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Sensitive Viral Safety Testing for Adventitious Virus Detection with the QIAseq xHYB MAP/HAP/RAP Panel

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Introduction

Products of biological origin, such as cytokines, monoclonal antibodies and subunit vaccines, as well as genetically engineered viral vectors and viral vector products, must undergo viral safety evaluation, including adventitious agent testing to detect unintended viral contaminants and viral clearance studies to demonstrate that the manufacturing process can remove or inactivate viruses. This has traditionally been tested through various methods, such as in vitro assays utilizing indicator cell lines or in vivo assays, such as antibody production tests against the viral contaminant. However, these approaches can be time-consuming, exhibit reduced sensitivity or require specialized conditions depending on the contaminant (1). Also, these methods are in conflict with the principles of the 3 R's (reduce, refine or replace).

There are more modern approaches that apply molecular biology methods for adventitious agent testing, such as qPCR/dPCR and metagenomic sequencing. While qPCR/dPCR exhibit high sensitivity, they are limited in that each target requires a separate design, which may need to be updated periodically as circulating variants change. Depending on the library prep method, metagenomic sequencing can identify all nucleic acids within a sample. This is done by cDNA synthesis, randomly fragmenting RNA/DNA, ligation of adapters, library amplification and sequencing. However, this approach can be limited in sensitivity unless ultra-deep sequencing is performed, which is associated with increased cost per sample.

Targeted sequencing offers higher sensitivity with fewer reads than shotgun, and at a lower cost per sample, since more samples can be added per sequencing run. Hybrid capture is especially useful for viral detection due to the tolerance of the probes to mismatches. Therefore, it can enrich for non-annotated variants without requiring constant updates to probe designs.

ICH MAP/RAP/HAP

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) released a set of guidelines (2) with the aim to harmonize the regulatory expectations across regions worldwide. With the adoption of these guidelines, the benefits include safe, effective, high-quality medicines for patients and the industry as a whole. Part of the guidelines are virus-screened in rodent antibody production assays, which are divided into three groups: Mouse Antibody Production (MAP) test, Rat Antibody Production (RAP) test and Hamster Antibody Production (HAP) test. Below are the targets for each test, on which the QIAseq MAP/RAP/HAP panel is based.

Table 1. Viral targets included in the QIAseq xHYB MAP/HAP/RAP panel based on ICH MAP, RAP and HAP guidelines

MAP viruses	Taxon ID	NCBI name
Ectromelia Virus	12643	Ectromelia virus
Hantaan Virus	3052480	Orthohantavirus hantanense
K Virus (Kilham rat virus)	12441	Kilham rat virus
Lactate Dehydrogenase Virus (LDM)	11048	Lactate dehydrogenase-elevating virus
Lymphocytic Choriomeningitis Virus (LCMV)	3052303	Mammarenavirus choriomeningitidis
Minute Virus of Mice	10794	Minute virus of mice
Mouse Adenovirus (MAV)	10530 931972 573199	Murine adenovirus 1 Murine adenovirus 2 Murine adenovirus 3
Mouse Cytomegalovirus (MCMV)	10366	Murid betaherpesvirus 1
Mouse Encephalomyelitis Virus (Theilers, GDVII)	12127	Theiler's murine encephalomyeltits virus GDVII
Mouse Hepatitis Virus (MHV)	11138	Murine hepatitis virus
Mouse Rotavirus (EDIM)	70865	Murine rotavirus EDIM
Pneumonia Virus of Mice (PVM)	11263	Murine pneumonia virus
Polyoma Virus	1561702 1578134 1561703 1561704 1561705 1891767	Bovine polyomavirus 1 Bovine polyomavirus 2 Bovine polyomavirus 2a Bovine polyomavirus 2b Bovine polyomavirus 3 Betapolyomavirus macacae
Reovirus Type 3 (Reo3)	538123	Mammalian orthoreovirus 3
Sendai Virus (SeV)	3052731	Respirovirus muris
Thymic Virus (TV)	2419785	Murid herpesvirus 3
RAP viruses	**Taxon ID**	**NCBI name**
Hantaan Virus	3052480	Orthohantavirus hantanense
Kilham Rat Virus (KRV)	12441	Kilham rat virus
Mouse Encaphalomyelitis Virus (Theilers, GDVII)	12127	Theiler's murine encephalomyeltits virus GDVII
Pneumonia Virus of Mice (PVM)	11263	Murine pneumonia virus
Rat Coronavirus	31632	Rat coronavirus
Reovirus Type 3 (Reo3)	538123	Mammalian orthoreovirus 3
Sendai Virus	3052731	Respirovirus muris
Sialoacryoadenitis Virus (SDAV)	92931	Rat sialodacryoadenitis coronavirus
Toolan's H-1 Virus	10799	H-1 parvovirus
HAP viruses	**Taxon ID**	**NCBI name**
Lymphocytic Choriomeningitis Virus (LCM)	3052303	Mammarenavirus choriomeningitidis
Pneumonia Virus of Mice (PVM)	11263	Murine pneumonia virus
Reovirus Type 3 (Reo3)	538123	Mammalian orthoreovirus 3
Sendai Virus	3052731	Respirovirus muris
(Simian virus 5) SV5	2560580	Mammalian orthorubulavirus 5

