. Compared to FAMA the sensitivity of VZV-TRFIA for post-vaccine antibody was 100%; however, specificity could not be reliably computed due to the low number (n = 5) of negative samples. Likewise, compared to FAMA the sensitivity of commercial gpEIA was 84·0%.

The rate of seroconversion in HCWs shown by FAMA to be VZV IgG negative at pre-vaccination was 93·7% as determined by FAMA compared to 95·8% as determined by VZV-TRFIA and 70·8% as determined by commercial gpEIA.

Post-vaccination VZV serological status of HCWs at 12–18 months follow-up

Results for FAMA, VZV-TRFIA and gpEIA were available for 73/110 HCWs at 12–18 months follow-up. By FAMA (Table 1), 21·9% sera had no VZV IgG detected, 13·7% sera had FAMA titre = 2, 26·0% sera had FAMA titre = 4, 27·4% sera had FAMA titre = 8 and 11·0% sera had FAMA titre >8. By VZV-TRFIA and gpEIA there were 19 (26·0%) and 38 (52·0%) sera, respectively, which tested VZV IgG negative. The mean VZV IgG level by VZV-TRFIA was 800·9 (range 78–3346) mIU/ml. For the commercial gpEIA a mean VZV IgG value could not be computed as VZV IgG levels >810 mIU/ml were not quantitated.

There were 13 HCWs who tested VZV IgG negative by FAMA at 12–18 months follow-up, despite being FAMA positive 6 weeks following the second dose of vOka. The serological profiles of these HCWs, who appeared to lose VZV IgG over the longer term according to FAMA, are shown in Table 2. Two HCWs appeared to lose VZV IgG which appeared to have been present pre-vaccination, one (study no. 1100) had a FAMA titre = 2 and one (study no. 1046) had a FAMA titre = 4. Compared to FAMA the sensitivity and specificity of VZV-TRFIA at 12–18 months post-immunization was 91·2% and 87·5%, respectively and for commercial gpEIA the sensitivity and specificity was 61·4% and 100%, respectively.

Overall comparison of VZV-TRFIA and gpEIA with FAMA

Both VZV-TRFIA and gpEIA correlated well with FAMA, with Pearson r values of 0·84 and 0·83, respectively. The VZV-TRFIA geometric mean VZV IgG level increased from 71 mIU/ml at pre-vaccination to 619 mIU/ml at 6 weeks post-vaccination and then declined to 301 mIU/ml at 12–18 months follow-up (Table 1). Likewise, for FAMA the geometric mean VZV IgG titre increased from 1·7 to 6·6 and then decreased to 3·8 pre-vaccination, 6 weeks post-vaccination and 12–18 months follow-up, respectively. The commercial gpEIA geometric mean VZV IgG level increased from 23 mIU/ml at pre-vaccination to 315 mIU/ml at 6 weeks post-vaccination and then declined to 128

Table 1. *Results of FAMA, VZV-TRFIA and gpEIA at pre-vaccination, 6 weeks post-2 doses of vOka, and at 12–18 months follow-up*

Assay	Parameter	Pre-vaccination	6 weeks post-2 doses vOka	12–18 months follow-up
FAMA numbers of sera by titre per test group	Titre <2	61	5	16
	Titre 2	16	8	10
	Titre 4	13	15	19
	Titre 8	7	36	20
	Titre >8	3	22	8
FAMA titre/test group	Geometric mean titre	1·7	6·6	3·8
VZV-TRFIA	Geometric mean VZV IgG (mIU/ml)	71	619	301
Sensitivity	Value % (95% CI)	74·4 (57·9–87·0)	100 (95·5–100)	91·2 (80·7–97·1)
Specificity	Value % (95% CI)	93·4 (84–98·1)	60·0 (14·6–94·7)	87·5 (61·6–98·4)
PPV	Value % (95% CI)	87·9 (71·8–96·6)	97·6 (91·6–99·7)	96·3 (87·2–99·5)
NPV	Value % (95% CI)	85·1 (74·3–92·6)	100 (29·2–100)	73·7 (48·8–90·8)
gpEIA	Geometric mean VZV IgG (mIU/ml)	23	315	128
Sensitivity	Value % (95% CI)	46·2 (30·1–62·8)	84·0 (74·1–91·2)	61·4 (47·6–74)
Specificity	Value % (95% CI)	100 (94·1–100)	80·0 (28·4–99·5)	100 (79·4–100)
PPV	Value % (95% CI)	100 (81·5–100)	98·5 (92·2–100)	100 (90–100)
NPV	Value % (95% CI)	74·4 (63·6–83·4)	23·5 (6·8–49·9)	42·1 (26·3–59·2)

FAMA, Fluorescent-antibody-to-membrane-antigen; TRFIA, time-resolved fluorescence immunoassay; gpEIA, glycoprotein enzyme immunoassay; PPV, Positive predictive value; NPV, negative predictive value; CI, confidence interval.

mIU/ml at 12–18 months follow-up. The geometric mean VZV IgG levels detected by commercial gpEIA were significantly lower ($P < 0.01$) compared to VZV-TRFIA. Based on comparison of the proportions of positive results, at pre-vaccination, post-vaccination and 12–18 months follow-up there was no significant difference ($P < 0.01$) between VZV-TRFIA and FAMA. The lower results by commercial gpEIA compared to FAMA and VZV-TRFIA were reflected in the quantitative and qualitative results (Table 1) with the difference in the proportion of positive results significantly lower at each visit ($P < 0.01$).

