

Accelerating Bulk Harvest Release Testing using the Rapid Adventitious Virus detection Blazar® CHO Animal Origin Free Virus Panel

Background

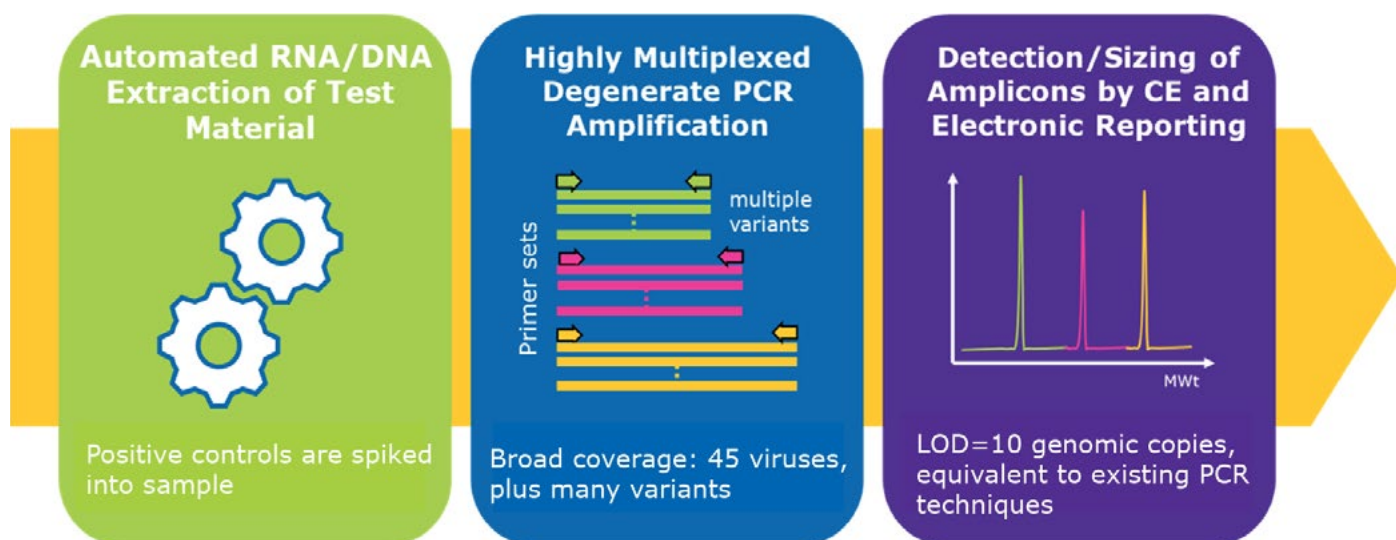
Adventitious agent testing is often a rate-limiting step in downstream processing. Traditionally, most testing at the bulk harvest stage is performed using time-consuming culture-based methods, which rely on amplification of the contaminating agent. Alternative methods that offer a more rapid turnaround are needed to relieve this bottleneck and address the increasing pressures on manufacturing speed.

Molecular methods can meet this industry need. For the detection of viruses, the use of nucleic acid-based technologies is supported by regulatory guidelines such as the ICH Q5A document. While next generation sequencing technologies use a sequence-agnostic approach to detect all viral sequences, they often lack the speed required for a rapid bulk harvest lot release package. In contrast, standard PCR-based methods take just days, but typically target only a single virus. For manufacturing processes that use CHO cells and animal origin-free components, Rapid Adventitious Virus detection: Blazar® CHO AOF panel offers the ideal combination of speed, sensitivity and breadth of detection to serve as an alternative to the *in vitro* virus assay. By employing degenerate PCR methodology, the Blazar® CHO AOF panel achieves broad spectrum virus detection in less than two weeks.

Method Overview

- Multiplexed degenerate PCR, allowing detection of multiple virus families and variants in a single reaction. Primers are designed against conserved target regions within each family
- Nested PCR approach achieves a high degree of sensitivity and specificity, and enables the detection of emerging viruses and variants that share the same conserved regions
- Viruses that cannot be cultured, or do not produce detectable effects in culture, can also be detected by this method
- Positive controls are spiked into each sample to demonstrate extraction recovery and PCR amplification at the detection limit in both the DNA and RNA panels

The assay uses a 3-step workflow as illustrated below:



Key Benefits

The Rapid Adventitious Virus detection: Blazar® CHO AOF panel offers:

- Rapid results – a 12-day turnaround for CHO bulk harvest samples to replace and/or supplement *in vitro* assay results
- An accelerated bulk harvest testing package for animal origin-free processes, in combination with other rapid methods
- Expected sensitivity and breadth of detection (including emerging variants) through use of degenerate primer design and nested PCR methodology
- An established technology, familiar to regulators. The use of molecular methods such as the Blazar® platform is in line with the evolving regulatory landscape (such as the ICH Q5A revision)
- Reduced sample requirements compared with traditional methods

Technical Specifications

Assay code	399003GMP.BSV (Rockville, US) 399003GMP.BUK (Glasgow, UK)
Sample format	2x1ml of test article
Compliance	GMP
Total turnaround time	12 days
Virus coverage	15 virus families, including 5 DNA virus families (Adenoviridae, Anelloviridae, Circoviridae, Parvoviridae, Polyomaviridae) and 10 RNA virus families (Bornaviridae, Caliciviridae, Coronaviridae, Hepeviridae, Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Reoviridae, Rhabdoviridae, Togaviridae)
Internal controls	Spike recovery: DNA and RNA virus each spiked at detection limit in the test article prior to extraction, to demonstrate extraction and PCR efficiency
Sensitivity	10 genomic copies per reaction*
False positive rate	<1%
True positive rate	>99%

* 100 genomic copies per reaction for polyomaviruses

Other Applications

In addition to its primary application for virus detection in bulk harvest samples from animal origin-free processes, the Rapid Adventitious Virus detection: Blazar® CHO AOF panel can serve as a valuable tool for investigation purposes. It can also be used for rapid pre-screening of cell banks prior to entry into a GMP manufacturing facility.

Learn more at: SigmaAldrich.com/Blazar

Contact Us

Europe:

Todd Campus
West of Scotland Science
Park Glasgow,
G20 0XA, Scotland
Tel: +44 (0)141 946 9999
Biosafety@merckgroup.com

North America:

14920 Broschart Road
Rockville, MD 20850-3349 USA
Tel: 301 738 1000
Biosafety@merckgroup.com

Singapore:

#2 Science Park Drive
#04-01/12 Ascent Building,
Tower A Singapore 118222
BiosafetyAP@merckgroup.com

China:

15-18F, No.3, Building C,
The New Bund World Trade
Center(Phase II) Lane 227
Dongyu Road, Pudong
New District,
Shanghai, China
BiosafetyCN@merckgroup.com

We have built a unique collection of life science brands with unrivalled experience in supporting your scientific advancements.

Millipore® **Sigma-Aldrich®** **Supelco®** **Milli-Q®** **SAFC®** **BioReliance®**