Material and methods

Probes were designed according to the ICH guidelines for MAP/HAP/RAP using the proprietary standardized panel design of QIAGEN for viral genotyping, which utilizes all complete viral genomes in the NCBI Virus database. This allows a comprehensive design that takes into account viral genome diversity. Additional probes targeting Human respirovirus 1 (human parainfluenza virus 1) and Human adenovirus 2 were included as positive controls for the library construction and hybrid capture workflow when these viral targets are spiked in.

Total nucleic acid was extracted from cultured Jurkat cells using QIAGEN AllPrep DNA/RNA Mini Kit following the manufacturer's instructions. Viral nucleic acid from Human adenovirus 2 (VR-846DQ), Human parainfluenza virus 1 (VR-94D), Reovirus 3 (VR-824DQ) and Sendai virus (VR-907DQ) were obtained from ATCC. The copy number for each virus was determined by the QIAGEN dPCR Microbial DNA Detection Assay. Each viral nucleic acid was spiked into Jurkat total nucleic acid at concentrations ranging from 10 to 10,000 copies per 50 ng of Jurkat total nucleic acid.

Library construction and hybrid capture targeted enrichment followed the QIAseq xHYB Viral and Bacterial Panel Handbook. Briefly, total nucleic acid was reverse-transcribed, and second-strand synthesis was performed to generate double-stranded cDNA. This was purified with QIAseq beads. Libraries from purified double-stranded cDNA and genomic DNA were constructed using the QIAseq FX library construction kit, which utilizes UDIs. After size selection with QIAseq beads, four libraries were pooled and concentrated using a SpeedVac. The dried-down libraries were resuspended in hybridization mix and combined with the QIAseq xHYB MAP/HAP/RAP probe panel, along with One-4-All Blocking Solution and One-4-All Blocking Oligos. This was incubated overnight, and the probe-bound libraries were captured using streptavidin-coated magnetic beads. The streptavidin beads were then washed to remove unbound or loosely bound libraries. The remaining post-capture library was amplified. After library quantitation, sequencing was performed using the Illumina NextSeq2000 instrument with a P1 flowcell kit.

CLC Microbial Genomics Workbench with the QIAseq xHYB Viral DNA Panel workflow was used for data analysis. A custom TaxPro and Find Best Reference database was created for the MAP/HAP/RAP panel. Each of the samples was downsampled to 2 million paired-end reads before classification so that the analysis could be compared across samples.

Results and discussion

To determine the sensitivity and specificity of the QIAseq MAP/HAP/RAP panel, reads were classified using CLC TaxPro, in which each individual read is assigned to the lowest common ancestor. The number of on-target classified reads correlated with the initial viral copy number spiked into Jurkat total nucleic acid. The panel was able to detect viral targets down to 10 copies, with at least 600 reads to confidently identify the target virus.

The performance of the hybrid capture panel was compared to shotgun sequencing. The same libraries used for hybrid capture were also used for shotgun sequencing, and fold-enrichment was calculated. The maximum enrichment factor was 233,000-fold, demonstrating a significant increase in sensitivity with hybrid-capture targeted sequencing. The QIAseq xHYB MAP/HAP/RAP panel efficiently enriches viral targets, even when shotgun sequencing fails to detect viruses due to low copy number.

