



## Research article

# Evaluation of flow cytometry-based fluorescent antibody to membrane antigen test to measure the humoral immunity against the varicella-zoster virus

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## ABSTRACT

The fluorescent antibody to membrane antigen (FAMA) test is the gold standard for measuring the immunity induced by varicella vaccines with high sensitivity and specificity. However, certain aspects of the FAMA test, such as time consumption, non-automation, and subjective interpretation by observers using fluorescence microscopy, are obstacles to handling large amounts of samples. To overcome these hurdles, flow cytometry was adopted to analyze and compare the flow FAMA titer with the classic FAMA titer. In addition, to save time in FAMA antigen preparation and reduce lot-to-lot variation, the stability of the FAMA antigen stored in liquid nitrogen was investigated. The FAMA test was performed on sera from 229 children, and antibody titers were analyzed using fluorescence microscopy (classic FAMA) and flow cytometry (flow FAMA). For comparison, glycoprotein enzyme immunoassay (gpEIA) titer was also measured. A strong correlation was found between the flow and classic FAMA titers, and the flow FAMA and gpEIA titers, with Pearson's  $r$  of 0.9316 and 0.8588, respectively. Between the classic FAMA and gpEIA titers, the Pearson's  $r$  value was 0.8156. The positive percent agreement, negative percent agreement, and area under the curve of the flow FAMA against classic FAMA were 95.0 %, 86.8 %, and 0.909, respectively. And those of the flow FAMA against gpEIA were 80.0 %, 87.6 %, and 0.838, respectively. The FAMA antigen stored in liquid nitrogen was stable for up to 12 months. Based on the above data, flow FAMA has the potential to be used as an alternative to classic FAMA. Moreover, pre-made FAMA antigen may reduce the preparation time and lot-to-lot variation of FAMA test.

## 1. Introduction

Varicella-zoster virus (VZV) causes varicella (chickenpox) with primary infection and herpes zoster (HZ) in older or immunocompromised individuals by reactivation of the latent virus [1]. Fever and rashes are the main symptoms of varicella in children, and severe complications such as encephalitis, pneumonia, or even death can occur [2,3]. Moreover, primary varicella infections in adolescents and adults increase the risk of complications and mortality [4].

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In 1974, a live-attenuated Oka strain varicella vaccine was developed in Japan, and the vaccine strain has been used worldwide [5–7]. Another live-attenuated MAV/06 strain varicella vaccine was developed in Korea in 1994 [8]. In Korea, varicella vaccine has been used since 1988 and has been included in a national immunization program with a single-dose schedule since 2005. However, mild breakthrough infections sometimes occur in vaccinated children. Therefore, developing more effective vaccines and determining appropriate doses for protection are important. Attenuated varicella vaccine is known to induce approximately 10-fold lower immunity levels than in natural infection [9,10]. Therefore, measuring the immunogenicity induced by the varicella vaccine should be more sensitive than the diagnostic methods for the natural immunity induced by varicella infection.

The fluorescent antibody to membrane antigen (FAMA) test is a cell-based assay that uses VZV glycoproteins expressed on the VZV-infected cell surface as an antigen [11]. The FAMA test is considered the gold standard method for measuring protective antibodies against VZV with high sensitivity and specificity and has been used as a reference method to evaluate the performance of other assays [12–16]. However, the FAMA test has several disadvantages. First, it requires enormous labor and time to prepare the FAMA antigen and skillful techniques to perform the test. Second, the FAMA test results are visually observed with a fluorescence microscope; therefore, the results are subjective and cannot be expressed quantitatively. Therefore, experts are required. Third, because the FAMA test uses unfixed VZV-infected cells (FAMA antigens), these antigens are freshly prepared immediately before performing the test. Fourth, because VZV is a typical cell-associated virus, it is difficult to accurately quantify virus titration. Therefore, the outcomes of FAMA antigens may vary, even though the same ratio of normal cells to VZV-infected cells was used to prepare FAMA antigens in each experiment. These aspects are obstacles to handling numerous samples in a short period, while precluding the subjectivity of the observer. Therefore, improvements to the FAMA test are indispensable.

