Cell Acquisition Method Validation by Flow Cytometry for MCTI CIMATEC HDT RNA Vaccine Immunogenicity Evaluation Against SARS-CoV-2

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The COVID-19 pandemic highlighted the importance of developing and improving techniques that can help diagnose and evaluate the disease's immunological response. Standardizing methods is necessary to ensure the confidence and reproducibility of the results. Therefore, the objective of this work was to carry out a concurrent validation to compare the reference methods for acquiring data used in the evaluation of the immunogenicity of the Vaccine. RNA MCTI CIMATEC HDT, performed by flow cytometry. From the analysis of the cellular profile through the evaluation of cell size and complexity and expression of immunophenotypic markers, no significant differences were found, demonstrating that the methodology used in the BD FacsMelody cytometer at SENAI/ CIMATEC is suitable for obtaining the results.

Keywords: Flow Cytometry. Validation. COVID-19.

Introduction

According to the World Health Organization, the coronavirus pandemic caused by SARS-CoV-2 was responsible for the deaths of more than six million people around the world [1]. Under these circumstances, there was a significant investment in developing diagnostic tests capable of detecting possible infection by the virus and an escalation in the production of vaccines that would reduce the risk of complications and the mortality rate [2,3].

Several techniques can be used to quantify immunological responses to vaccines, including flow cytometry, which consists of quantifying cells marked with antibodies coupled to a specific fluorochrome for a marker of interest [4]. During this pandemic, this technique gained more space, exerting a strong influence on published data [5]. This fact demonstrates the need to ensure that operational procedures can guarantee data robustness and reproducibility. The preparation and storage of samples, the calibration of equipment, and the standardization and optimization of the protocols used help to improve processes, ensuring better performance of functions and contributing to more excellent reliability of the data generated.

Therefore, this work aimed to validate the reference methods for data acquisition used in the immunogenicity assessment carried out by flow cytometry of the MCTI CIMATEC HDT RNA Vaccine.

Materials and Methods

A concurrent validation was carried out, which refers to a combination of retrospective and prospective validation applied in the case of a process similar to another previously validated.

Blood samples were collected and subjected to the density gradient separation technique (Ficoll-Hypaque, Histopaque/Sigma-Aldrich) to obtain peripheral blood mononuclear cells (PBMC) through centrifugation, which were subsequently cryopreserved until the use. The cells were cultivated in 96-well plates under the following conditions:

1. Only with RPMI-1640 culture medium (Gibco®- Thermo Fisher Scientific);
2. Stimulated with phytohemagglutinin (PHA - Phytohemagglutinin) as positive control at concentrations of 5 µg/mL and 10 µg/mL;
3. With Spike, a SARS-COV-2-specific protein, at a concentration of 2.5 µg/mL.

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The cells were incubated in the culture oven for 24h at 37°C and 5% CO_2, and four hours before the end, monensin and brefeldin were added to all wells. We used surface marker antibodies to perform cell labeling (CD3 - APC-H7, CD4 - Krome Orange, CD8 - PerCP-Cy 5.5, CD107a - FITC, CD69 - BV786, CD137 - PE, CD40 - PE-Cy7, CD19 - FITC, CD30 - BV421). Cell permeabilization was performed to label intracellular antibodies (IL-2 - BV421, TNF-α - BV750, IFN-γ - PE-Cy7, IL-5 - PE, IL-13 - APC, Granzyme B - Alexa Fluor 647) and then fixing. Several washing steps were performed, and the cells were subsequently maintained in 1X PBS until acquisition on the cytometers.

As an evaluation criterion, analyses carried out on the BD LSRFortessa flow cytometer from FIOCRUZ-Ba were used, comparing those carried out by the BD FACSMelody cytometer from SENAI/CIMATEC, evaluating the cellular profile (size and granularity) as well as the frequency of expression of immunophenotypic markers. The acceptable variation is ± 5% as a criterion for accepting values.

Results and Discussion

During the coronavirus pandemic, the need to develop and improve techniques to assist in the rapid and accurate diagnosis of immunological responses to vaccination became even more evident. The standardization of experiments is necessary to eliminate the possibility of variables that could compromise the results, making the technique reproducible and reliable [6]. In addition, validating a process or equipment certifies that the result found is what was expected [7].

Based on the results found in the analysis of the cellular profile through the evaluation of cellular size and complexity and expression of immunophenotypic markers from the data acquired on the two cytometers LSFortessa from FIOCRUZ-Ba and the BD FacsMelody from SENAI/CIMATEC, it was verified that there are no significant differences between the data, thus indicating that the methodology used to acquire data on the SENAI/CIMATEC BD FacsMelody cytometer is suitable and can continue to obtain the results (Figure 1).

