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AVDTIG Spiking Study 4 - Evaluation of Long-Read Sequencing for Adventitious Virus Detection in a Low Complexity Viral Background

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The ICH Q5A(R2) revision recognizes High-Throughput Sequencing (HTS, also known as Next Generation Sequencing) as an alternative method to some of the classical safety assays for adventitious virus detection in biologics. Most HTS applications for viral safety have focused on using short-read sequencing technologies, while there is limited experience of the performance of long-read sequencing. To address this gap, in 2020, the Advanced Virus Detection Technologies Interest Group (AVDTIG), sponsored by the Parenteral Drug Association (PDA), launched an international collaborative study referred to as "AVDTIG Spiking Study 4". This study involved twelve laboratories from regulatory agencies and national institutes, biologics manufacturers, academia, and contract research organizations.

The main aim of the study was to evaluate the application of long-read sequencing to detect adventitious viruses in a high viral titer background. The experimental design mimicked a virus seed or a viral vector, and was similar to a previous short-read sequencing study ("AVDTIG Spiking Study 2B"). Samples were spiked with 5 viruses developed by CBER and adopted by the WHO as International Reference Reagents for adventitious virus detection by HTS. Each participant tested at least 2 spike levels of the 5 model viruses in a fixed amount of Human adenovirus 5 (109 GC in 200µL) and the non-spiked adenovirus background, using a common protocol for virus dilutions and spiking levels. To process and sequence samples, each participating lab applied either its individual, or a common protocol shared within the team. Data analysis was performed by individual bioinformatic workflows using both targeted and non-targeted approaches.

The study will present preliminary results, share overall findings from the targeted bioinformatic analyses, and provide an initial assessment of the long read sequencing performances. The findings will offer insight into the ability of long-read HTS to detect viral contamination across different viral species.