In this study, to avoid the subjectivity of observers to the FAMA test results, flow cytometry was adopted for analyzing the results (flow FAMA). Furthermore, to reduce the lot-to-lot variation of FAMA antigens and the time and labor required to prepare FAMA antigens, the stability of FAMA antigens stored in liquid nitrogen was evaluated for up to 12 months.

2. Materials and methods

2.1. Sera

Serum samples from 229 children were used for the FAMA and gpEIA tests (Table 1). Children’s sera were divided into two groups: Group 1 sera were obtained before and  $5 \pm 1$  weeks after varicella vaccination (Suduvax®, GC Biopharma Corp., Yongin-si, Korea) with a single dose at 12 to 15-month-olds (IRB no. YUH-13-0330-016) and comprised 36 pre- and 41 post-vaccination samples (Table 1). Group 2 sera were obtained from 152 healthy children aged 2–6 years (IRB no. PCR-09-23) (Table 1). Of these, 25 (16.4 %) experienced varicella, and 4 had varicella before 12 months (Table S1). WHO International Standard VZV Immunoglobulin (Cat. W1044, NIBSC, UK) was used as the positive control. VZV panel plasma supplied by the Korea Ministry of Food and Drug Safety (KMFDS) were used as a negative control and samples for the FAMA antigen stability test (Table 2). All samples were stored at  $-70^{\circ}\text{C}$  before use.

2.2. Ethics and consent

This study was approved by the Institutional Review Board of Yeungnam University Medical Center. IRB no. PCR-09-23 was approved on May 21, 2009, and YUH-13-0330-016 was approved on April 4, 2013. Written informed consent was obtained from the guardians of all enrolled children.

2.3. Preparation of FAMA antigen

MRC-5 (medical research council cell strain 5) cells obtained from the European Collection of Authenticated Cell Cultures (ECACC, England, RRID: CVCL2622) were cultured and infected with the VZV Ellen strain (ATCC, Manassas, USA) at a multiplicity of infection of 0.005. They were harvested when the cells showed a 70 % cytopathic effect. Freshly harvested VZV-infected cells were resuspended in Dulbecco’s phosphate-buffered saline (DPBS) and used as the FAMA antigen. For the long-term stability study of the FAMA antigen, VZV-infected cells were stored in liquid nitrogen after 24 h chilled down in freezing container at  $-70^{\circ}\text{C}$ .

Table 1  
Sera information.

Group	Status	Age (year)	Number
1	Pre-vaccinated	1	36
	<sup>a</sup> Post-vaccinated	1	41
2	Healthy children	2–6	152
Total			229

<sup>a</sup> Post-vaccination sera were obtained 4–6 weeks after varicella vaccination.

**Table 2**  
Antibody titers of varicella-zoster virus panel plasma.

Panel <sup>a</sup> No.	Classic FAMA titer <sup>b</sup>	gpEIA titer <sup>c</sup> (mIU/mL)
Panel 1	Negative	5.6
Panel 2	Negative	5.9
Panel 5	8	53.1
Panel 8	16	84.1
Panel 10	32	139.9
Panel 12	64	229.8

Abbreviations: FAMA, fluorescent antibody to membrane antigen; gpEIA, glycoprotein enzyme immunoassay.

<sup>a</sup> Varicella zoster virus panel plasma was supplied by the Korea Ministry of Food and Drug Safety.

<sup>b</sup> Classic FAMA titer was defined when the panel plasma was supplied.

