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Pharma & Biopharma Manufacturing & Testing Services

White Paper

Rapid Biosafety Testing Enables the Future of Manufacturing

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The safety of biologic medicines relies, in part, on a robust biosafety testing program applied across the biomanufacturing process to evaluate samples for the presence of adventitious agents. For batch mode production processes, such as those used for monoclonal antibodies (mAbs), biosafety testing takes place along the entire manufacturing process. The main testing points are just after the bioreactor, called bulk harvest lot release testing (BHLRT), as well as at the end of the process before and after fill-finish. The characterization and biosafety testing of raw materials are also required, and in particular for the manufacturing cell line (**Figure 1**).

Traditional methods for adventitious agent testing are well established and typically rely on biological amplification and detection, which can take several weeks. This time-consuming approach is not compatible with novel treatment modalities such as modified T-cell therapy which requires a rapid time-to-result, nor with antibody manufacturing processes which are undergoing intensification in the move towards semi-continuous and continuous processes.

This whitepaper explores the factors driving the evolution toward faster biosafety testing and describes rapid approaches for adventitious agent testing during cell line characterization and BHLRT that are more aligned with current and future manufacturing trends.

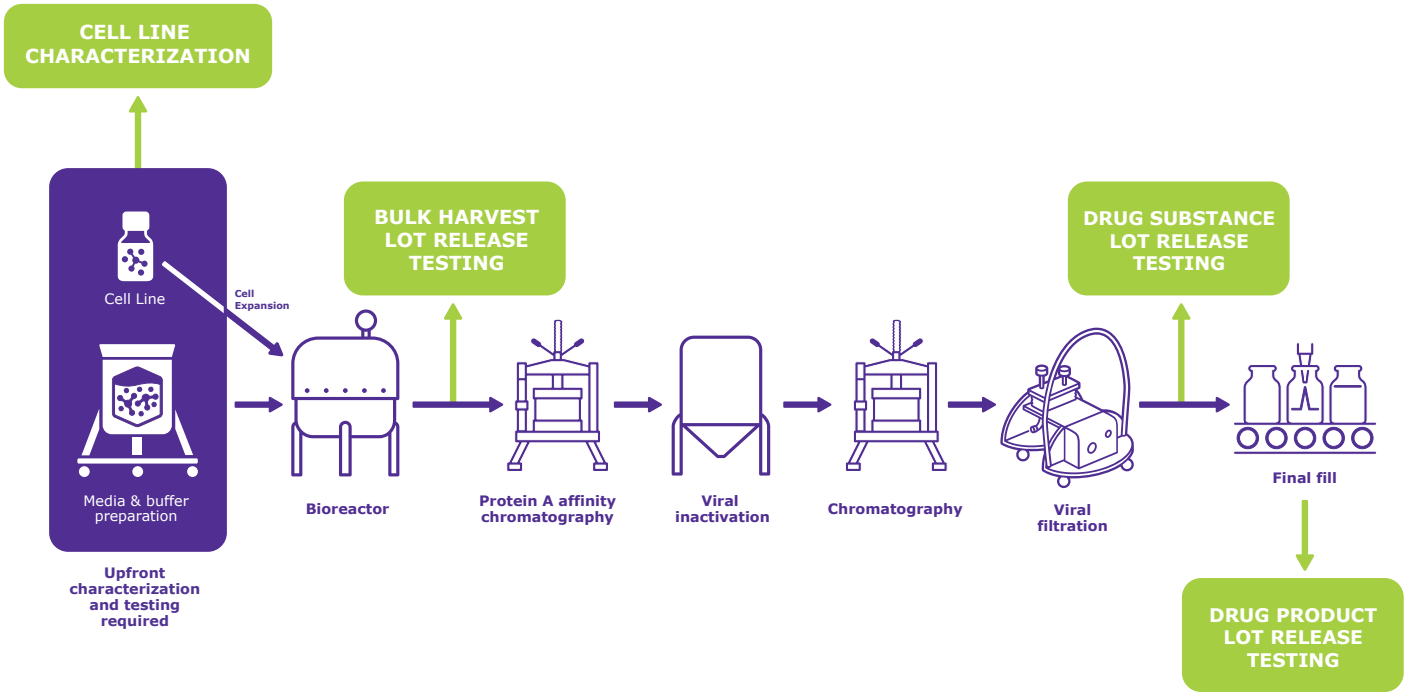

Figure 1. Current manufacturing processes utilize batch operations that require biosafety testing across the whole process

