Are new options needed for primary packaging? It is time to address particulates and fractures.

S. Werner, A. Shields, K. Aoki, M. Eitner, M. Said
BioLife Solutions, Bothell Washington

Abstract

Primary containers for Cell and Gene Therapies (CGT) are critical components of manufacturing that carry high potential risk to CGT product safety and performance. The presence of particulates in CGT products has been a challenging issue to address during the early stages of the CGT industry evolution. Significant challenges remain in addressing detection, identification, prevention, and resolution. While recent industry experience demonstrates that the presence of particulates is now a focus for developers and regulators, it is unclear how developers can appropriately define and mitigate this risk. Particulates in this industry can be introduced from extrinsic aspects of the manufacturing process (for example environment, culture ware and processing equipment) or be intrinsic to the therapy itself (cells, aggregates, scaffolds, etc.). This makes it clear that the traditional standard of essentially free from visible particulate cannot be the appropriate standard for some of these treatment modalities. However, validation of the process, including analysis of the source and identification of particulates due to the consumables and systems used, of CGT manufacturing is the starting point to characterize the risks and begin to identify effective control strategies. One known source of particulates in the CGT industry is often the primary container. In this study, we evaluated a range of commercially available primary containers for the presence of visible and subvisible particulate. Of the available options, soft-sided bags were found to carry the highest risk of visible particulate contamination while cryovials had a lower risk. Newer primary packages were found to have substantially lower presence of particulates along with improved ability to visually inspect. Fracture risk was also evaluated as fracture at cryogenic temperatures remains a risk during transport and distribution. Of the packaging options evaluated, rigid containers manufactured with cyclic olefin-based polymers were significantly easier to handle and carried reduced fracture risk. These data suggest that new advancements in primary packaging should assist CGT developer mitigation of these critical risks.

Materials and Methods

Commercially available bioprocessing containers were purchased either directly from suppliers or distributors. Containers were filled with cryomedia or LAL water. For freezing studies of CryoCase, units were willed with tryptic soy broth under aseptic conditions. The BioLife Solutions CryoCase is a late-stage prototype of a novel, rigid container primarily of COC and EVA materials designed for cryogenic storage of volumes between approximately 15ml and 75ml.

The clinical basis for particulate control is generally broken into several distinct possible sequelae. However, generally, it has been difficult to assess level of risk even though the potential hazards can be significant. Associated risks may include:

- Embolization
- Host response
- Biological or chemical reactivity
- Microbial contamination

Risk analysis of the intended use should be taken into consideration when determining specifications and requirements.

> Applicants should consider these clinical risk factors when developing their quality target product profile and in establishing an appropriate control strategy and acceptance criteria for visible particulate.
>
> Draft guidance from FDA (Docket FDA-2021-D-0241)
>
> Ultimately, the safety considerations related to particulate matter in injections must be assessed for each drug product, intended patient population, and method of administration. No single set of inspection criteria can adequately anticipate all potential risks to the patient
>
> USP <1790>
>
> Despite the publication of these chapters, factors such as the probabilistic nature of visible particulate detection, complex patient risk assessments, difficult to inspect products, and the current good manufacturing practice (CGMP) implications of certain types of visible particulate matter contamination pose significant challenges to the implementation and execution of a robust visible particulate prevention and detection program.
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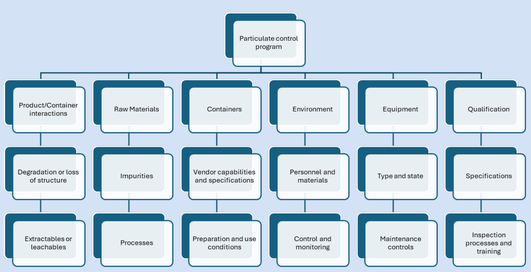
> PDA JPST May 2020, 74 (3) 359-366

Sources and evaluation of particulates



Evaluation of Commercially Available Containers

Multiple commercially available bioprocessing or cryostorage containers were filled with a clear, colorless solution and evaluated for the presence of visible particulates. Containers were evaluated at an inspection station with white and black fields for 5 seconds in front of each field.

Particulate Evaluation		
Bag Type	Number of Bags Filled	% Particulate Rejects
EVA Bag 1	38	30%
LDPE Bag 1	40	50%
EVA Bag 2	10	30%
LDPE Bag 2	10	100%
FEP Bag	10	60%
ULDPE	15	53%
Fluoropolymer	>50	<10%

Evaluation of the BioLife CryoCassette

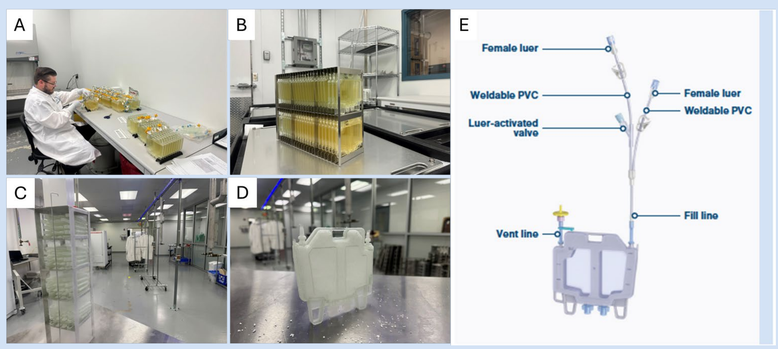
Prototype units of the BioLife Solutions CryoCase were evaluated under two inspection conditions. First, 30 units were inspected for 5 seconds in front of a white background and 5 seconds in front of a black background. In the second condition, 10 units were inspected for 60 seconds in front of a black background. Under the conditions of the study, no particulates of fibers were identified.