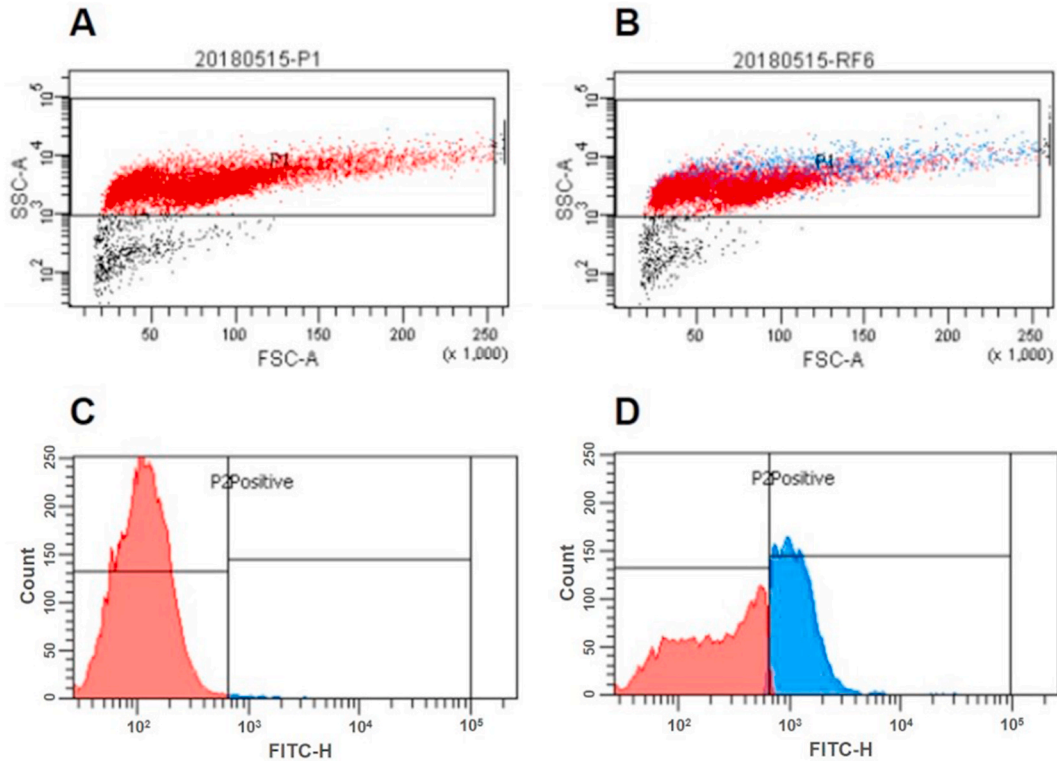
<sup>c</sup> gpEIA titer was determined using the VaccZyme VZV glycoprotein IgG low-level enzyme immunoassay kit when the panel plasma was supplied.

**2.4. FAMA reaction**

All FAMA experimental procedures were performed as previously described [11,17]. All sera were 2-fold serially diluted with DPBS. Diluted WHO International Standard VZV Ig to 7.8 mIU/mL (designated as RF6 in this experiment), VZV panel P1 (VZV negative plasma), and DPBS were used as positive, isotype, and negative controls, respectively. Two-fold serially diluted serum and control samples were used for primary antibody reactions. Alexa Fluor 488 goat anti-human IgG (Molecular Probes, New York, USA) was used as a secondary antibody.

**2.5. Classic FAMA analysis**

After the FAMA reaction, cells were smeared onto 14-well slides and air-dried. After mounting, classic FAMA results were analyzed using a fluorescence microscope. The results were graded as negative (–), weak (w), 1+, 2+, or 3+ according to the intensity of the fluorescence band structure on the cell surface [17]. The grades were determined using the following criteria: no fluorescence or



**Fig. 1.** Gating strategy for the positive cutoff of flow FAMA test. After excluding cell debris with a rectangular gate (P1) in dot plots (A, B), isotype control cutoff level (P2) was set using classic FAMA negative plasma (C). Positive cutoff level was set by the percentage of positive control RF6 (D) in histograms.

nonspecific fluorescence as negative, specific, very weak fluorescence as weak, clear fluorescence with a complete thin ring structure as 1+, bright fluorescence with a complete ring structure as 2+, and very bright fluorescence with a complete thick ring structure as 3+. The classic FAMA antibody titer was determined by the final dilution factor which represents 1+. According to our previous data, the cutoff value for a positive level of the classic FAMA antibody titer was set at 16 [17].

## 2.6. Flow cytometry-based FAMA (flow FAMA) analysis

After the FAMA reaction, the cells were analyzed using a BD FACS Canto II flow cytometer and BD FACS Diva software (BD Biosciences, USA). A total of 10,000 events were collected and analyzed for each sample. To assign a quantitative value that corresponded to humoral immunity, two gates were created: P1 gate excluded cell debris (Fig. 1A and B), and P2 gate was set by isotype control using classic FAMA-negative plasma (panel 1) (Fig. 1C). The event percentage of RF6 (which represents 1+ in the classic FAMA) was used as a positive cutoff value (Fig. 1D). The flow FAMA antibody titer was determined as the final dilution factor whose event percentage exceeded the positive cutoff value.