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Current Approaches to Biosafety Testing

Establishing that the manufacturing cell line is free from adventitious agents such as viruses and microorganisms is a central part of the characterization process. The traditional approach has been to use a range of predominantly cultivation-based methods. **Figure 2A** summarizes the traditional approach to adventitious agent testing for a typical CHO cell line. For the detection of microorganisms, cultivation methods include a direct incubation approach, such as sterility testing for bacteria and fungus, and incubation with solid and broth media as well as mammalian cells for mycoplasma detection. The detection of adventitious virus traditionally requires the use of a cell culture-based system with three permissive cell lines, referred to as an *in vitro* screen. Visual examination for cytopathic effect, heamadsorption and heamagglutination are the traditional endpoints, but others may also be required to determine the presence of viral contamination. In addition to the *in vitro* screen, animal models are often used to test for viral contamination. This approach includes the use of mice and embryonated eggs for general viral detection, or antibody production in hamsters and mice for identification of specific rodent viruses. Other species-specific viral risks may be identified with other molecular methods, such as PCR. These rapid molecular methods typically deliver results in about one week; the timeline for other cell line characterization tests, however, can stretch to several weeks.

A similar and aligned testing strategy is also leveraged for BHLRT. As discussed, this testing is used after the bioreactor step, but prior to any downstream purification steps. This is because downstream purification steps will remove adventitious agents and testing at this stage allows for the best opportunity to detect any contamination. Again, testing for adventitious agents traditionally relies on cultivation-based methods for the detection of microorganisms and viruses. Specific viral threats may also be detected through the use of molecular methods such as PCR. Depending on the application, retrovirus quantification by examination with electron microscopy may also be required (**Figure 2B**). As with cell line characterization, these methods are quite time-consuming.

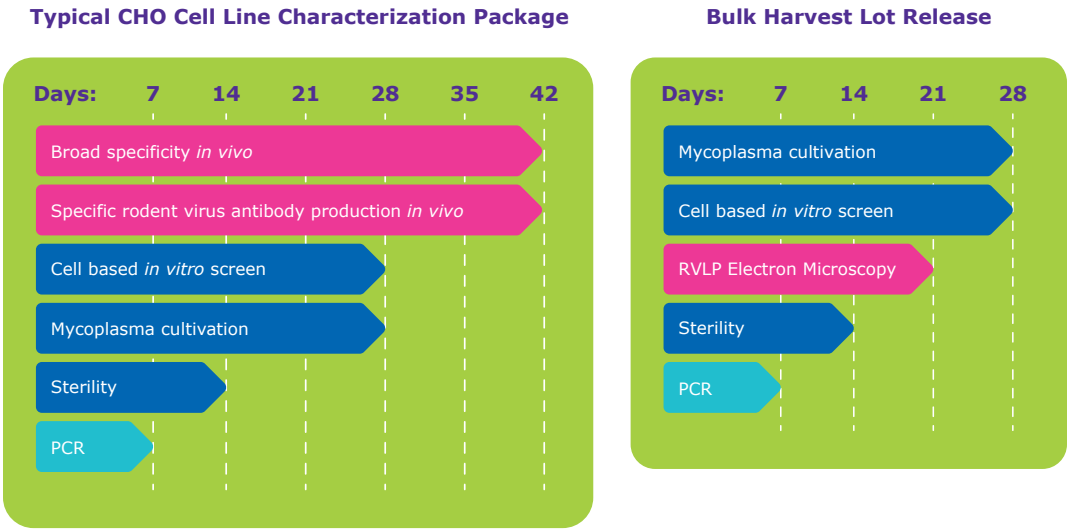

Figure 2. Traditional methods for adventitious agent testing rely on biological amplification which can take several weeks of lab time for both cell line characterization (A) and bulk harvest lot release (B). Set-up and read-out of the tests add to the timeline.

