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White Paper

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

The biologics industry is continually developing new manufacturing approaches designed to improve yield, reduce processing time and costs, and harness the power of digitalization to increase process efficiency. In line with these advancements, regulators are publishing guidance on the use of new technologies for adventitious agent detection, which can support progress in biomanufacturing by offering increased breadth of detection, improved sensitivity, and faster timelines. This white paper describes the Blazar® CHO Animal Origin Free (AOF) panel for rapid adventitious virus detection, a targeted molecular method designed to detect virus families. In appropriate situations, the panel can be used for testing of bulk harvest material derived from CHO cells and can be combined with other rapid methods to significantly reduce the overall turnaround time for a bulk harvest testing package.

Prior to the start of downstream processing, bulk harvest testing must be performed to detect the presence of any adventitious agents that have the potential to affect the quality of the drug and present a safety risk for patients. For bulk harvest samples derived from CHO cells, this testing typically consists of cultivation-based methods for mycoplasma and sterility, *in vitro* detection of adventitious agents, quantitation of retrovirus-like particles (RVLP) by transmission electron microscopy and species-specific PCR assays e.g. for mouse minute virus (MMV). With conventional methods, this testing can require up to five weeks, creating a rate limiting bottleneck for the release of bulk harvest to the downstream process. While rapid methods are now available to accelerate testing for mycoplasma, sterility and RVLPs, a faster method for broad specificity virus detection is necessary to replace the *in vitro* cultivation technique for detection of adventitious agents.

The Blazar® CHO AOF Panel is designed to accelerate bulk harvest testing and potentially replace slower culture-based *in vitro* virus detection methods. The panel is a targeted molecular method with broader coverage than traditional PCR-based detection methods. The Blazar® CHO AOF Panel degenerate primers are designed to target specific viruses while also detecting related viral variants and newly emerging viruses. The combination of the Blazar® panel with rapid methods for mycoplasma, sterility and RVLP detection offers the potential to reduce the overall turnaround time for CHO bulk harvest testing from 35 days to 14 days (**Figure 1**).

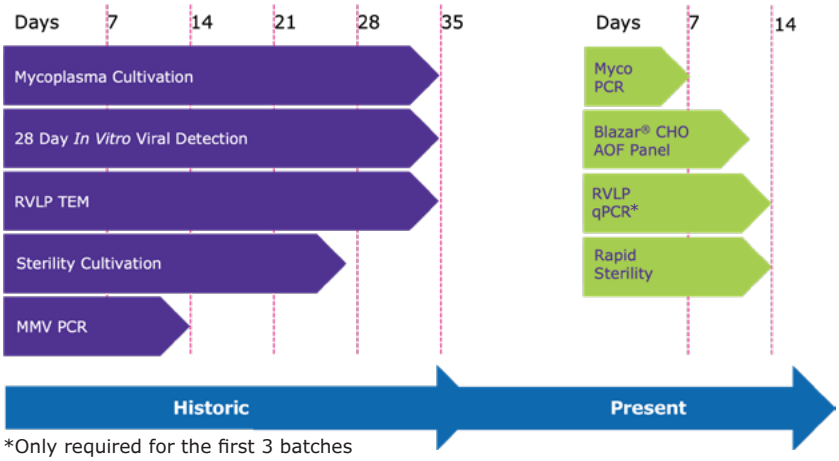


Figure 1. Accelerating CHO bulk harvest testing requires a full set of rapid methods. Timelines reflect the typical minimum total assay turnaround time.

Selecting an appropriate method for virus testing of bulk harvest

Several methods are available for biosafety testing of bulk harvest material. Traditional *in vitro* cultivation for detection of adventitious viruses is a broad detection assay. However, the test can require a month for adventitious agents to manifest themselves in cell culture and only those viruses that produce an effect in one of the end-point tests (cytopathic effect, haemadsorption or haemagglutination) will be detected.

In recent years, next generation sequencing has become an accepted method for broad spectrum detection of adventitious agent sequences. This is an unbiased method as it enables identification of any nucleic acid present in a sample. While the technology is highly robust, it generates a significant amount of data that requires sophisticated analytical tools and extensive analysis to translate raw sequence data and determine whether any adventitious agents are present.