## 2.7. Glycoprotein enzyme immunoassay (gpEIA)

Antibody titer was measured using the VaccZyme™ VZV gpEIA low (detection limit: 10–810 mIU/mL) and screening (detection limit: 0.5–10 IU/mL) kits (Binding Site, Birmingham, UK), which measures IgG antibodies specific to viral envelope glycoproteins. Samples over the detection limit of the low kit were measured again using screening kit. Procedures were performed according to the manufacturer's instructions. Based on previous studies [17], the protective level cutoff value was set at 50 mIU/mL.

## 2.8. Statistical analysis

The linear correlations between the flow and classic FAMA titers, the flow FAMA and gpEIA titers, and classic FAMA and gpEIA titers were verified using the Pearson correlation coefficient, and the stability of the pre-made FAMA antigen was verified using repeated-measures ANOVA with GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Concordance between tests was assessed by positive percent agreement (PPA) and negative percent agreement (NPA). The receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were obtained to determine the cutoff values of the flow FAMA antibody titer using MedCalc Software version 20 (MedCalc, Belgium).

# 3. Results

## 3.1. The FAMA and gpEIA geometric mean titers (GMT)

Classic and flow FAMA antibody titers and gpEIA titer were analyzed using sera from 229 children. The classic FAMA GMT of pre- and post-vaccination G1 group were 2.1 and 47.2, respectively (Table 3). That of G2 was 18.5. The flow FAMA GMT were 3.3 and 44.9 in pre- and post-vaccination G1 group, respectively and that of G2 was 14.5 (Table 3). The gpEIA GMT of pre-vaccination G1, post-vaccination G1, and G2 groups were 14.0, 71.0, and 88.4 mIU/mL, respectively (Table 3).

## 3.2. Determination of cutoff value for flow FAMA antibody titer

To determine the cutoff value of the flow FAMA titer, the PPA, NPA, and area under the ROC curve were measured with three different cutoff levels of flow FAMA titer (8, 16, and 32) with a fixed classic FAMA cutoff of 16. When the cutoff level of the flow FAMA titer was set to 16, the PPA and NPA of flow FAMA were 95.0 % and 86.8 %, respectively (Table 4). And the area under the ROC curve were the highest such as 0.909 (95 % CI, 0.864–0.943) (Table 4). When the flow FAMA cutoff was set to 8, the PPA and NPA were 74.2 % and 100.0 %, respectively. They were 100.0 % and 74.5 %, respectively, when the cutoff value was set to 32 (Table 4). Compared to gpEIA (cutoff 50 mIU/mL), the PPA and NPA of flow FAMA were 80.0 % and 87.6 %, respectively, and the area under the ROC curve was the highest at 0.838 (95 % CI, 0.784–0.883) at the flow FAMA cutoff of 16 (Table 4). When the flow FAMA cutoff was set to 8, the PPA and NPA were 62.3 % and 97.4 %, respectively. They were 87.5 % and 79.0 %, respectively, when the cutoff value was set to 32 (Table 4). Therefore, a cutoff level of 16 was the most appropriate for flow FAMA.

**Table 3**  
The FAMA and gpEIA geometric mean titers.

Group	Status	Classic FAMA GMT	Flow FAMA GMT	gpEIA GMT (mIU/mL)
1	Pre-vaccinated	2.1 ± 1.3	3.3 ± 2.0	14.0 ± 1.8
	<sup>a</sup> Post-vaccinated	47.2 ± 2.8	44.9 ± 2.8	71.0 ± 2.2
2	Healthy children	18.5 ± 5.4	14.5 ± 4.8	88.4 ± 5.7

Abbreviations: FAMA, fluorescent antibody to membrane antigen; gpEIA, glycoprotein enzyme immunoassay; GMT, geometric mean titer.

<sup>a</sup> Post-vaccination sera were obtained 4–6 weeks after varicella vaccination.

Table 4

PPA, NPA, and AUC from different cutoff values of the flow FAMA test compared to the classic FAMA test and gpEIA.