Rapid methods do exist for the detection of potential contaminants for BHLRT such as mycoplasma PCR, assessment of retrovirus-like particles using qPCR and rapid sterility approaches (**Figure 3**). Given the availability of these methods, what is holding the industry back from adoption? An alternative for the broad detection of viral contamination afforded by *in vitro* screening is clearly an issue that inhibits wholesale adoption. In addition, existing documentation processes such as preparing the batch record documentation may limit the desire to move away from these slower incumbent methods. There is little incentive to make a painful switch that includes a general change control as well as potential regulatory approval when other parallel processes such as documentation and review may have similar extended timelines.

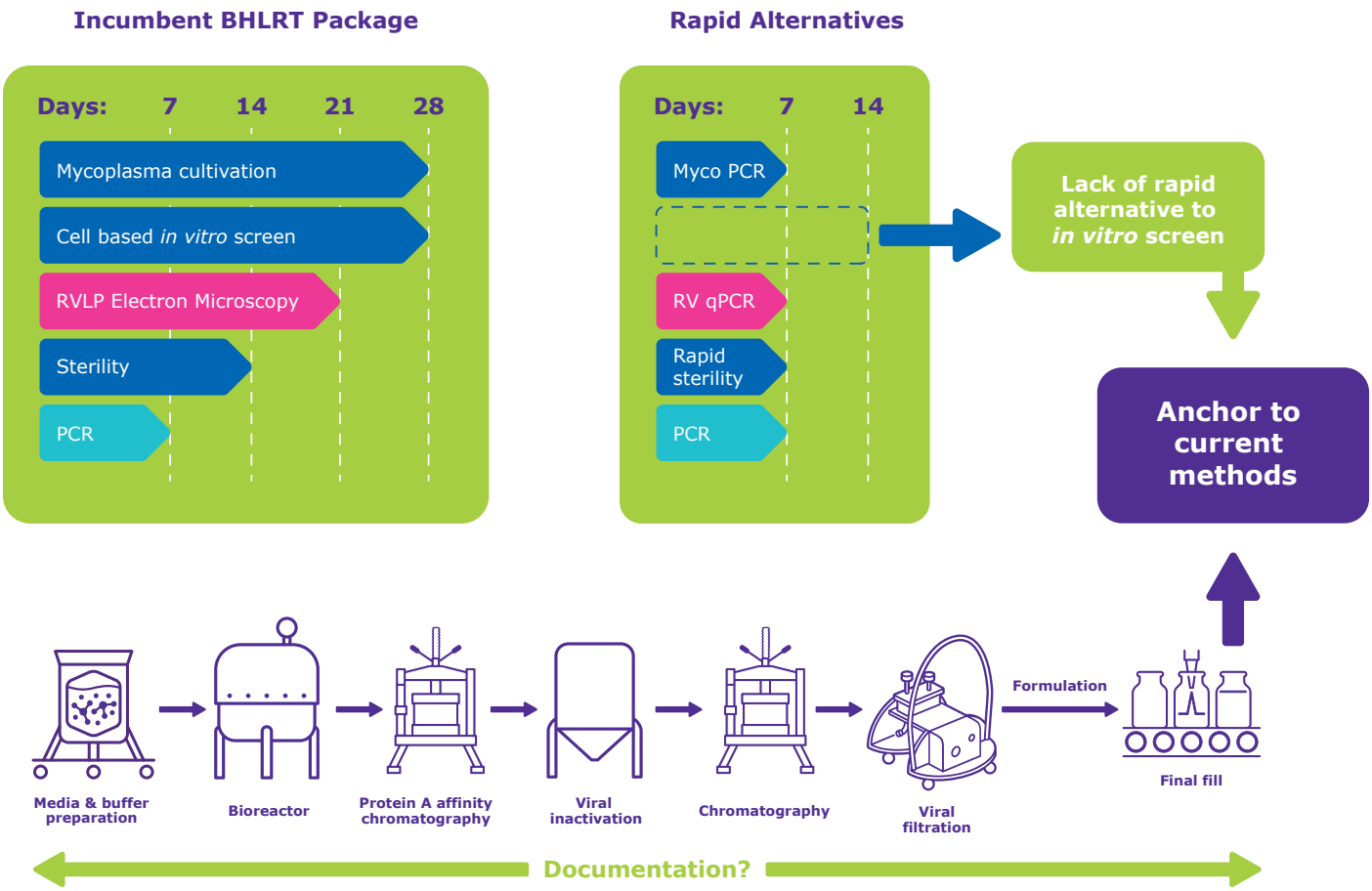

Figure 3. Rapid bulk harvest release methods exist but might not be adopted due to a lack of alternatives for all methods. The time-consuming process of generating quality documents doesn't align well with rapid methods and may lead to continued use of slower approaches.

Drivers of Change

Change is never easy, especially within the GMP regulated environment of biopharmaceutical manufacturing. However, there are certain drivers which are pushing the industry towards more rapid biosafety testing (**Figure 4**).

Figure 4. Several trends are influencing and accelerating the adoption of rapid biosafety testing.

Openness of Regulatory Authorities

It may seem that there is little regulatory drive for new and rapid technologies; the fact that legacy methods and technologies are suggested within regulatory documents certainly helps to reinforce this perception. For example, the antibody production test is suggested as a method for the detection of specific viral risks for rodent cells within ICH Q5A.[1] However, ICH Q5A also indicates that suggested assay protocols "is not all-inclusive or definitive" and that "alternative techniques…may be acceptable." The guidance also notes that "PCR may be appropriate for detection of… human…as well as for other specific viruses". Perhaps in acknowledgement of these guidelines being implicated for slowing adoption, the International Council for Harmonisation has called for ICH Q5A to be updated such that the use of alternative technologies, such as PCR, are given higher prominence as viable alternatives to more traditional cultivation-based methods.[2] These updates are expected in 2020.