Particulate Evaluation		
CryoCase	Number of Bags Filled	# of particulates identified
5 Second evaluation	30	0
60 second evaluation	10	0

The BioLife CryoCase is a new rigid container intended to be an alternative cryo-container for storage of cellular material or other high value samples that require storage at temperatures down to -196 °C.

The container is designed to have the following characteristics:

- Improved particulate profile
- Fracture resistance at cryotemperatures
- Improved handling and Inspectability
- Consistent freezing profiles
- Compatibility with automated systems
- Reduced storage footprint
- Elimination of metal storage cassette



A) Sealing fill and vent tubes of CryoCases filled to 75ml with Tryptic Soy Broth. B) Filled CryoCase containers loaded into a rack for cryopreservation in a CRF or storage in -80 °C freezer. C, D) CryoCase containers immediately after removal from liquid nitrogen storage. E) Features of the fill and vent lines of the CryoCase container.

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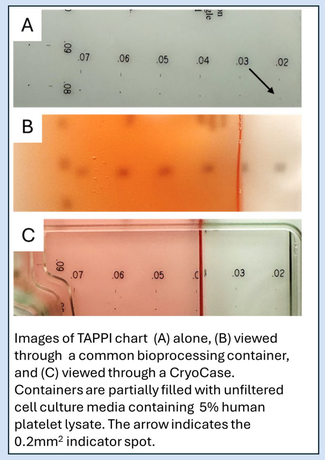
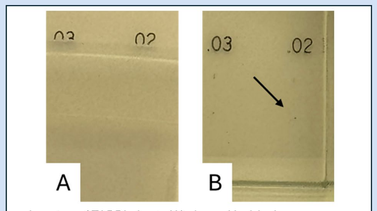
Inspectability

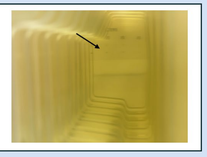
Manual visual inspection is not expected to capture 100% of defects.
Visible particles are generally considered to be particles >100 microns (<0.02 mm^2)
Reliability of human detection in single use consumables approaches 70% at ~500microns (~0.2mm^2)(ref.)



Images of TAPPI chart (A) alone, (B) viewed through a common bioprocessing container, and (C) viewed through a CryoCase. Containers are partially filled with unfiltered cell culture media containing 5% human platelet lysate. The arrow indicates the 0.2mm^2 indicator spot.



Images of TAPPI chart (A) viewed behind a bioprocessing container or (B) the CryoCase. The containers are filled with a 90% DMSO solution. The arrow indicates the 0.2mm^2 indicator spot.

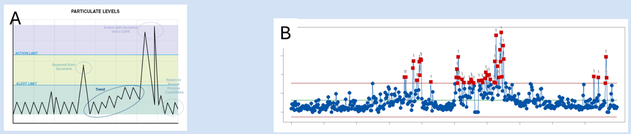


TAPPI chart viewed through 10 CryoCase inspection windows. Containers were filled with Tryptic Soy Broth. Arrow indicates 0.2mm^2 indicator.

Inspection of cellular products

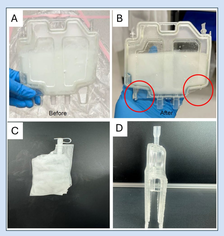
Cellular suspensions are a difficult to inspect product in the absence of obscuring factors from the primary container. A) MSC cell suspension at 16x10^6 cells/ml in a CellSeal cryovial. B) MSC cell suspension at 0.8x10^6 cells/ml stored in a CellSeal CryoCase.

Particulate Control Program: Life Cycle Approach



Monitoring particulates throughout the use of a single use consumable. A) Conceptual model of particulate changes over time. There can be cycles of particulates changes over time, moving trends, and unique problems. Action and alert limits should be specified (adapted from 2020 Particulate Paper, BPSA). B) A control chart over 24 months showing variable particulate levels detected in fluid filled single use consumables monitored over time.

Addressing fracture risks



The Cyclic olefin co-polymer used for CellSeal vials and the CellSeal CryoCase is fracture resistant at -196 °C. A) CryoCase immediately after removal for srtorage in vapor phase liquid nitrogen. B) 10 frozen CryoCases were dropped from approximately 2 meters. The only observed failures were at the protective shock absorption legs. C) 5 cryobags were dropeed from approximately 2 meters. All 5 units exhibited catastrophic failures. D) Cross sectional view of the COC walls and weld bead of the CryoCase.