Efficacy measurement	Cut-off of Flow FAMA compared to classic FAMA (cutoff = 16)			Cut-off of Flow FAMA compared to gpEIA (cutoff = 50mIU/mL)		
	8	16	32	8	16	32
PPA (%)	74.2	95.0	100.0	62.3	80.0	87.5
NPA (%)	100.0	86.8	74.5	97.4	87.6	79.0
AUC (95 % CI)	0.871 (0.820–0.911)	0.909 (0.864–0.943)	0.873 (0.822–0.913)	0.798 (0.741–0.848)	0.838 (0.784–0.883)	0.832 (0.778–0.878)
Youden index J <sup>a</sup>	0.7417	0.8182	0.7452	0.5969	0.6760	0.6648

Abbreviations: FAMA, fluorescent antibody to membrane antigen; gpEIA, glycoprotein enzyme immunoassay; PPA, positive percent agreement; NPA, negative percent agreement; AUC, area under the curve; CI, confidence interval.

<sup>a</sup> The cutoff value of flow FAMA was determined using the Youden index.

3.3. Concordance between classic and flow FAMA tests and between gpEIA and FAMA tests

From the above PPA, NPA and AUC results, positive cutoff value was set to 16 for flow FAMA test. In Group 1, none of the 36 pre-vaccinated sera were positive for classic FAMA. However, three of them (8.3 %) were positive for flow FAMA (Table 5). Those three pre-vaccinated samples (Nos. 11, 29, and 42) were all negative for gpEIA titer (Table 6). Among the 41 post-vaccinated sera from Group 1, 38 (92.7 %), 37 (90.2 %), and 27 (65.9 %) were positive for classic FAMA, flow FAMA, and gpEIA, respectively. In Group 2 (152 healthy 2–6-year-old children), 74 (48.7 %), 60 (39.5 %), and 68 (44.7 %) children were positive for classic FAMA, flow FAMA, and gpEIA, respectively (Table 5). Compare to classic FAMA, 21 samples among the 229 sera (five false positives and 16 false negatives) showed discrepancy in flow FAMA (Table 6). Except for three pre-vaccinated samples, the 18 discrepant samples represented 2-fold different antibody titers (Table 6). Compare to gpEIA, 14 sera (5 false positive and 9 false negative) were discrepant in flow FAMA (Table 6). The Pearson correlation coefficients between the flow and classic FAMA titers, between the flow FAMA and gpEIA titers, and between classic FAMA and gpEIA titers of the 229 sera were 0.9316 ( $p < 0.0001$ , 95 % CI, 0.9121–0.9469, Fig. 2A), 0.8588 ( $p < 0.0001$ , 95 % CI, 0.8205–0.8894, Figs. 2B), and 0.8156 ( $p < 0.0001$ , 95 % CI, 0.7670–0.8548, Fig. 2C), respectively.

3.4. Stability of pre-made FAMA antigen

The FAMA antigens were stored in liquid nitrogen for 12 months to confirm their stability during long-term storage. Classic and flow FAMA tests were performed at 0 (freshly harvested), 1, 3, 6, 9, and 12 months using VZV panel plasma (Table 2). The variation in antibody titers according to the storage period of the FAMA antigen was analyzed using repeated-measures one-way ANOVA, and there were no statistical differences ( $p > 0.05$ ). Antibody titers of the positive control RF6 were 64 in both classic (Fig. 3A) and flow FAMA (Fig. 3B) during the entire period. Moreover, the antibody titers of the negative controls (P1 and P2) were 2 in both classic and flow FAMA for up to 12 months. The other panel plasma were all stable and in the acceptable error range of the FAMA test ( $\log_2^{\pm 1}$ ) in classic FAMA up to 12 months. Although the flow FAMA titer varied in one plasma (P8), the titer did not decrease during the later period.