Regulators are also actively participating in the assessment of alternative, rapid technologies. The Advanced Virus Detection Technologies Interest Group (AVDTIG) is an example of a cross industry group with regulatory input that are investigating alternatives.[3] Other examples of interest groups also interested in alternative rapid methods are Biophorum Operations Group (BPOG) and Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB).

Technological Advances

One of the rapid molecular methods that continues to generate a great deal of interest within biosafety testing applications is Next Generation Sequencing (NGS). This technology offers a number of advantages including:

- Delivery of unbiased and unselected analyses enabling detection of a very broad range of adventitious agents.
- The ability to handle difficult and complex samples and not be subject to the vagaries that a cell-based system would be in terms of toxicity or inhibition.
- Relatively rapid and direct identification.
- Familiarity throughout the industry via interest groups such as AVDTIG and known to regulators.

Despite these advantages, challenges do remain and include:

- Sensitivity can be variable.
- NGS instruments are relatively complex with higher complexity in terms of sample preparation.
- Detection of highly novel contaminants can be a challenge. An example of this is the discovery of a novel rhabdovirus which latently infects Sf9 cells.[4] Ultimately, the detection of this virus required deep sequencing and extensive analysis, which may be challenging to implement routinely.
- Current NGS approaches may not be fast enough for all applications.

To this end, well-established PCR technology, qPCR and more recently, digital PCR, are still of high importance in the evolution of biosafety testing. These methods are both rapid and highly sensitive. However, the issue with PCR is that the primer design restricts the breadth of detection. One of the ways to broaden the detection ability of PCR is to use a degenerate primer approach to target and detect virus families.

The Blazar™ platform is designed as a rapid assay that uses a highly multiplexed degenerate primer design to enable the detection of more than 5000 viral variants to a validated sensitivity of 10 genomic copies. By amplifying multiple targets within a conserved region of the viral family genome, the platform detects a much broader range of adventitious viruses as compared to traditional PCR methods. For example, the Blazar™ rodent virus panel is able to detect the previously unknown, emergent virus MKPV that was reported in 2018.[5]

Certainly, technological advancements as well as the permissiveness of the regulators can help facilitate change within the biosafety industry. On their own, however, these factors are insufficient to motivate manufacturers to adopt rapid methods. In contrast, the factors outlined below are important drivers of change.