PCR is an extremely robust technique for detection of nucleic acids and enables high sensitivity with use of specific primers. This method is biased, however; it will only detect viruses containing the target region to which the primers were designed. To address this limitation, regulators have recommended use of degenerate PCR in biosafety testing to extend detection capability across a much larger spectrum of adventitious agents[1-3]. The Blazar® CHO AOF panel for rapid adventitious virus detection is a targeted molecular method that combines speed, sensitivity, and breadth of detection, and is ideally suited for reducing the turnaround time of bulk harvest testing.

Design and application of the Blazar® CHO AOF panel

In contrast to single-plex PCR in which two primers target a single sequence, the Blazar® platform uses multiplex degenerate PCR in which multiplex primer sets are used for detection of many viruses as well as the internal controls (**Figure 2**).

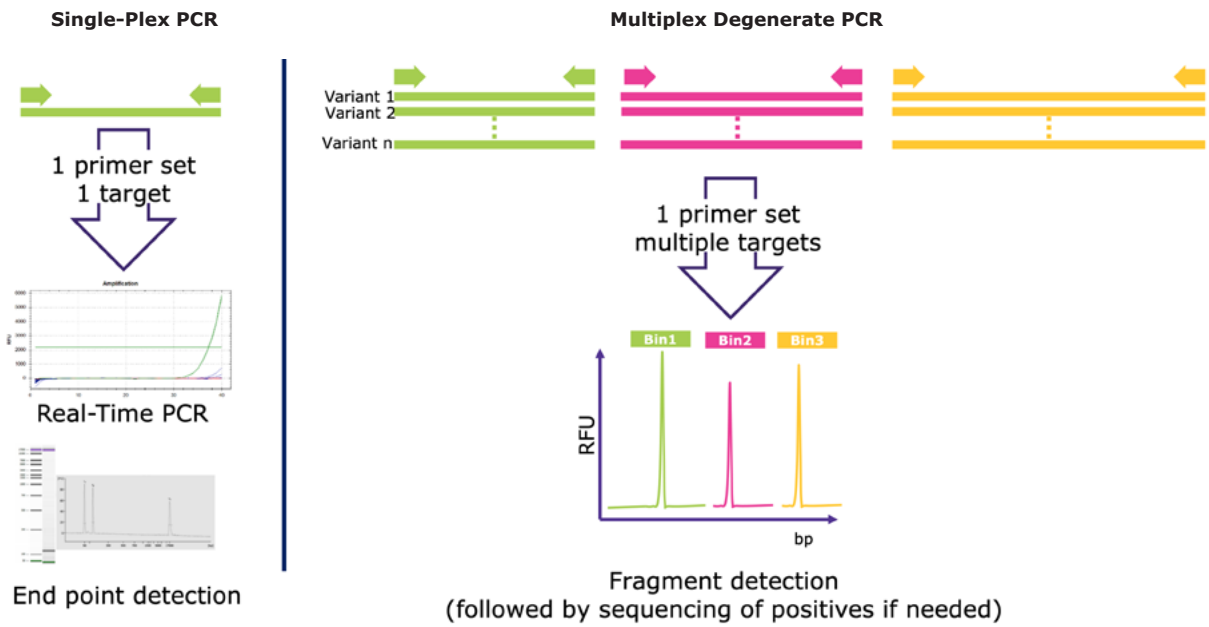


Figure 2. Use of degenerate primers provides broad specificity in the Blazar® platform.

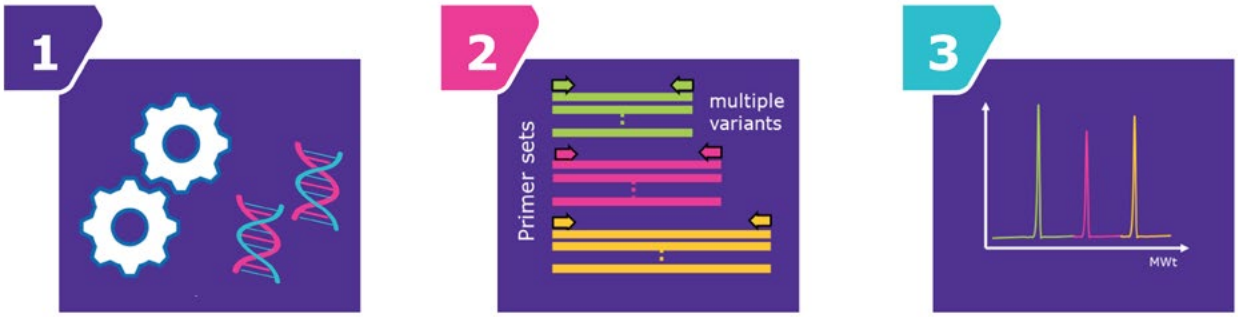


Figure 3. The Blazar® CHO AOF virus panel workflow uses a 3-step method for virus detection.