4. Discussion

Enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), FAMA test, and gpEIA are currently being used to detect antibodies against VZV [18]. IFA is a highly sensitive method, but it does not represent a protective antibody against glycoproteins present in the cell membrane because the cell membrane is destroyed during fixation. Although ELISA is a well-commercialized, high-throughput automated method, it also does not represent the protective antibody since it uses the entire VZV antigens to capture VZV antibodies. For these reasons, IFA and ELISA are mainly used for the diagnosis of varicella infection or for the surveillance of seroprevalence [19]. Although the gpEIA procedure is similar to that of ELISA, gpEIA uses VZV envelope glycoproteins to capture antibodies and is considered a suitable method for evaluating varicella vaccine immunogenicity [17,20–22]. The gpEIA kit developed by Merck is not commercially available [16,23]. Two gpEIA kits have been developed by Binding Site (VaccZyme VZV gpEIA kit) and Virion/Serion (SERION ELISA classic VZV IgG kit) [17], but these kits have not been fully validated for varicella vaccine evaluation. We used the VaccZyme gpEIA kit in this study, but unfortunately the VaccZyme gpEIA kit is currently no longer

Table 5

Positive rates of VZV antibody in each group when analyzed by classic and flow FAMA tests, and gpEIA.

Assay	Group 1		Group 2	Total (n = 229)
	Pre-vaccinated (n = 36)	Post-vaccinated (n = 41)	Healthy (n = 152)	
Classic FAMA <sup>a</sup>	0 (0.0 %)	38 (92.7 %)	74 (48.7 %)	112 (48.9 %)
Flow FAMA <sup>b</sup>	3 (8.3 %)	37 (90.2 %)	60 (39.5 %)	100 (43.7 %)
gpEIA <sup>c</sup>	1 (2.8 %)	27 (65.9 %)	68 (44.7 %)	96 (41.9 %)

<sup>a</sup>, <sup>b</sup> The cutoff values of both classic and flow FAMA tests were set to 16.

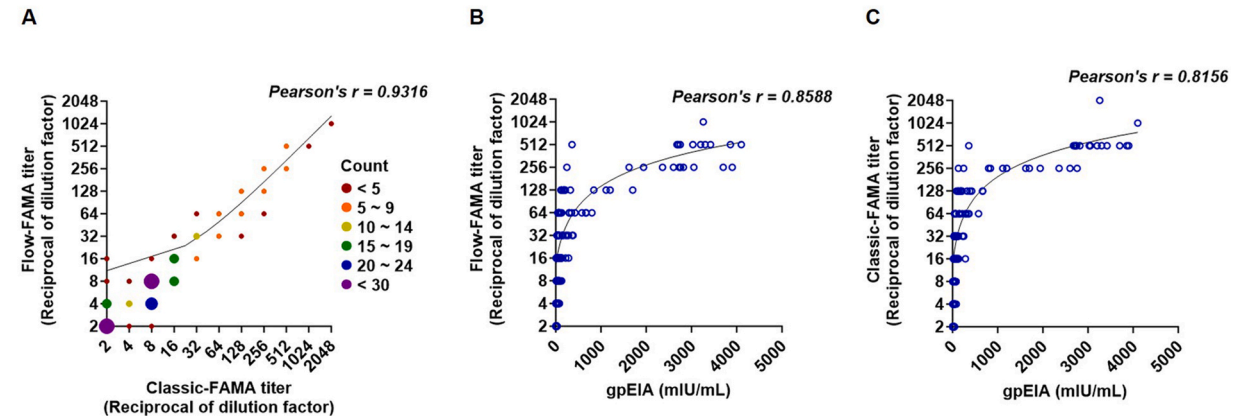
<sup>c</sup> The cutoff value of gpEIA was set to 50 mIU/mL.

Abbreviations: FAMA, fluorescent antibody to membrane antigen; gpEIA, glycoprotein enzyme immunoassay.

**Table 6**  
False positive and false negative samples of flow FAMA test.

Sera sample No.	Classic FAMA titer	Flow FAMA titer	gpEIA titer (mIU/mL)
11 (Pre-vaccinated)	2	16	13.3
29 (Pre-vaccinated)	2	16	25.9
42 (Pre-vaccinated)	2	16	9.5
81	8	16	17.2
358	8	16	44.1
22	16	8	57.9
30	16	8	87.9
43	16	8	37.2
109	16	8	25.6
116	16	8	130.2
136	16	8	38.0
159	16	8	17.0
249	16	8	45.1
251	16	8	66.3
288	16	8	94.9
293	16	8	61.3
295	16	8	59.9
341	16	8	51.1
364	16	8	48.6
367	16	8	75.2
485	16	8	32.0

Abbreviations: FAMA, fluorescent antibody to membrane antigen; gpEIA, glycoprotein enzyme immunoassay.



