BioReliance®
Contract Testing Services

Reference Guide

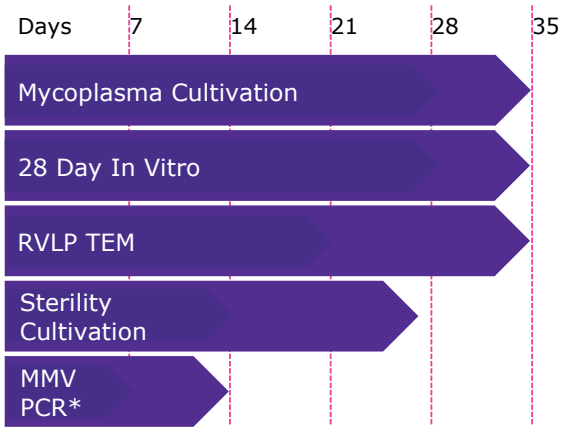
Accelerating Bulk Harvest Release Testing: A Complete Set of Rapid Methods for Adventitious Agent Detection

Background

Adventitious agent testing is often a rate-limiting step in downstream processing. Traditionally, most biosafety 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 faster turnaround time are needed to relieve this bottleneck and address the increasing pressures on manufacturing speed and efficiency.

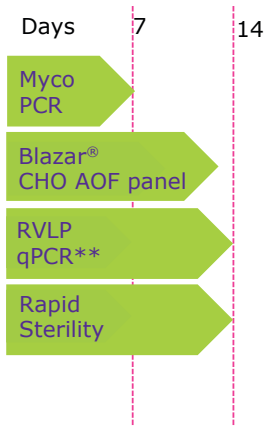
In line with current regulatory guidance, we have developed a suite of methods for rapid and sensitive adventitious agent detection in bulk harvest material from CHO-based manufacturing processes. These methods encompass detection of bacteria and fungi, mycoplasma, viruses, and retrovirus-like particles. By using a combination of validated assays, a full set of results can be obtained in less than two weeks. As indicated, this significantly reduces the overall timeline for bulk harvest biosafety testing:

Traditional package (35 days)



Timelines illustrate the minimum total assay turnaround time

Accelerated package (14 days)



* MMV is included as a target in the Blazar® CHO AOF panel (separate PCR not required in accelerated package)

** Needed for the first 3 batches only

Rapid Methods Overview

- **Sterility:** Use of the BacT/ALERT® platform enables rapid detection of bacteria and fungi through detection of any CO_2 produced by these organisms. Samples are monitored automatically at regular intervals throughout the incubation period, removing the need for manual observations.
- **Mycoplasma:** A PCR-based method targeting the 16S rRNA gene of mycoplasma species (with capability to detect >100 strains). The assay is validated according to pharmacopeial requirements for nucleic acid testing approaches.

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- **Adventitious viruses:** The Blazar® CHO AOF panel is a targeted molecular method that can detect 15 virus families, including multiple variants in a single reaction. Viruses that cannot be cultured, or do not produce detectable effects in culture, can also be detected by this method. Designed for animal origin-free (AOF) processes.
- **Quantitation of retrovirus-like particles (RVLPs):** A PCR-based method for accurate quantitation of C-type particles, which has been optimized for testing bulk harvest material. Although RVLPs are not typically infectious when derived from CHO cells, it is a regulatory requirement to enumerate them in a minimum of three production batches.

Key Benefits

Each of the rapid assays provides benefits over the incumbent method.

	Sterility		Mycoplasma		Adventitious virus		RVLP quantitation	
	Traditional	Accelerated	Traditional	Accelerated	Traditional	Accelerated	Traditional	Accelerated
Method	Culture-based, compendial	BacT/ALERT® platform	Culture-based, compendial	PCR	*In vitro*	Blazar® CHO AOF panel	Transmission electron microscopy	PCR
Detection limit	≤ 100 CFU/ml	≤ 6 CFU/ml	10-100 CFU/ml	10 CFU/ml	1-10 $TCID_{50}$/ml	≤ 1 $TCID_{50}$/ml	~1 x 10^5 RVLP/ml	1 x 10^3 RVLP/ml
Turnaround	26 days	14 days	35 days	7 days	35 days	12 days	35 days	14 days
Sample requirements	20 ml	20 ml	15 ml	4 ml	15 ml	1 ml	20 ml	2 ml

When used in combination the rapid methods package offers:

- Rapid results – all assays completed in 14 days or less
- Equivalent or better sensitivity compared to traditional methods
- Established technologies, familiar to regulators. The use of targeted molecular methods such as the Blazar® platform is in line with the evolving regulatory landscape (such as the ICH Q5A (R2) document)
- Reduced sample requirements compared to traditional methods

Factors to Consider

Suitable use of the rapid methods package is subject to the following (3) criteria being met:

1. Test samples must be bulk harvest material derived from CHO cells
2. The production process must use animal origin-free (AOF) materials
3. The cell banks (including MCB and WCB, plus EOPC where available) must be fully characterized through broad spectrum and specific virus testing (rodent, bovine, and porcine viruses)

All assays are GMP-compliant and offered at multiple locations in our state-of-the-art testing network. Early discussions with regulators are recommended prior to implementing and filing with new assay methods. Our expert regulatory consultancy team is available to help.

Contact us to learn more, or visit **SigmaAldrich.com/Blazar**

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