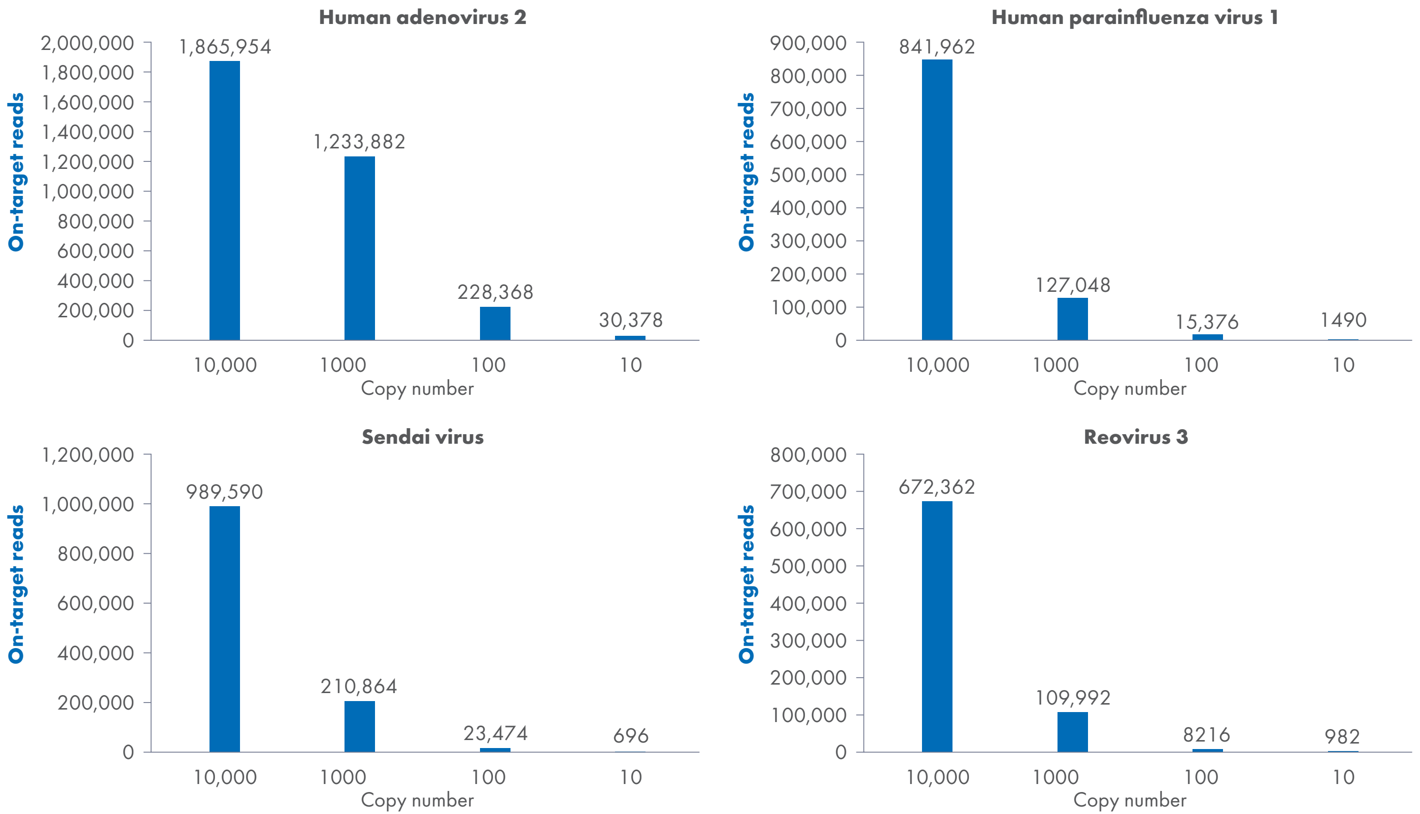

Figure 1

All samples were downsampled to 2 million reads before analysis. Each read was then classified using TaxPro, and the number of on-target reads classified at the species level was counted.