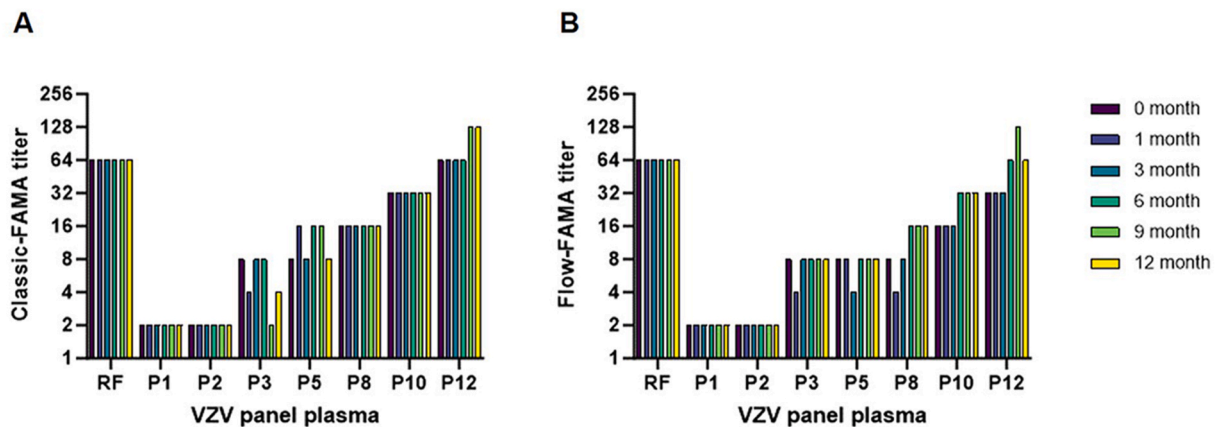
**Fig. 2.** Correlation between three different antibody measurement tests. Correlation of flow and classic FAMA titers (A). Numerical values are expressed according to the size and color of the dot (A). Correlations of flow- (B) and classic-FAMA (C) to gpEIA titers.  $r$ : Pearson's correlation coefficient. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

available.

The FAMA test is a method for measuring protective antibodies that react with VZV glycoproteins, such as gE, gB, gH, gI, and gC, on the surface of VZV-infected cells, with high sensitivity and specificity [11]. In the conventional FAMA test (classic FAMA), the results are visually observed using a fluorescence microscope. Hence, the results may depend on the subjectivity of the observer and cannot be expressed quantitatively. Despite these disadvantages, the FAMA test is considered the gold standard for evaluating the immunogenicity of varicella vaccines [16,24,25]. Therefore, in this study, flow cytometry was adopted to reduce the subjectivity of the observer and to obtain quantitative results of the FAMA test.

We observed a strong correlation between flow and classic FAMA tests (Pearson's  $r = 0.9316$ ). The AUC of flow FAMA was 0.909, which is similar to the AUC of the previously reported flow cytometry-adapted FAMA (0.9125) compared with the standard FAMA results using 62 sera by Lafer et al. [26]. They reported 95.25 % sensitivity and 80.0 % specificity for flow cytometry-adapted FAMA. In this study, the PPA and NPA of the flow FAMA were 95.0 % and 86.8 %, respectively, showing similar results. Compared to classic FAMA, 21 discrepancies among 229 sera were observed, including five false positives and 16 false negatives (Table 6). Therefore, the discrepancy rates between classic and flow FAMA were also similar in both studies by Lafer et al. and us (9.68 % and 9.17 %, respectively). Three of the five false-positive samples were pre-vaccinated, and two were healthy sera. The classic FAMA titers of the three pre-vaccinated sera were all 2 and gpEIA titers were also negative level (Table 6). The appearance of the four of five false-positive sera was cloudy. Although examining cloudy samples by classic FAMA is very difficult, FAMA experts can distinguish haziness and positive signal. However, flow cytometry recognizes haziness as a positive signal, leading to false positives. Therefore, despite the high





**Fig. 3.** Stability of pre-made FAMA antigen was analyzed using classic (A) and flow (B) FAMA tests. FAMA antigens were stored for 0, 1, 3, 6, 9, and 12 months. WHO International Standard VZV Immunoglobulin diluted to 500 mIU (RF) and VZV panel plasma were used for primary antibodies. The titers of all samples were compared to those in the 0 month.