Replacement of Animal Models

As described above, cell line characterization for CHO cell lines has traditionally been dependent on the use of *in vivo* methods. There is a clear impetus to move away from the use of animal models where possible. This is driven both by a longstanding regulatory push, as well as corporate ethical initiatives around the "3Rs". These assays typically require the longest duration within a characterization package which also helps to drive their replacement. It is also increasingly recognized that more rapid cell line characterization can help companies access the clinic faster for first-in-human trials, potentially inferring significant commercial advantage.

For well characterized cell lines, such as CHO, a strong argument can be made for the replacement of the broad specificity *in vivo* methods with a NGS approach, which is a broad-specificity molecular technology. In addition, the lengthy antibody production assay that is typically used to identify specific rodent viruses can be replaced with a directed molecular approach, such as the PCR-based alternative Blazar™ rodent virus panel. This platform combines the breadth of detection of NGS with the speed and sensitivity of PCR and provides accurate and highly sensitive viral detection in just days, versus weeks for the *in vivo* based method.

Novel Treatment Modalities

Another significant driver of the need for more rapid testing is the emergence of novel treatment modalities. One such example is autologous CAR-T therapy, in which T cells are removed from the patient, transformed by a virus and reinfused into the patient. Testing and characterization are applied to the transforming virus as well as to the final CAR-T product, prior to infusion. For patients who are waiting for an infusion of these potentially lifesaving therapies, the length of time required for conventional biosafety testing is simply too long, as four to six weeks is typically needed for a full biosafety testing package. In addition to the time considerations for testing, limited sample volume can also be a challenge; this is in stark contrast to the large volumes available from batch processing of monoclonal antibodies. Another consideration that necessitates an alternative approach is the compatibility of the raw material with the various assays.

All of these factors mean that in many cases only alternative technologies are feasible for the detection of viral contamination in these novel treatment modalities.

CAR-T manufacturers are also incorporating strategies to minimize the risk of adventitious agent introduction through use of closed processes; this approach allows a more risk-based strategy for testing the cells prior to infusion into patients.

Evolution of Manufacturing Models

The move from conventional batch processes towards process intensification in the manufacturing of biologics is also a major driver of change (**Figure 5**).