The Blazar® CHO AOF panel includes 15 virus families identified by internal experts, key opinion leaders, and industry consultants with regulatory experience as being relevant to a CHO manufacturing process using animal origin-free materials (**Table 1**).

Virus Family	Type
Adenoviridae	dsDNA
Anelloviridae	ssDNA
Circoviridae	ssDNA
Parvoviridae	ssDNA
Polyomaviridae	dsDNA
Bornaviridae	ssRNA
Caliciviridae	ssRNA
Coronaviridae	ssRNA
Hepeviridae	ssRNA
Orthomyxoviridae	ssRNA
Paramyxoviridae	ssRNA
Picornaviridae	ssRNA
Reoviridae	dsRNA
Rhabdoviridae	ssRNA
Togaviridae	ssRNA

Table 1. The Blazar® CHO AOF panel targets 15 virus families.

The design of the Blazar® CHO AOF panel makes it suitable for use when the following requirements are satisfied:

- The bulk harvest material is derived from CHO cells
- The production process uses only animal origin-free materials
- The master and working cell banks (and end of production cells, where available) have been fully characterized and tested for the presence of adventitious viruses, including broad and species-specific virus tests that cover potential risks from animal-derived materials (such as those of bovine/porcine origin) that may have been used at some point in the history of the cell line development

Meeting the above criteria allows a risk-based approach to be adopted in terms of virus selection for the panel. The list is therefore focused on viruses that are particularly relevant for CHO bulk harvest derived from animal origin-free processes, and reflects viruses identified in previous CHO cell contamination events, viruses for which CHO cells are permissive, and/or have the potential to infect human cells, as well as emerging viruses that may pose future risk. Due to the degenerate nature of the primers, the Blazar® CHO AOF panel has been predicted to detect over 26,000 RNA and over 2,000 DNA viral target sequences.

Blazar® CHO AOF panel validation and equivalence study

The Blazar® CHO AOF panel has been validated according to ICH Q2 (R1) principles including specificity and detection limit. Positive reference controls were used in three validation runs (**Table 2**). An additional run confirmed the same detection limit in the presence of CHO-K1 nucleic acid, demonstrating the ability of the assay to tolerate complex matrices such as the CHO material.

DNA

Virus family	Detection Limit (copies/PCR)
Internal control	10
Adenoviridae	10
Circoviridae	10
Anelloviridae	10
Circoviridae	10
Adenoviridae	10
Circoviridae	10
Parvoviridae	10
Parvoviridae	10
Anelloviridae	10
Circoviridae	10
Parvoviridae	10
Polyomaviridae	100
Adenoviridae	10

RNA

Virus family	Detection Limit (copies/PCR)
Internal control	10
Picornaviridae	10
Rhabdoviridae	10
Caliciviridae	10
Paramyxoviridae	10
Orthomyxoviridae	10
Reoviridae	10
Paramyxoviridae	10
Caliciviridae	10
Togaviridae	10
Reoviridae	10
Paramyxoviridae	10
Bornaviridae	10
Hepeviridae	10
Coronaviridae	10

Table 2. DNA and RNA virus families and detection limits used to validate the Blazar® CHO AOF panel.

The robustness of the Blazar® CHO AOF virus panel was evaluated by our R&D team through testing of CHO bioreactor material spiked with five hallmark viruses for biosafety testing (parainfluenza virus 3, simian virus 5, mouse minute virus, reovirus 3 and porcine circovirus 1). Viruses were spiked at 1 $TCID_{50}$/mL, which is equivalent to or below the limit of detection (LOD) of these viruses in the *in vitro* cultivation assay. Spiked samples were tested in parallel in the Blazar® CHO AOF virus panel as well as the traditional *in vitro* method. Each of the five viruses was detected using both methods at 1 $TCID_{50}$/mL (**Figure 4**), demonstrating at least equivalent performance of the Blazar® platform compared to the legacy technique.