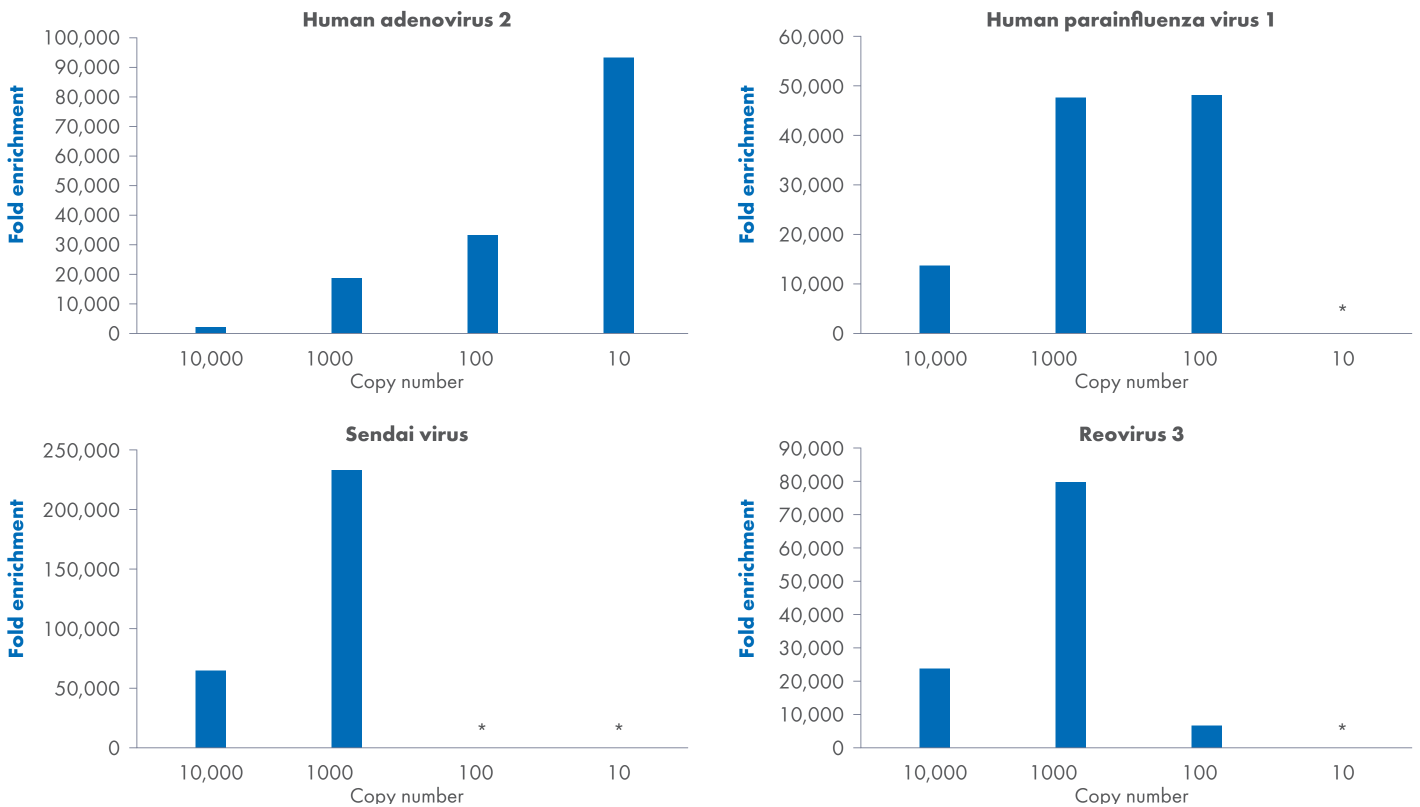

Figure 2

For shotgun sequencing, each library was sequenced with 11.4–17.6 million reads per sample. Fold enrichment was calculated as the hybrid-capture on-target rate divided by the shotgun sequencing on-target rate. Asterisks (*) indicate samples in which no on-target reads were detected by shotgun sequencing.

Genome coverage and uniformity following hybrid capture were assessed across viral targets and input copy numbers. Greater than 90% genome coverage at ≥20× depth was maintained down to 100 copies for three of the four viruses tested, with Reovirus 3 being the exception.

The uniformity of coverage also remained high across all viruses down to 100 copies, with >89% of the genome covered at >0.2x mean coverage.

