White Paper

Mitigating Risks Associated with Cell Culture Media Preparation and Handling

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The preparation of media for use in biopharmaceutical processes can be complex and may carry risks that must be identified, assessed and mitigated to assure consistency of performance and minimize likelihood of contamination. There are risks throughout the media preparation life cycle, which can be associated with raw material sourcing, preparation, filtration, transfer, storage, and point of use. Without the proper risk mitigation, problems can arise which may ultimately compromise product quality, shutdown production or potentially impact patient safety.

This whitepaper describes considerations for selecting a vendor to support your media preparation workflow, and will discuss approaches to minimize variability and de-risk steps, including:

- Sourcing raw materials
- Preparation and mixing
- Filtration
- Storage and transfer

Selecting a Trusted Partner

Media formulations should be selected and developed with the full process in mind: banking in, banking out, seed train and scale-up, and ultimately production. The goal is to achieve the highest viable cell densities which deliver the desired yield. Whether the medium is an off-the-shelf, plug-and-play formulation or customized from individual components, selecting a supplier that can deliver consistent quality and supply, and offer the flexibility in media delivery is paramount.

During evaluation of possible media providers, it is recommended to assess the robustness of the supplier's supply chain by asking specific questions including:

- Has the supply chain been reliable in the past?
- How does the supplier manage the hundreds of components that can be used in various media formulations?
- How does the supplier ensure raw materials have not been tampered with or adulterated during the shipping process?
- How readily available is the necessary supporting regulatory information?

Suppliers of cell culture media and supplements should have expertise in cell culture formulation requirements, a deep history with the right suppliers for quality raw materials, and processes to control and manage those raw materials. They should leverage scalable technology to support batch sizes from clinical phase to commercial, and ensure lot-to-lot consistency to manage and minimize variability. A company that demonstrates a strong track record in dry powder cell culture media manufacturing at an industrial scale should have the necessary in-house competency and experience to provide consultation services in all aspects of media customization. In addition, using a company that offers a broad portfolio of media formulations and supplements simplifies optimization of culture conditions during process development.

Sourcing Raw Materials

Sourcing cell culture media requires a thorough evaluation and risk analysis of the options that are available. Areas for consideration include:

- Adherence of the supplier to quality guidelines
- Availability of media formats and sizes compatible with unit operations
- Assurance of the desired product attributes

Cell culture media regulations are not mature, but manufacturers should align with their customer's needs* and comply with voluntary standards such as ISO 9001. At Merck, we establish specifications for each raw material including identification and appearance upon receipt, and a variety of analytical and bioassays to confirm the raw material is appropriate quality for use in biopharmaceutical production. We only select suppliers who can supply the right documentation that ensures the quality of each raw material.

As raw materials move into our facility and processes, they are identified and tracked throughout the weighing and manufacturing process as part of a batch record. When the final cell culture media product is packaged, we place the media in a container with a tamper-evident seal. The customer receives containers which have been sealed and closed at our facility.

Production and packaging of cell culture media by the vendor should be in a format compatible with the customer's unit operations and align with how it will be used. This approach helps to reduce risks by streamlining workflow. Examples include right-sized weighing in which materials are weighed based on the target required for hydration, and transfer bags which allow a sanitary connection to the entrance port of the hydration vessel. With this plug-and-play approach, material can be moved from containers directly to hydration. Packaging solutions should also be available in customized sizes to fit height and weight requirements of the bioproduction site.

The supplier should also have a complete and detailed understanding of media components and how the product is made, to ensure the necessary product attributes including:

- Appropriate flow characteristics to enable free flow delivery into the hydration vessel
- Appropriate milled product to prevent aggregates or insoluble components in the hydration vessel
- A highly solubilized formulation which allows for proper dissolution in the most economical and easy fashion

If there are challenges with media dissolution, compaction can be used to decrease the amount of air space between molecules within a cell culture medium formulation; when the compacted formula comes into contact with water, it solubilizes more readily. If hydration volumes are limited, concentrated media solutions are an option.