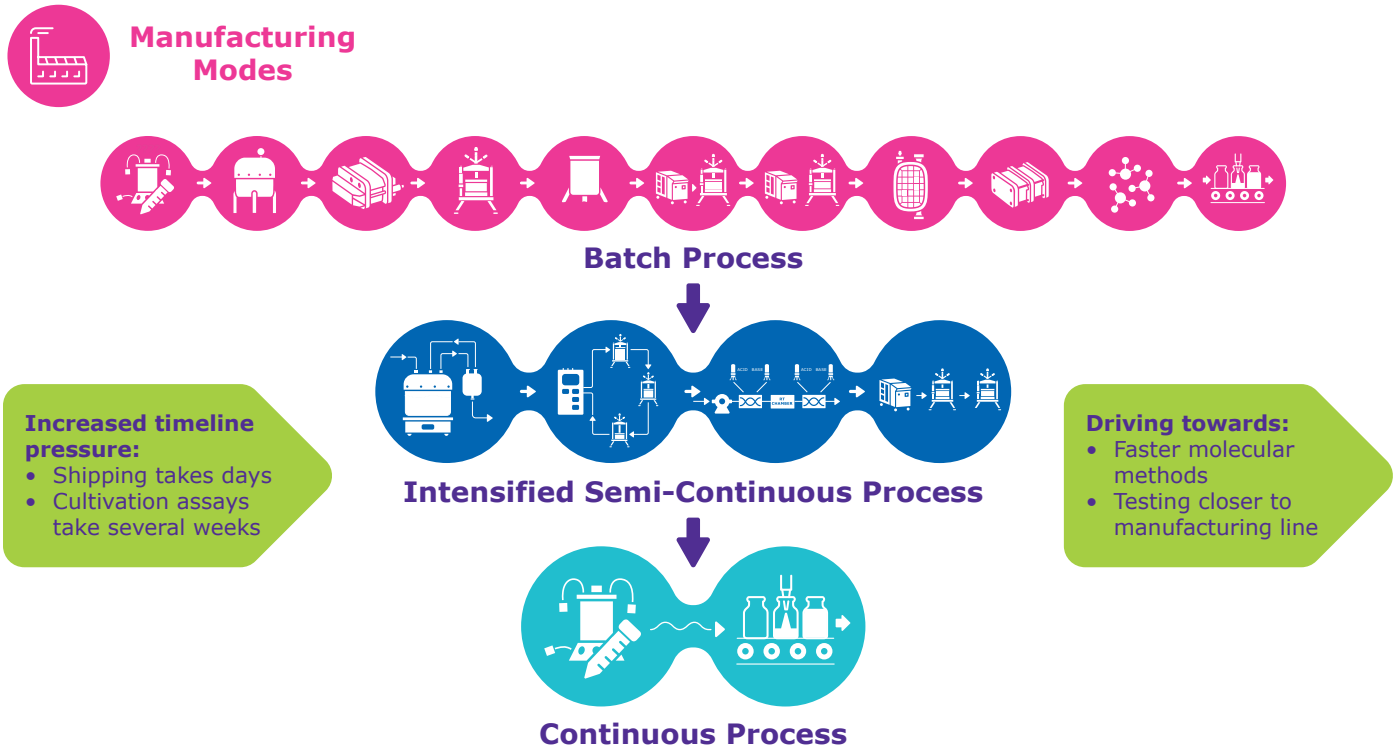

Figure 5. The move from batch processes towards process intensification and continuous processes are driving the need for more rapid biosafety testing.

Process intensification is focused primarily at driving manufacturing costs down. However, intensification also creates increased pressure on timelines, which is not compatible with the traditional cultivation assays, which take weeks. If a manufacturer were to establish a fully continuous process, any idle times within this process, such as shipping for off-site testing, would need to be minimized or eliminated. These shortcomings are therefore driving the use of faster molecular methods and will ultimately bring testing closer to the manufacturing line.

The trend towards greater proximity of testing to the manufacturing line ranges from a near-line scenario to fully integrated in-line analysis:

- Near-line testing is accomplished using kits or solutions that can be run close to the manufacturing line. This is not necessarily within a dedicated QA or QC lab, but is much closer to the manufacturing line than current methods.
- At-line approaches require testing methods and technologies be brought into the manufacturing suite. While the testing is separate from the manufacturing itself, it is run within the manufacturing suite. Successful at-line testing likely requires a higher level of automation and simplification of the process itself.
- On-line testing involves a sample being taken directly from the bioreactor or from the process itself and measured automatically.
- In-line testing is accomplished via a sensor or detection device placed within the bioreactor itself.

Ultimately, not all technologies are suitable for in-line testing, nor do they need to be. Examples of on-line approaches are mass spectrometry and Raman spectroscopy,[6] which are used effectively to identify adventitious contaminations within the process itself. Other technologies such as PCR or rapid sterility may only be suited for an at-line solution within the manufacturing suite.

One commonality among these advanced strategies is the management of the data. Indeed, for some of these technologies, it needs to be confirmed how their output, such as a spectroscopic profile, is connected with biosafety risk. Currently, that connection is not clear. In addition, questions must be answered regarding detection of silent latent risks, such as the rhabdovirus within Sf9 cell lines mentioned earlier.

Conclusion

The need for more rapid biosafety testing is being addressed on a number of fronts. Novel technologies such as the BioReliance® Blazar™ platform that replaces lengthy assays such as the *in vivo* or *in vitro* cell-based assays can help facilitate adoption of rapid methods. However, it is the utilization of these technologies to address specific customer problems that will truly accelerate the adoption of rapid biosafety methods. Corporate ethical objectives, as well as continuous processing and new treatment modalities such as CAR-T therapies are helping to drive the industry as well as regulatory acceptance. Indeed, it is highly likely that these technologies will migrate closer to the production line, further accelerating testing while helping to ensure process and patient safety.

References

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