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BIOANALYTICAL TOOLS FOR THE QUANTIFICATION OF IN-PROCESS AND FINAL PRODUCT SAMPLES OF A ROTAVIRUS VACCINE

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Rotavirus A infection is a global leading cause of severe acute gastroenteritis associated with most of life-threatening diarrhoea episodes in infants and young children. However, the disease burden is being reduced namely due to wider access to rotavirus vaccines and their inclusion into National Immunization Programs of several countries. Besides being live-saving, rotavirus vaccination has an important economic impact, being cost-effective or even cost-saving in some countries. Nevertheless, vaccines are not yet routinely available in all countries. There is a demand to expand the rotavirus vaccination programs and improved analytical tools for high-throughput in-process product quality control and vaccine manufacturing will be important in this context. Moreover, this can be also useful for current marketed vaccines to support their life cycle.

In this work, we present the development of two analytical tools for the quantification of rotavirus particles contained in a licensed vaccine. In-process and drug product samples were evaluated using biolayer interferometry analysis, applied on an Octet platform, and flow cytometry, respectively.

On the Octet, the binding of rotavirus proteins to distinct glycoconjugate receptors and a monoclonal antibody was explored. The quantification is based on the binding response of rotavirus to coated biosensors. The best approach was achieved using the antibody and presented a linear response range within $2.5x10^6$ to $1.6x10^8$ rotavirus particles.mL^{-1}, and a limit of detection (LOD) and limit of quantification (LOQ) of $2.5\ x10^6$ and $7.5x10^6$ rotavirus particles.mL^{-1}, respectively. Moreover, the binding specificity to the biologically active form of rotavirus particles was also confirmed by evaluating the binding to free VP7 protein, which was neglectable. Method suitability for quantification of in-process samples was validated using samples from different stages of the vaccine manufacturing process. Rotavirus titers obtained using this octet approach were comparable with the approved CCID(50) method. This cell-free based method enables a fast and high throughput analysis, compatible with time constraints during bioprocess development.

The flow cytometry-based immuno-titration assay established enabled the rapid and accurate determination of rotavirus infectious units (IU) in drug product samples. This titration method counts infected cells by measuring the expression of viral protein VP7 in individual MA104 cells after staining with a specific antibody. The titers can be quantified starting from $1.42x10^4$ IU.mL^{-1}. Assay development revealed that the seeding time before infection, sample dilution and post-infection time at which viral protein expression is evaluated, are critical parameters for the precision, robustness, and accuracy of IU titer determination. Results were also validated in parallel by CCID(50) assay which generated similar titers when testing different vaccine formulations.

Overall, the methods described here present many advantages over standard virus titration approaches, such as CCID(50) or plaque assay, namely due to the high throughput capacity, rapidness, accuracy and reproducibility. Ultimately, both have the potential to have a high impact on bioprocess development and batch release of rotavirus vaccines. Moreover, the developed tools may be adapted to other viral particles-based drug products.