PPA (95.0 %) of flow FAMA, the NPA (86.8) of flow FAMA was low when cloudy negative sera were included. The classic FAMA titers of 16 false-negative samples were all 16 (cutoff level); in contrast, the flow FAMA titers were all 8. Since the FAMA test used 2-fold serially diluted serum, the results of classic FAMA titer 16 and flow FAMA titer 8 for the same sample are within the acceptable error range of  $\log_2^{\pm 1}$ . However, the FAMA titer 8 and 16 are important criteria that distinguish between positive and negative, additional research will be necessary for setting equivocal range.

Since VZV is not released into the culture media as a cell-free virus, this cell-associated property leads to frailty in the FAMA test. During FAMA antigen preparation, VZV-infected MRC-5 cells are inoculated into normal MRC-5 monolayer, and the number of virus particles inside the infected cells is not the same. Thus, variations in infectivity might occur in each experiment, which could affect the antibody titer in the FAMA test. In this study, to reduce the variation in infectivity and the labor and time required for FAMA antigen preparation, FAMA antigen were prepared and stored in liquid nitrogen for up to 12 months to evaluate their long-term stability. The FAMA titers obtained using the same lot of pre-made FAMA antigens were stable for up to 12 months (within  $\log_2^{\pm 1}$ ) in classic FAMA. However, there were certain variations in the flow FAMA titers in two panel plasma. Notwithstanding, the flow FAMA titers did not decrease until 12 months. Consequently, the variation in infectivity, the labor and time required for FAMA antigen preparation can be reduced by using FAMA antigens stored in nitrogen for up to 12 months in the classic FAMA test.

Based on these results, the flow FAMA test can be an alternative to the classic FAMA test. Unlike the classic FAMA test, which requires expertise in the analysis process, flow FAMA using flow cytometry can be accessible to beginners. Another advantage is that it quantifies the results to eliminate subjectivity and increase their reliability. However, flow FAMA test has certain weaknesses in discriminating between haziness and positive signal when using cloudy negative samples. Long-term storage of the pre-made FAMA antigen is available for FAMA analysis, which saves time in preparing the FAMA antigen and reduces lot-to-lot variation. We believe further improvements in flow FAMA test can make it a useful tool for evaluating the immunogenicity of varicella vaccines. There is a limitation regarding the cellular immune response. Only sera were obtained from all subjects, no cellular immunity were evaluated in this study.

#### Institutional Review Board statement

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Yeungnam University Medical Center (IRB no. YUH-13-0330-016, IRB no. PCR-09-23).

#### Declaration of ethics statement

This study was reviewed and approved by the Institutional Review Board of Yeungnam University Medical Center, with the approval numbers: YUH-13-0330-016 and PCR-09-23. Written informed consent was obtained from the guardians of all enrolled children.

#### Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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## CRediT authorship contribution statement

**Junmo Lim:** Writing – original draft, Validation, Methodology, Formal analysis. **Yunhwa Kim:** Writing – review & editing, Visualization, Resources, Methodology, Formal analysis. **Ji Young Hwang:** Visualization, Resources, Formal analysis. **Kyung Min Lee:** Resources, Formal analysis. **Hosun Park:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Abbreviation table

FAMA	fluorescent antibody to membrane antigen
gpEIA	glycoprotein enzyme immunoassay
PPA	positive percent agreement
NPA	negative percent agreement
AUC	area under the curve
VZV	varicella-zoster virus
HZ	herpes zoster
MRC-5	medical research council cell strain 5
DPBS	Dulbecco's phosphate-buffered saline
ROC	receiver operating characteristic
CI	confidence interval
ELISA	enzyme-linked immunosorbent assay
IFA	immunofluorescence assay
GMT	geometric mean titer

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e36614>.

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