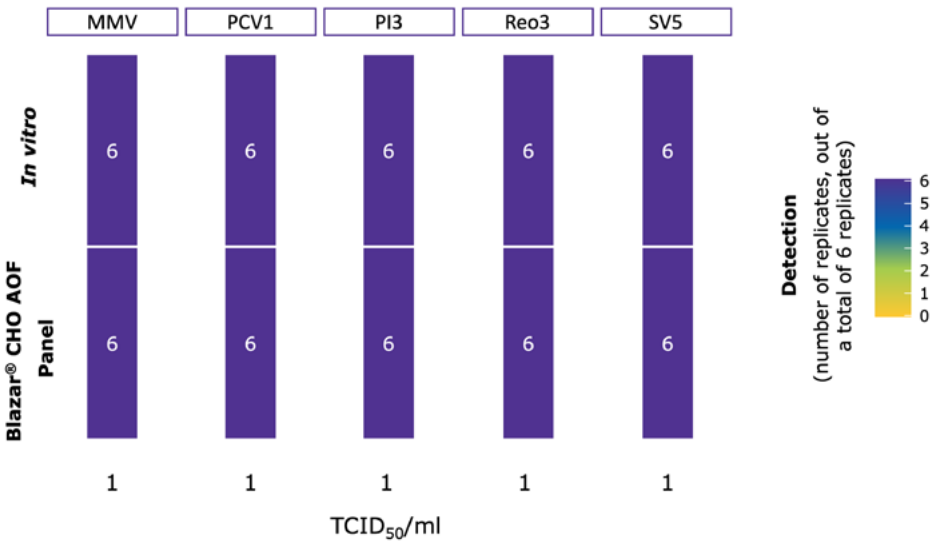


Figure 4. Equivalence study: Blazar® CHO AOF Panel vs *in vitro* results

Regulatory expectations for virus testing of bulk harvest material

The Blazar® CHO AOF virus panel was designed to meet regulatory expectations and recommendations (**Table 3**). Regulatory authorities are increasingly encouraging and accepting the use of state-of-the-art technologies to replace traditional methods for virus testing. The ICH Q5A[1] revision indicates that nucleic acid testing (NAT) methods are suitable for use at the bulk harvest stage as an alternative to *in vitro* methods and states "Methods, such as NAT or targeted NGS, may also be appropriate for detection of specific viruses (e.g., minute virus of mice) or virus families, based on risk assessment for potential introduction of contaminants. Such rapid test methods can facilitate real-time decision making."

The Blazar® technology has already been successfully included in regulatory submissions in multiple geographical regions, in the form of the Blazar® rodent virus panel, which is designed for cell line characterization of rodent cell banks. The Blazar® CHO AOF virus panel employs a similar multiplex degenerate primer-based strategy for PCR amplification for viral detection and is validated in accordance with the ICH Q2 guidance. A biologics master file (BMF) has been filed with the United States Food and Drug Administration (FDA). Regulatory guidance requires demonstration of new assays to be fit for their intended use and this has been accomplished by spiking studies, including the equivalence study outlined above.

In accordance with the expectations of the regulatory agencies, adventitious agent testing should be routinely applied to each unprocessed bulk harvest. The testing may include classical cell culture based assays or non targeted NGS. However, assays such as NAT or targeted NGS may also be appropriate for detection of specific viruses or virus families. Such testing can facilitate rapid decision making. The Blazar® CHO AOF panel incorporates nucleic acid based strategies.

Early discussions with regulators are recommended prior to implementing and filing with new assay methods. Our expert regulatory consultancy team is available to provide support for such discussions.

