Mitigating Risk for Transfer of Living Materials into Closed Systems

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Introduction

A critical factor for cell and gene therapy manufacturing is microbiological contamination control, for both patient safety and regulatory compliance. Clean, bio-decontaminated work areas and a closed process are common expectations in the regulatory guidance which is intended to reduce the risk of contamination entering a medicinal product. Scaling up from clinical development to commercial manufacture offers an opportunity to build a pragmatic process design which provides robust, and validated contamination control measures.

Simplicity in design has a positive influence on contamination control. Particularly as cell therapy manufacture has a complex process flow. The FDA Guidance for Human Somatic Cell Therapy and Gene Therapy emphasizes the importance of preventing adventitious contamination when handling human cells. Many of the manual steps that are or used to be performed in single or multiple biosafety cabinets with multiple manual transfers of materials can now be done using closed systems designed to 'close the process' to reduce the risk of contamination entering the critical process. The Pharmaceutical Inspection Co-operation Scheme (PIC/S), Good Manufacturing Practices for Advanced Therapy Medicinal Products, and European Commission (EC) Good Manufacturing Practice for ATMPs, Volume 4, provide guidance for closed systems. Furthermore, FDA Guidance for Industry: Sterile Drug Products states that the "use of an isolator system further enhances product protection".

Manual activities have a high risk for introducing contamination, so performing them in biosafety cabinets (not built for a closed system) within cleanrooms may not be the best choice. Advanced technologies exist that have assisted in improving the design of the complex process.

Isolators are the best practice expectation for performing sterility testing in a QC laboratory, or fill/finish activities in a production environment. The intended outcome of this design is microbial contamination control. The same consideration is used for the process steps in advanced therapy manufacture. Closing and automating this process adds value to the manufacturing operation and can save development and production time.

Isolator technology provides significant benefits in the contamination control of biopharmaceutical processes:

- A fixed barrier between the critical product/process and the external environment (including the operator) addresses the most common cause of Grade A microbial recoveries, which are typically from human commensal species.
- A validated, automated, 6-log sporicidal bio-decontamination of the surfaces of the processing environment and materials loaded into chamber.

A critical factor in maintaining an aseptic environment in an isolator is the transfer of living materials (human cells) into the system and monitoring their viability. Automated bio-decontamination of living materials may not be possible due to their sensitivity to the sporicidal agent used for bio-decontamination (hydrogen peroxide vapor) which may penetrate into the container, particularly if it has a porous membrane to facilitate the exchange of gases. Also, the temperatures achieved during the bio-decontamination process may affect living cells. Therefore, other methods may be required to transfer living materials into closed systems.

> **"The hazards introduced from entry or removal of items during processing should be minimized and supported by high capability transfer technologies or validated systems that robustly prevent contamination and are appropriate for the respective technology."** EU Annex 1 2022 Section 4.18

References

1. EU GMP Annex 1 2022 Section 4.3
2. EU GMP Annex 1 2022 Section 4.18
3. Effects of residual H2O2 on the growth of MSCs after decontamination - PMC (nih.gov)
4. https://www.hindawi.com/journals/omcl/2018/4081890
5. FDA Guidance for Human Somatic Cell Therapy and Gene Therapy (March, 1998)
6. Pharmaceutical Inspection Co-operation Scheme (PIC/S) Good Manufacturing Practices for Advanced Therapy Medicinal Products (Annex 2A, February 2022)
7. European Commission (EC) Good Manufacturing Practice for ATMPs, Volume 4 (November, 2017)

Methods for transfer of living materials into an isolator

Manual transfer with manual application of disinfectant (e.g. 70% isopropyl alcohol, IPA)

Benefits

- IPA does not damage living cells like H_2O_2 can
- A hierarchy of material transfer controls can be implemented to enable sample transfer whilst protecting the isolator from the surrounding environment, including:
  - Transfer hatch with interlocking doors
  - Pressure cascade to protect the hatch from the surrounding environment
  - Localized Grade A unidirectional downflow with validated air cleanup time before exposing the isolator to the transfer hatch

Risks

- The transfer hatch is not bio-decontaminated, relying on the operator not to touch the internal surfaces to prevent entry of contamination into the aseptic environment
- IPA is not sporicidal so still has a risk of bringing contamination into the aseptic environment

Rapid Transfer Ports (RTPs)

- RTP beta containers can be autoclaved empty and the living material/sample transferred into the container under unidirectional air flow (laminar flow hood)
- Alternatively, if the living material is a liquid product, it is possible to keep the bag of material outside of the isolator and transfer it in via a port (e.g. Aseptic Technologies Port) with use of a peristaltic pump. Alternatively, a capped tube can be passed into an isolator through a tri-clover fitting, the isolator bio-decontaminated, and the tube cut to remove the cap for the liquid to be delivered either by gravity or squeezing the bag. This may be beneficial for living materials which can be damaged by passing through a peristaltic pump.

Use of hydrogen peroxide vapor or 6% peroxide wipes

Despite the sensitivity of living materials to hydrogen peroxide, use of hydrogen peroxide bio-decontamination may be possible depending on the level of ingress into the sample, the resistance of the container to hydrogen peroxide and the resistance of the specific cell line to hydrogen peroxide. Ingress testing can be performed by the hydrogen peroxide vapor supplier to assess risk to the sample from H_2O_2.

Then it needs to be determined what concentration level is acceptable based on the specific cell lines. One study has shown that H_2O_2 concentrations above 0.5 ppm can be damaging to mesenchymal stem cells (MSC) cell lines; but different cell lines have different tolerances. For example, stem cells can be more resistant to oxidative stress from H_2O_2 than mature cells.

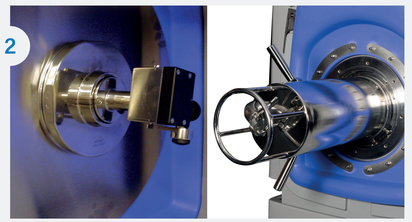
Conclusion

The options for transfer of living materials include:

1

Manual disinfection of containers carries a higher risk of introducing contamination into an isolator due to IPA lacking sporicidal activity but is typically quicker than an automated bio-decontamination process and avoids the risk of damaging living materials.

2



The use of **Rapid Transfer Ports (RTPs)** provides a lower contamination risk but may not be practical as it includes an extra step of sample transfer under unidirectional air flow. Keeping the material outside of the isolator and transferring it in through a sterile liquid connection offers a very low risk of introducing contamination into the process, so it should be the ideal process to strive for.

3

The **automated bio-decontamination** solution is the most robust method for contamination control but may not be possible. Ingress testing should be conducted to determine whether the container is compatible with hydrogen peroxide vapor bio-decontamination.

Keep in mind that process design alone cannot ensure robust contamination control. Most engineering controls require a robust infrastructure and ongoing training and oversight of operator activities. Taking the right approach to preventing microbial contamination is inclusive of stepwise training and learning. It is significantly important that operators must be trained appropriately to perform with consistent aseptic skill and behavior which will ensure consistency for a stable and effective contamination control program.