Media Preparation at the Biopharmaceutical Site

Preparation of media presents a number of opportunities for risk exposure. An assessment should be conducted to prioritize risks and develop mitigation plans – ranging from procurement of raw materials, how many different materials are needed, the quality attributes necessary for each material, and the method to confirm the materials received match the specification requirements. Once formulation begins, the process should remain as closed as possible to minimize bioburden ingress and timely to prevent possible proliferation.

After the medium has been prepared and sampled to ensure it meets specifications, it is ready to be filtered and then transferred for use or stored. The most common challenges faced at this point in the workflow include:

- Maintenance of sterility throughout transfer and storage, and determining when media should be filtered and what is the most appropriate filter
- Complicated sub-aliquoting into smaller storage volumes from a large batch size
- Lack of footprint availability for storage
- Stability of the solution during storage period

Media preparation operations can be performed using stainless steel or single-use equipment with the choice of technology dependent on manufacturers' facility, preference and scale. The time, labor and costs for set-up and preparation, cleaning and sterilization, qualification and validation, processing, tear down, turn-around time, and disposal all need to be considered in the cost analysis.

* We apply voluntary standards as requested by customers.

Additionally, the following questions should be addressed and factored into the cost analysis:

- Is it a single product facility or multi-product facility?
- What type of process is being run (e.g. mAb, vaccine, plasma, ADC, etc), and are there any special requirements such as containment?
- What scale is the process or specific unit operation being run at?
- What phase of manufacturing is the process in? Will it eventually need to be scaled up or scaled out to meet future demand?
- Is it a batch or continuous process?

At processing scales below 3000 liters, it is generally more efficient and cost effective to use single-use for media preparation. Adoption of single-use technologies helps minimize and control bioburden due to the fact that the systems typically come pre-sterilized, enable closed processing and are discarded after every use. The advent of aseptic and sterile connectors has enabled efficiencies in preparation, whether with stainless steel technology, a hybrid approach or fully single-use. Having the ability to make sterile connections without the use of a laminar flow hood or biosafety cabinet has added efficiency, enabled closed processing, and improved bioburden control.

Single-use plastic bags have specific handling requirements including everything from receipt in the warehouse and storage, through to unpackaging, installation and use. Many suppliers provide training, to ensure operators understand the proper techniques to minimize the risk of damage to the bags.

Mixing

One of the most common process tasks in the biopharmaceutical industry is the mixing of media solutions for use in upstream unit operations. This step increasingly utilizes single-use mixing systems to improve operational flexibility, decrease validation and process duration, and minimize the risk of contamination. Mixing of floating and sinking powders especially at high volume, however, presents substantial challenges in getting good dispersion and dissolution of particles.

The creation of a vortex and abundant surface movements are keys to success in mixing difficult to incorporate ingredients. Axial and radial flow patterns should allow for quick distribution of sinking powders, minimizing settling at the bottom of the vessel. Floating powders should be drawn into the vortex, allowing for effective wetting and distribution throughout the entire vessel volume. With the appropriate impeller and motor design, mixing time for even the most difficult to mix powders can be significantly reduced.

Unlike traditional stainless-steel mixers, single-use mixers reduce downtime because they don't need to undergo clean-in-place and steam-in-place operations. Single-use systems typically come pre-sterilized.

Filtration

There are no specific regulations or guidelines for sterile filtration of media. The level of bioburden reduction or process protection implemented is generally guided by internal risk assessments and will be based on experience, risk profile and the types and source of raw materials in the cell culture process. As cell culture media offer a rich environment for bioburden, it is critical to mix and filter the solutions as fast as possible to minimize the possibility of bioburden proliferation. Process validation for media preparation will define the pre-filtration hold time which should be sufficient to allow the solution to be batched, mixed and filtered, but not so long that there is an opportunity for bioburden proliferation.