Overall, compared to FAMA the combined sensitivity and specificity of VZV-TRFIA for detecting VZV IgG post-vaccination and at 12–18 months follow-up ($n = 159$) were 96·4% (95% CI 91·7–98·8) with a PPV of 97·0% (95% CI 92·7–99·2) and 80·9% (95% CI 58·1–94·5) with a NPV of 77·3% (95% CI 54·6–92·2), respectively. Likewise, the sensitivity and specificity of commercial gpEIA for detecting VZV IgG post-vaccination and at 12–18 months follow-up were 74·6% (95% CI 66·5–81·6) with a PPV of 99·0% (95% CI 94·8–100) and 95·2% (95% CI 76·2–99·9) with a NPV of 36·4% (95% CI 23·8–50·4), respectively.

DISCUSSION

Few laboratories have been able to replicate the reference FAMA described by Gershon and colleagues; for example, Wutzler and colleagues [22, 27] when assessing the serological detection of VZV IgG by purified glycoprotein and whole cell lysate assays used an 'in house' modification of FAMA. Unfortunately, comparative data benchmarking their modified FAMA against the reference FAMA [11, 12] methodology does not appear to have been presented. A particular challenge with FAMA is the need to read slides while cells are still in suspension which is important to retain the presentation of functional epitopes. Technical expertise, subjectivity in reading, and interpretation of results are important issues with FAMA and to address these factors, in our study, a quality control test panel of 64 sera was jointly tested by author S.P.S. at New York and both sets of results were shown to be comparable. The FAMA described in our study showed 94% agreement with the reference FAMA performed at New York. FAMA, a technically demanding technique that is not easily automatable, is not suited for screening large numbers of samples although, recently, potential automation of the FAMA has been explored using a flow cytometry-adapted assay [28]. For small numbers of sera the high sensitivity and specificity of the visualization of membrane fluorescence confirms the utility of FAMA; however, according to our knowledge, there are no laboratories in the USA where FAMA is being performed.

This study highlights a number of potential pitfalls when estimating the response of adults to vOka

Table 2. *Serological profiles of healthcare workers who initially seroconverted following two doses of vOka and then tested negative by FAMA VZV IgG at 12–18 months follow-up*

Study no.	Pre-vaccination					6 weeks post-2nd dose vOka					12–18 months follow-up				
	FAMA titre	TRFIA (mIU/ml)	TRFIA interpn	gpEIA (mIU/ml)	gpEIA interpn	FAMA titre	TRFIA (mIU/ml)	TRFIA interpn	gpEIA (mIU/ml)	gpEIA interpn	FAMA titre	TRFIA (mIU/ml)	TRFIA interpn	gpEIA (mIU/ml)	gpEIA interpn
1001	<2	130	S	11	S	8	229	P	14	S	<2	87	S	9	S
1003	<2	48	S	12	S	4	256	P	29	S	<2	101	S	43	S
1019	<2	21	S	<10	S	4	326	P	150·6	P	<2	15	S	<10	S
1025	<2	23	S	<10	S	8	322	P	173	P	<2	65	S	26	S
1035	<2	37	S	<10	S	4	370	P	169	P	<2	111	S	60	S
1041	<2	44	S	<10	S	2	232	P	62	S	<2	108	S	13	S
1046	4	397	P	n.t.	n.t.	4	2192	P	104	S	<2	687	P	59	S
1047	<2	30	S	<10	S	8	553	P	163	P	<2	118	S	48	S
1069	<2	26	S	<10	S	8	783	P	230	P	<2	144	P	29	S
1092	<2	25	S	<10	S	2	250	P	96	S	<2	48	S	<10	S
1098	<2	22	S	15	S	8	381	P	198	P	<2	47	S	59	S
1100	2	30	S	19	S	4	478	P	210	P	<2	112	S	91	S
1102	<2	17	S	18	S	n.t.	347	P	154	P	<2	126	S	49	S

FAMA, Fluorescent-antibody-to-membrane-antigen; TRFIA, time-resolved fluorescence immunoassay; gpEIA, glycoprotein enzyme immunoassay; interpn, interpretation; S, VZV IgG susceptible, P, VZV IgG positive, n.t., not tested.

vaccination. First, there is a need to reliably select adults who are truly non-immune to VZV. Selection of adult VZV susceptibles has been shown to lack specificity when using recall of a negative history of chickenpox although specificity can be improved by subsequent laboratory testing [29, 30]. The outcome of this approach is dependent on the sensitivity and specificity of laboratory test methodology used. In our study a commercial EIA using purified VZV whole cell extract was initially used to confirm VZV susceptibility in HCWs; however, 39%, 29% and 18% were VZV IgG positive by FAMA, VZV-TRFIA and commercial gpEIA, respectively. These findings underline the importance of using assays of similar sensitivity and specificity to FAMA if accurate determinations of those in need of vOka vaccination are to be made.