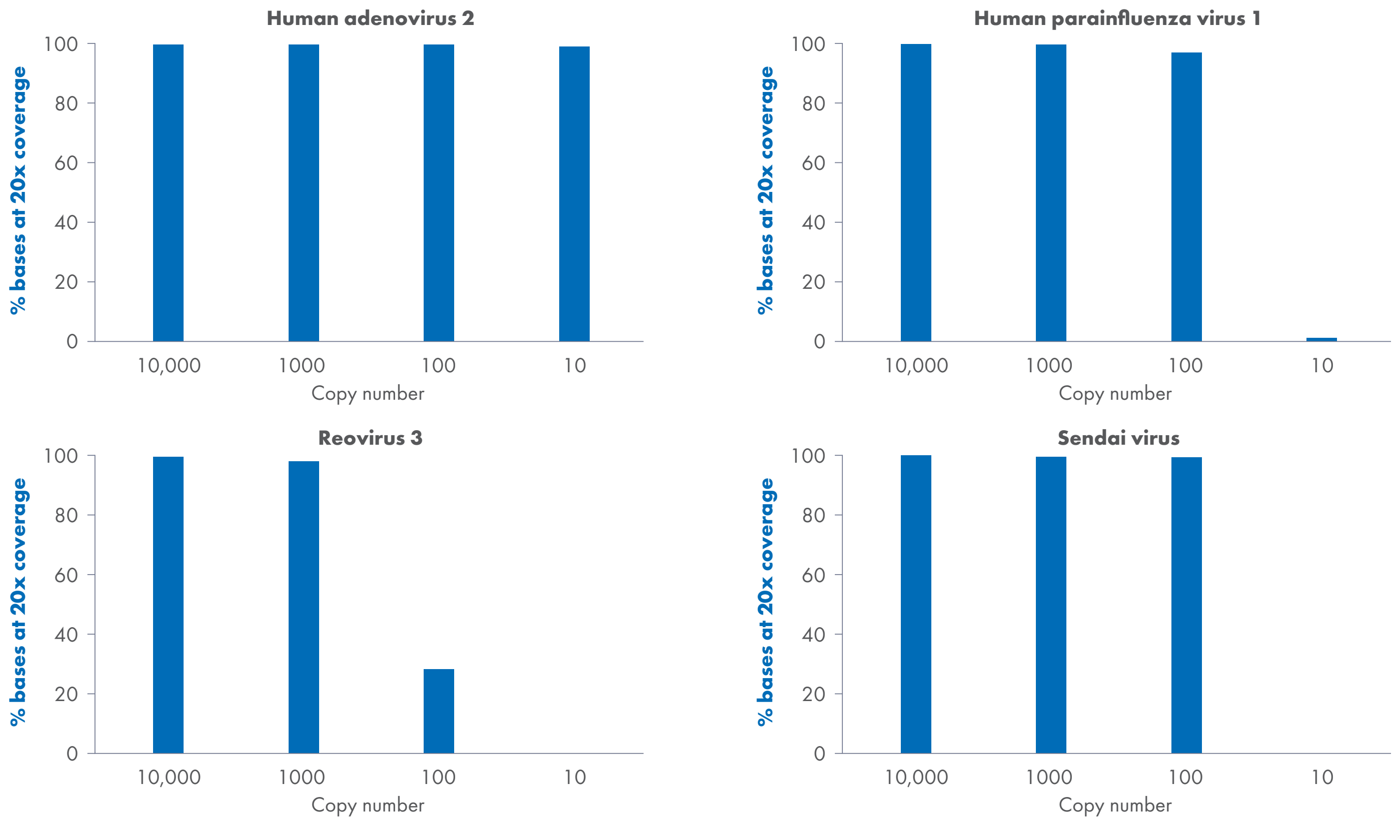

Figure 3
MAP/HAP/RAP Panel maintains sufficient coverage as determined by % of the genome at 20x coverage.

