Establishment of a high throughput fluorescent antibody to membrane antigen assay to measure the humoral immunity against the varicella-zoster virus

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Abstract/ Introduction

Varicella-zoster virus (VZV) is a highly contagious alpha-herpesvirus that infects more than 90% of people worldwide. The primary infection with VZV results in Chickenpox (varicella), predominantly affecting children. Reactivation of latent VZV leads to shingles (herpes zoster), which mainly occurs in people aged 50 years and above and is often accompanied with intense neuralgia. It is estimated that about one third of those who have had chickenpox will develop shingles. To date, there is still no specific cure for VZV-induced diseases, thus VZV infection is still prevalent and accounts for a significant disease burden worldwide.

Vaccination is among the most cost-effective ways for preventing chickenpox and shingles. Therefore, measuring the specific immunogenicity induced by the varicella vaccine is very crucial. 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. The FAMA test is regarded as the gold standard for measuring protective antibodies against VZV due to its high sensitivity and specificity and serves as a reference method to evaluate the performance of other assays.

However, the standard FAMA procedure is semi-quantitative, low-throughput, and labor-intensive, and requires subjective evaluation by trained technicians, which limits its widespread application. To overcome the disadvantages, expand the application, and avoid the subjectivity of observers to the FAMA test, a high content screening (HCS) based FAMA assay has been developed. 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 has also been evaluated.

Method / Experimental design

Preparation of FAMA antigen: MRC-5 cells were cultured and infected with the VZV Ellen strain at a multiplicity of infection of 0.005. They were harvested when the cells showed a 70% to 80% 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 or the fixed slides/plates were stored at − 70 ◦C.

FAMA assay: VZV-infected cells were fixed to 12-well slides or 96-well plate with cold acetone for 15 to 30 minutes and washed 3 times using phosphate-buffered saline (PBS). Collected sera were serially diluted, mixed with VZV-infected cells, incubated for 30 minutes at 37°C, and washed 3 times using PBS. A secondary antibody, goat Anti-Human IgG H&L (FITC), was added to mixtures, incubated for 30-40 minutes at 37°C, and washed 3 times using PBS. Assessment for a fluorescent ring was analyzed using a fluorescence microscope or using the HCS operetta.

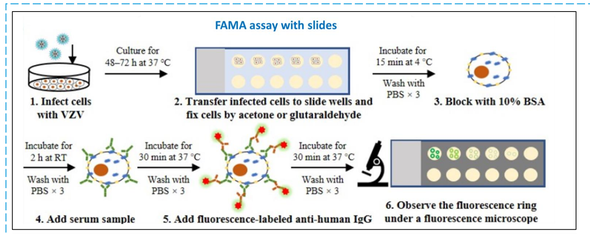

Figure 1. Schematic of the process of FAMA assay with classic slides

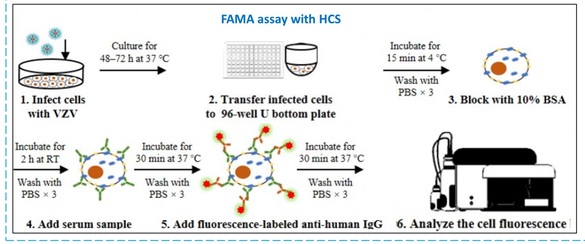

Figure 2. Schematic of the process of FAMA assay with HCS

Results

1. Establish the FAMA assay with both classic slides and HCS methods. FAMA assay aims to determine the presence of antibodies specific to viral proteins that distribute on the surface of VZV-infected cells, which correlate with protection from disease. When the viral proteins (e.g., VZV glycoproteins) distributed on the surface of VZV infected cells bind to their specific antibodies in the serum samples, ring-like fluorescence patterns are typically observed. The highest dilution that can still cause a positive ring-like fluorescence reaction is taken as the FAMA titer of the serum sample. As shown in Fig. 3, the ring-like fluorescence can be observed in both the slides and HCS method, while there was no ring-like circle in the negative control.

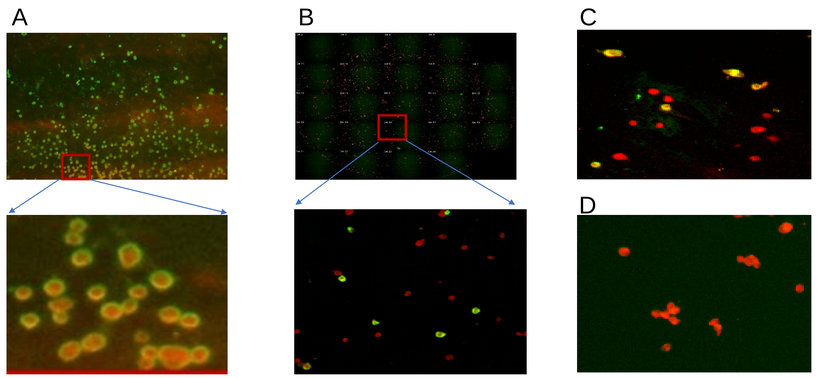

Figure 3. Fluorescence in FAMA assay. (A) Fluorescence picture under microscope analysis. (B) Fluorescence picture with HCS. (C) Positive control. (D) Negative control.

2. Concordance between classic slides and HCS methods. To compare the concordance of the two methods, several human serum samples were subjected to both methods, and the FAMA titer was consistent between the two methods as the variation was below one-step dilution as shown in Fig. 4A.

To reduce the variation in infectivity and the labor and time required for FAMA antigen preparation, FAMA antigen were prepared and stored at − 70 ◦C to evaluate their long-term stability. As shown in Fig. 4B, the FAMA titer was stable for both the two methods, and the longer stability will be further analyzed.

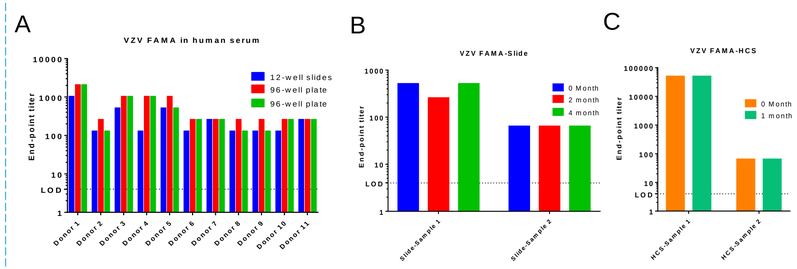

Figure 4. Comparison between classic slides and HCS measurement tests. (A) VZV positive human serum were used for FAMA analysis. (B&C) Stability of pre-made FAMA antigen was analyzed using slide and HCS tests.

Conclusions/ Summary

We aim to develop a versatile, high throughput fluorescent antibody to membrane antigen assay to evaluate the humoral immunity of VZV. Based on these results, the HCS FAMA test can be an alternative to the classic FAMA test. Unlike the classic FAMA test, which requires expertise in the analysis process, HCS FAMA quantifies the results to eliminate subjectivity and increase their reliability. 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 HCS FAMA test can make it a useful tool for evaluating the immunogenicity of VZV.

References

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