At 6 weeks following the second of two doses of vOka, 94·2% of HCWs tested positive for VZV IgG by FAMA. VZV-TRFIA correlated well with FAMA when applied to samples collected 6 weeks following the second dose of vOka with 100% of samples testing VZV IgG positive in both assays. The rate of seroconversion determined by FAMA was 93·7% and by VZV-TRFIA and commercial gpEIA was 95·8% and 70·8%, respectively. The rates of seroconversion detected by FAMA (93·7%) and VZV-TRFIA (95·8%) are consistent with that expected for immunocompetent adults receiving two doses of vOka 4–8 weeks apart [31].

Point cut-offs for VZV-TRFIA of VZV IgG of 93·3 mIU/ml based on mixture modelling [32] and 100 mIU/ml [25] based on protection following exposure to chickenpox have been proposed for VZV-TRFIA when applied to naturally infected populations. In this study a cut-off VZV IgG level of 100 mIU/ml has been used to grade HCWs pre-vaccination as either susceptible or immune and this approach is backed by recent guidance from Public Health England (www.phe.gov.uk, briefing note serial number 2015/067, 18 September, 2015). For VZV-susceptible individuals receiving vOka, a cut-off VZV IgG of 130 mIU/ml has been used in this study. The adoption of this cut-off is based on a previous study [24] in which avidity data was used to grade immune response to vOka as either primary or secondary and ROC analysis was used to generate a cut-off of VZV IgG of 130 mIU/ml. The FAMA data generated in this study further justifies the use of a 130 mIU/ml cut-off for VZV immunity in adults following two doses of vOka. Two important caveats

need to be applied as a consequence of the proposed adoption of the 130 mIU/ml cut-off. First, it should not be applied to children or immunocompromised adults as no data has been generated for these populations. Second, VZV-TRFIA should continue to be evaluated in vaccinees who develop breakthrough varicella after a known exposure. On the basis of the data gathered in this study we cannot recommend application for measuring response to vOka vaccination of the particular commercial gpEIA we have tested.

The immune response of adults compared to children is lower following vOka vaccination, which may be a consequence of a diminished capacity of circulating peripheral blood T lymphocytes to recognize VZV antigens after primary sensitization *in vivo* [33]. In our study, a significant reduction in VZV IgG antibody levels was seen at 12–18 months follow-up (Table 1). By FAMA, 78·1% of HCWs had VZV IgG detected compared to 74·0% determined by VZV-TRFIA and 47·9% determined by gpEIA. The seropositivity rates determined by FAMA and VZV-TRFIA are similar to those reported by Gershon and colleagues [34] who reported 94% seropositivity 1–3 months after two doses of vaccine and 70% seropositivity by FAMA 2 years after the last dose of vaccine. Long-term serological data from immunized HCWs using methods other than FAMA or Merck gpEIA are limited. Saiman and colleagues have performed a similar study [35] to ours, in which sera from HCWs were tested by FAMA, commercially available latex agglutination assay and enzyme-linked immunosorbent immunoassay (ELISA). In comparison with FAMA, they reported the sensitivity and specificity of latex agglutination to be 82% and 94%, respectively and ELISA demonstrated a sensitivity and specificity of 74% and 89%, respectively.

In our study it has been shown in HCWs that for serum samples taken pre- and post-vOka vaccination there is good correlation between VZV IgG levels measured by VZV-TRFIA and FAMA. The lack of reliable commercially produced assays for measuring VZV IgG following vOka vaccination has resulted in a reliance on 'in house' assays which may be difficult to replicate outside their host laboratories. The disappointing results for the commercial gpEIA used in this study are specific to the assay used and modification [36] or application of other commercial gpEIAs may yield better outcomes. VZV-TRFIA is automatable and the reagents used are commercially available (www.perkinelmer.co.uk). VZV-TRFIA appears a suitable alternative to FAMA for measuring post-vaccination immunity and further studies are required to confirm a long-term correlate of protection.

DECLARATION OF INTEREST

A.A.G. has acted as a consultant for GlaxoSmith Kline and Merck for which she holds an additional service contract. The remaining authors declare no conflict of interest.

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