Establishing a standard operating procedure for sample preparation and data acquisition improves results by ensuring that possible discrepancies are not due to erroneous procedures that could cause deviations in results [7,8]. In this way, validation provides robustness and effectiveness of the results generated, ensuring the quality of the process.

Final Considerations

This study provided that the method used to acquire data on the SENAI/CIMATEC BD FacsMelody cytometer can continue to be carried out since, in the validation process, it was considered suitable, the previously validated method.

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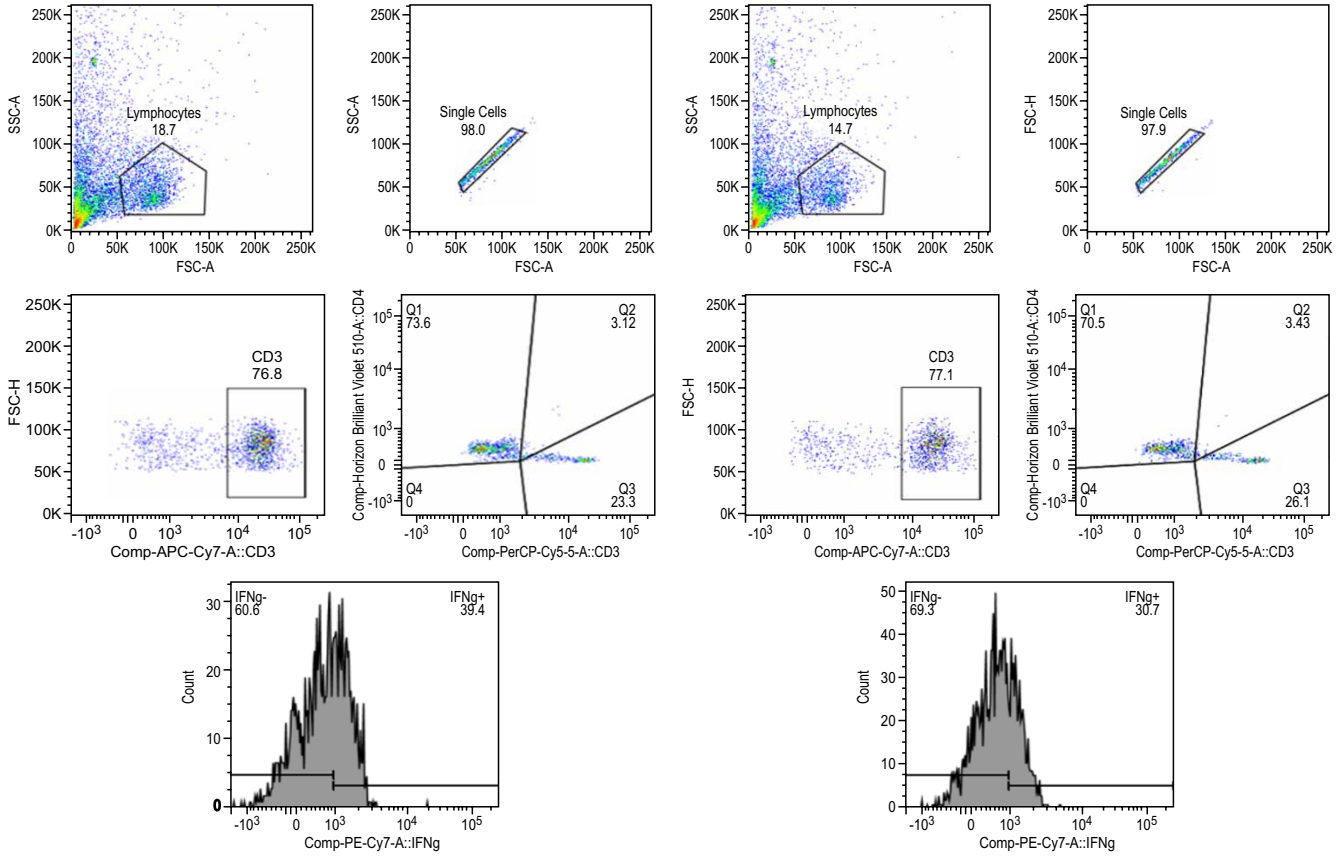
Figure 1. Analysis of the cellular profile on the LSRFortessa (FIOCRUZ-Ba) and BD FACSMelody (SENAI/CIMATEC) cytometers.



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