



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

### **Matrix effects on Limit of Detection for NGS-based Adventitious Virus Detection Assays**

A matrix specific evaluation of limit of detection for NGS-based Adventitious virus assays is outlined within the regulatory documents, most notable ICH Q5A and Ph Eur 2.6.41 DRAFT. This is typically accomplished by spiking well-characterized viruses into the sample matrix at varying concentrations to assess detectability of viruses with different physiochemical properties. The information gained from spiking studies allows the sponsor to model the specific limit of detection for the unique sample matrix and determine assay suitability through risk assessment.

Unlike other Molecular methods like PCR, a typical NGS assay does not employ the use of an exponential amplification step to boost low level signals to a detectable level above background. Instead, the NGS based approach generates the genomic profile of all nucleic acids present in the test sample, without distinguishing between background, product or adventitious agents. This means that sample matrices rich in extraneous nucleic acid composition (bulk harvest samples) may have challenges achieving a specific or suitable Limit of Detection (LOD) and LODs between different sample matrices may vary widely depending on the characteristics of the product and the matrix.

One advantage of a CRO is being able to assay many different products. In fact, limits of detection for NGS-based assays are relatively consistent across the industry but do vary between different matrix types. But to what extent and is there truly a universal representative matrix? This presentation will focus on the CRO experience in assessment of limits of detection in a wide variety of matrices. Data will be presented- demonstrating the need for LOD assessments for each different type of product. Along those lines, how can we apply the concept of "prior knowledge" when assessing suitability of a particular sample type and potentially apply this to platform-based technologies to expedite the use of NGS technology in testing.

