



International Alliance for
Biological Standardization

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

Frankfurt, Germany
December 3-5, 2024

Head-to-head comparison of NGS with in vivo animal assays and in vitro cell culture assays for adventitious virus detection

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This study has evaluated next generation sequencing (NGS) as an alternative method for adventitious virus detection by conducting a head-to-head comparison with the compendial in vivo animal assays and in vitro cell culture assays. Two viruses were selected from the WHO reference virus reagents, with distinct physical, chemical, and genomic properties, such that at least one was expected to produce a positive result in each of the different test assays. Based on infectious titer and genome copy number, different virus levels were spiked in CHO unprocessed bulk material to determine LOD in a complex biological matrix. To ensure a direct comparison of the different assays using the identical sample materials, spiked and unspiked samples were generated, blinded, aliquoted, stored at -80°C and distributed to each of the participating labs. Six spiked samples were selected based on pre-study results from ddPCR and in vitro cell culture assays. Two labs conducted the in vitro assays and three labs performed NGS. To reduce the number of animals used in the study, only one lab conducted the in vivo assays. Each lab followed their own workflow and assay protocols. Study results will be presented supporting the ICH Q5A(R2) guideline for considering NGS as an alternative method for broad adventitious virus detection to replace or supplement the conventional in vivo and in vitro adventitious virus detection assays.

This project (PC3.1-305) was supported by The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) by the Bill & Melinda Gates Foundation through funding from the Bill & Melinda Gates Foundation.