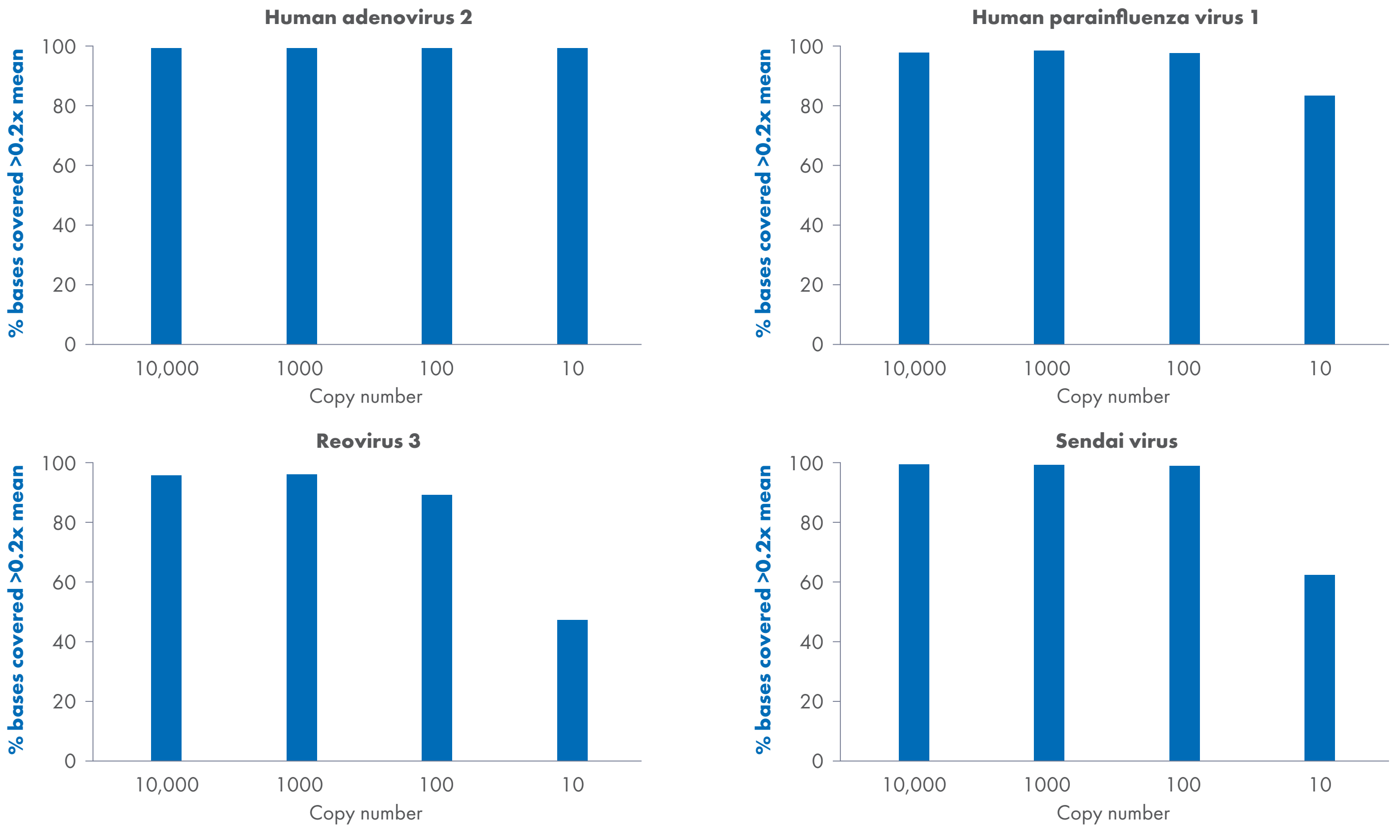

Figure 4
Uniformity of coverage determined by % of genome at >0.2x mean.

Base-by-base coverage distributions at 1000 copies for each viral target are shown in the plots. This demonstrates the high uniformity in coverage exhibited by the QIAseq MAP/HAP/RAP panel. Reovirus 3 is a segmented virus, and its genome was concatenated for mapping analysis. The regions that show a drop in coverage demarcate the boundaries of each segment.

Figure 5
Base-by-base coverage distribution.

The base distribution was plotted in CLC, which also shows SNPs that differ from the reference sequence used for mapping (vertical, color-coded bars). Human parainfluenza virus 1 and Sendai virus have many SNPs that differ from the reference sequence. However, the QIAseq MAP/HAP/RAP panel can still efficiently enrich both viruses with relatively high uniformity.

Conclusion

The QIAseq xHYB MAP/RAP/HAP panel enables sensitive and comprehensive detection of adventitious viral agents aligned with ICH MAP, RAP and HAP testing requirements. Hybrid capture–based targeted sequencing provides substantial enrichment and improved sensitivity compared to shotgun metagenomic approaches, enabling confident viral identification at low copy numbers while maintaining high genome coverage and uniformity.

The panel's tolerance to sequence variation supports robust detection of divergent viral strains, reducing the need for frequent assay redesign. Together, these results demonstrate that the QIAseq MAP/RAP/HAP panel is a powerful molecular alternative to traditional in vivo and in vitro assays for viral safety testing of biopharmaceutical products.

References

1. James Gombold, Stephen Karakasidis, Paula Niksa, et al. Systematic Evaluation of In Vitro and In Vivo Adventitious Virus Assays for the Detection of Viral Contamination of Cell Banks and Biological Products. *Vaccine*. 2014 May 19; 32(24):2916-26. doi: 10.1016/j.vaccine.2014.02.021. Epub 2014 Mar 25.
2. ICH Q5A(R2) Guideline on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.

Learn more about the **QIAseq xHYB MAP/RAP/HAP panel** and how targeted sequencing can support sensitive adventitious agent testing.

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