The goal of filtering upstream media components is to prevent microbial contaminants entering a bioreactor and potentially disrupting supply continuity. All media raw material components have some level of bioburden, and understanding the risks presented by individual cell culture media components will help determine the type of filtration solution required. At a minimum, cell culture media should be processed through a 0.2 µm sterilizing-grade filter which meets the bacterial-retention testing requirements outlined in ASTM® standards.

There are multiple sterilizing-grade filter options dependent on the specifics of the fluid stream being processed: for fluid streams with high concentrations of particulates, high capacity filters offer efficient processing, and some have integrated prefilters that further improve processing efficiency. Alternatively, high area sterilizing-grade filters offer high particulate capacity in a small filter footprint. For non-fouling streams such as buffers, high flow filters enable rapid processing in a small filter area. Although sterilizing-grade filters remove bacterial contaminants, they do not provide complete protection against mycoplasma or other challenging microbial contaminants that could be present in cell culture media, and filters with a nominal pore size of 0.1 µm are often selected to provide increased assurance against these types of contaminants. Further risk reduction for upstream processes against potential adventitious viral contamination is now possible with the recent

introduction of virus filters specifically designed for efficient processing of cell culture media.

Understanding the potential contamination risks of the raw materials and the details of the process helps guide selection of the appropriate filter for processing cell culture media. Once selected, optimization of filter performance can increase the operational efficiency of media filtration providing confidence that sterility will be assured and that the filter meets the operational needs of the process while maintaining the performance attributes of the media.

Storage and Transfer

Ready-to-use media can be held or stored in various container types, based on the user's specific needs. Storage containers come in all types of sizes and materials and can be stackable, collapsible, cleanable, and mobile. They can also be customized with specific features, like load cells, jackets for heating and cooling, and tamper proof with temperature tracking. The decision regarding which container types and sizes is made by evaluating preparation needs and operations as a whole:

- Which media are required to support the upstream process?
- What is the total volume of media required to support the process?
- What are the pre-filtration and sterile hold times for each of the media?
- What are the storage conditions for each media?
- How much space is available in each storage area?
- Are there height and or floor space footprint constraints?
- Do the storage containers need to be heated, cooled or mobile?

Other key considerations for storage include stability and extractables. It is important to ensure that the media being stored remain stable over time and that the components of the storage container aren't interacting with the solution at the specified storage temperature, over the entire hold time.

Moving media from storage to production area is generally not considered a sophisticated operation. Considerations when moving media include the weight of the container, whether the container needs to be temperature controlled, the need for and type of wheels, and the distance to travel.

Nevertheless, it is one of the riskiest steps, both for the media and the workspace. As such, this transport action needs to be as easy and safe as possible, robust, compliant with storage conditions and the production area, and cost effective. Additionally, for "ready-to-use" media solutions, shipping regulations apply and must be addressed.

As part of the process design, and as a best practice, the floor plan should be laid out using actual equipment dimensions and placing tape on the floor. This allows operators to provide input to material flows and efficiency, while providing engineering with the information needed to best design the single-use assemblies that will be used in the process. The next step is to create prototypes of the designs and repeat the exercise. This will help identify required design changes to the assemblies, identify where supporting brackets, tubing tracks or organizational systems are needed, and assist with operator training.

Conclusion

There are risks at many points along the bioprocess workflow from sourcing, preparation, and handling to storage and transport of cell culture media. Proactive identification of possible risks and implementation of strategies to mitigate those risks will improve consistency and reliability of the process, and ultimately help ensure product quality. Working closely with an experienced, trusted partner for all aspects of cell culture medium selection, preparation, and handling should limit exposure to risks, and ensure the quality and consistency of supply for your production process.

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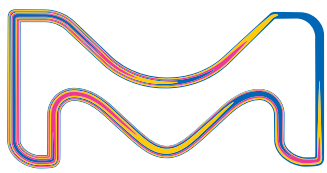

Lit. No. MK_WP3648EN Ver. 1.0
2019 - 20235
05/2019