

A HIGH-CONTENT IMAGING-BASED TECHNIQUE FOR DETECTING NEUTRALIZING ANTIBODIES IN SARS-COV-2 INFECTION

Mariana ULINICI

Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova

Corresponding author: Mariana Ulinici, e-mail: mariana.ulinici@usmf.md

Keywords:

HEK293/ACE2 cells, Pseudotyped SARS-CoV-2 lentivirus, GFP protein, neutralising antibodies, serum, plasma, DAPI staining, immune response, vaccines, therapies.

Introduction. The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had a profound impact on global health, with millions of confirmed cases and deaths worldwide. Conventional methods for detecting neutralizing antibodies rely on laborious and time-consuming plaque reduction neutralization tests (PRNT), which require live virus and biosafety level 3 (BSL-3) facilities. Alternative methods have been developed, such as ELISA and pseudovirus neutralization assays, but these methods also have limitations regarding sensitivity and specificity. Therefore, there is a need for more efficient and sensitive techniques to detect neutralizing antibodies in SARS-CoV-2 infection.

Aim. This study aimed to develop and evaluate a high-content imaging-based technique for detecting neutralizing antibodies in SARS-CoV-2 infection.

Material and methods. In this experiment, the Huh7 - hACE2 cells were infected with the pseudotyped SARS-CoV-2 lentivirus expressing GFP protein and then incubated with serum or plasma from individuals who had been infected with the virus or vaccinated against it. If the serum or plasma contained neutralizing antibodies, they would bind to the spike protein of the virus and prevent it from entering the cells, resulting in a lower number of GFP-expressing cells. The cells were then stained with DAPI, a fluorescent dye that binds to DNA and allows the visualization of cell nuclei. The number of GFP-expressing cells, the intensity of DAPI staining in each cell, and the presence and levels of neutralizing antibodies in the serum or plasma samples were determined using high-content imaging microscopy (Operetta). The images were analysed using Columbus Image Data Storage and Analysis System to quantify the number of cells infected with the pseudotyped SARS-CoV-2 lentivirus expressing GFP protein.

Results. We evaluated the performance of the high-content imaging-based technique by testing serum or plasma samples from 50 individuals with confirmed SARS-CoV-2 infection and 50 individuals who were not infected with the virus. The results showed that the technique had a sensitivity of 92% and a specificity of 98%, indicating high accuracy in detecting neutralizing antibodies. The technique also allowed us to quantify the levels of neutralizing antibodies in the samples, which varied among individuals.

Conclusions. This technique has the potential to provide a more accurate and efficient way of identifying individuals with neutralizing antibodies, which could be crucial for vaccine development and epidemiological studies. The technique offers several advantages over conventional methods, including high sensitivity and specificity. It also allows for the simultaneous detection of multiple types of antibodies, providing a more comprehensive understanding of the immune response to SARS-CoV-2 and the potential for high throughput. The development of this technique has important implications for understanding immune responses to SARS-CoV-2, developing effective vaccines and therapies, and ultimately controlling the spread of COVID-19.