



International Alliance for  
Biological Standardization

## 4th Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animal

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### Comparison of Non-Targeted and Broad-Spectrum Targeted NGS for Adventitious Virus Detection

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#### Introduction

Next generation sequencing (NGS) is a promising method to detect contamination by known and novel viruses.. Each step of the workflow can introduce a bias on the breadth of detection and impact the Limit of Detection ( LoD). We present a comparison of the LoD and breadth of detection using a non-targeted and targeted (VirCapSeq-VERT) approach. The VirCapSeq-VERT system is based on a comprehensive library of oligonucleotide probes that have been designed to bind and capture sequences to all known and novel vertebrate viruses.

#### Objectives

To compare the LoD and breadth of detection between the non-targeted mNGS approach and the VirCapSeq-VERT system.

#### Material and Methods

To determine the LoD and breadth of detection, we tested serial dilutions ( $10^6$  to  $10^1$  GE/ml) of the WHO international Reference Panel in a background of Ad5 DNA ( $10^9$  GE/ml) and pooled human plasma matrix.. For the targeted approach, viral nucleic acids were captured by hybridization after library preparation and re-amplified before sequencing.

To verify the breadth of detection we spiked 21 virus species at different TCID<sub>50</sub> in pooled human plasma.

Sequencing was performed using Illumina, with an average of 100, and 17 million reads per sample for non-targeted and targeted respectively. Bioinformatics were performed using PEI pipeline, and targeted analysis.

#### Results

Both systems were able to detect all the viruses of the WHO panel. Targeted approach exhibited lower LoD and longer coverage. Both approaches detected the 21 viruses from the PEI collection.





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### Conclusion

The use of a broad-spectrum targeted approach like the hybridization/capture VirCapSeq-VERT allows an increase of LoD between 100-1000 folds without affecting the breadth of detection. The use of these approaches can reduce the cost and time of sequencing. These advantages will allow a wider use of this technology in the interest of adventitious virus detection and public health.