Regulatory Expectations		Blazar® CHO AOF Panel
Alternate methods can be used, PCR can be used for viral detection	✓	Degenerate nested PCR-based broad detection of viruses; technology already in use for biosafety testing
Method should be validated	✓	Validated in accordance with ICH Q2 (R1) for the detection of specific viruses and variants within 15 virus families. Biologics Master File (BMF) filed with the U.S. FDA
Equivalent or better than established method	✓	Spiking and infection studies demonstrate equivalent or better sensitivity than the *in vitro* assay
Fit-for-purpose	✓	Spiking studies with representative DNA and RNA viruses with known 50% tissue culture infectious dose (TCID50) titers normalized to genomic copies, in bioreactor material, showed the limit of detection to be less than 1 $TCID_{50}$/ml per extracted sample
3Rs - reduce the use of animals and animal-based materials	✓	Nucleic acid-based method – no requirement for animal serum or erythrocytes as used in cell culture studies

Table 3. The Blazar® CHO AOF panel addresses important regulatory expectations for viral detection.

Blazar® CHO AOF virus panel specifications

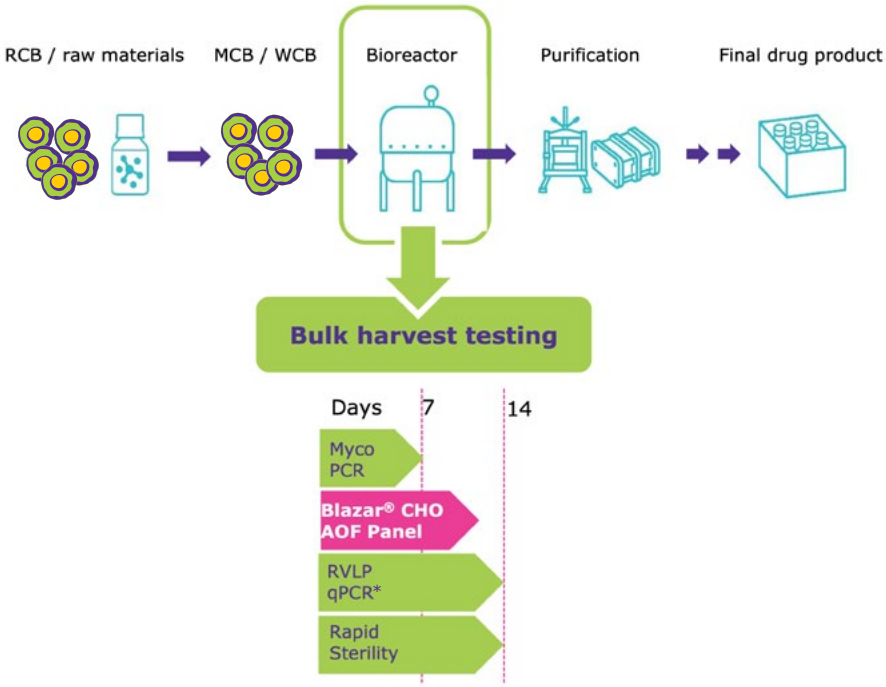
Table 4 summarizes the specifications for the Blazar® CHO AOF panel for rapid adventitious virus detection. Samples are submitted as two 1 mL tubes; one tube is used in the testing, and one serves as backup, representing a lower sample volume than the traditional *in vitro* assay. The total turnaround time for the panel is 12 days and 15 relevant virus families are covered. The sensitivity of the assay is 10 genomic copies per reaction with a false positive rate less than 1% and a true positive rate over 99%.

Sample format	2 x 1 ml of test article
Total turnaround time	12 days
Virus coverage	DNA and RNA viruses from 15 virus families
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%

Table 4. Blazar® CHO AOF panel specifications. * 100 genomic copies per reaction for polyomaviruses.

Conclusion

With use of the Blazar® CHO AOF panel for rapid adventitious virus detection in appropriate scenarios, conventional timelines for bulk harvest testing can potentially be reduced by three weeks. By accelerating biosafety testing in a manner that aligns with evolving regulatory recommendations, timelines are compressed, and therapies can reach patients more rapidly without compromising on safety. The Blazar® CHO AOF panel is part of a comprehensive set of rapid solutions for all aspects of bulk harvest adventitious agent testing (**Figure 5**).



*Only required for the first 3 batches

Figure 5. Implementation of rapid methods reduces timelines for CHO bulk harvest testing.

References

1. ICH Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (R2)
2. FDA Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials used in the Production of Viral Vaccines for Infectious Disease Indications (2010)
3. Ph. Eur. 5.2.14: Substitution of In Vivo Method(s) by In Vitro Method(s) for the Quality Control of Vaccines

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MK_WP12927EN Ver. 3.0
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06/2024