The average number of particles present in the units tested should not exceed 25/mL equal to or greater than 10 μm and should not exceed 3/mL equal to or greater than 25 μm. Also, total particle load should not exceed 6000 per container equal to or greater than 10 μm and should not exceed 600 per container equal to or greater than 25 μm.

Products that are used with a final filter during administration (in-line) are exempt from these requirements, providing that scientific data are available to justify the exemption. However, filtrates are expected to comply with the guideline. For products supplied or first reconstituted in <100 mL, and then diluted for infusion in a volume >100 mL, particle content should be assessed both before and after dilution and evaluated based on their final volume.

MICROSCOPIC PARTICLE COUNT TEST

As noted, the LO method is the preferred method for therapeutic protein injections and parenteral infusions. However, the microscopic method may be used when appropriate, such as determination of extrinsic and intrinsic particle types only. It should be demonstrated, however, that particular classes of particles (e.g., inherent) are also being counted when using this method. For the determination of product acceptability, apply the limits for the membrane microscopic test in general chapter ⟨788⟩. Because of the interference of some protein particles and their physical characteristics (fragile or translucent), the results of the *Microscopic Particle Count Test* are not equivalent to those of the *Light Obscuration Particle Count Test*, and the two methods cannot be considered interchangeable. For further guidance, see general information chapter ⟨1787⟩. ■1S (*USP37*)

Add the following:

▪⟨790⟩ VISIBLE PARTICULATES IN INJECTIONS

All products intended for parenteral administration must be visually inspected for the presence of particulate matter as specified in *Injections* ⟨1⟩. Dry solids, from which constituted solutions are prepared for injection, meet the requirements for *Constituted Solutions* in *Injections* ⟨1⟩ when they are prepared just prior to use. Where used in this chapter, the term *essentially free* means that when injectable drug products are inspected as described herein, no more than the specified number of units may be observed to contain visible particulates. Particulate matter is defined in *Particulate Matter in Injections* ⟨788⟩ as extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in solutions. Examples of such particulate matter include, but are not limited to, fibers, glass, metal, elastomeric materials, and precipitates. However, some products, such as those derived from proteins, may contain inherent particles or agglomerates; in such cases, requirements for visible particulates are specified in the individual monograph or in the approved regulatory application.

Where the nature of the contents or the container–closure system permits only limited capability for inspection of the total contents, the 100% inspection of a batch shall be supplemented with the inspection of constituted (e.g., dried) or withdrawn (e.g., dark amber container, suspensions, highly-colored liquids) contents of a sample of containers from the batch. The destructive nature of these tests requires the use of a sample smaller[1] than those traditionally used for non-destructive acceptance sampling after 100% inspection. While the tests described in this chapter may be useful during studies to examine product stability, this chapter is not intended to establish any new testing requirements for stability studies.

Inspection Procedure

Used along with 100% inspection during the manufacturing process, this procedure is sufficient to demonstrate that the batch is essentially free of visible particulates. A complete program for the control and monitoring of particulate matter remains an essential prerequisite.

Inspected units must be free from visible particulates when examined without magnification (except for optical correction as may be required to establish normal vision) against a black background and against a white background. Illumination at the inspection point is maintained at a minimum intensity between 2000 and 3750 lux. This can be achieved through the use of two 13-W or 15-W fluorescent lamps (e.g., F13/T5 or F15/T8). The use of a high-frequency ballast to reduce flicker from the fluorescent lamps is recommended. Alternative light sources (e.g., incandescent, LED) that provide illumination at the point of inspection within the specified minimum intensity range are acceptable. Higher illumination intensity is recommended for examination of colored solutions or product in containers other than clear glass.

Before performing the inspection, remove any adherent labels from the container, and wash and dry the outside. The unit under inspection should be gently swirled and/or inverted, ensuring that no air bubbles are produced, and inspected for approximately 5 s against each of the backgrounds. The presence of any particles should be recorded.

[1] The special level sampling plans described in ANSI/ASQ Z1.4–2008 or ISO 2859 are appropriate to guide the selection of sample size and acceptance criteria for this purpose.

Sampling at Batch Release (Following 100% Manufacturing Inspection)

Sample and inspect the batch using ANSI/ASQ Z1.4 or ISO 2859-1). General Inspection Level II, single sampling plans for normal inspection with an AQL of 0.65%. Alternative sampling plans with equivalent or better protection are acceptable. Not more than the specified number of units contains visible particulates.

Product in Distribution[2]

If it becomes necessary to evaluate product that has been shipped to customers (e.g., because of a complaint or regulatory concern), sample and inspect 20 units. If no particles are observed in the sample, the batch is considered essentially free of visible particulates. If available, additional units may be inspected to gain further information on the risk of particulates in the batch.

■1S (*USP37*)

[2] Testing outlined in *Product in Distribution* is permissible only if *Sampling at Batch Release (Following 100% Manufacturing Inspection)* has been successfully completed.

General Chapters

General Information

Add the following:

▪⟨1044⟩ CRYOPRESERVATION OF CELLS

INTRODUCTION

Cryopreservation is the process of cooling and storing cells, tissues, or organs at very low temperatures to maintain their viability. The purpose of cryopreservation is to bank the cells and allow their future use in in vitro or in vivo applications for which post-thaw function is sufficiently representative of the cells' prefreeze function. Cryopreservation also minimizes the risk of genetic mutation or development of subpopulations due to cell replication. Depending on the application, sufficient post-cryopreservation function may be assessed by the ability to divide, proliferate, differentiate, express genes, or to produce proteins, or by another specific functional property.

This chapter presents best practices for cryopreservation, maintenance, and use of a wide range of cells, cell therapy products, and cell banks derived from a variety of sources including human, animal, and microbial cultures (the chapter also contains an *Appendix* with additional guidance documents that are useful for particular cell types and applications). Cryopreserved cells provide a ready source of viable cells that can be used, either directly or indirectly, for the purposes of diagnostic tests, therapy, manufacture of drug products and vaccines, and for bioassays used to evaluate the potency of therapeutic drugs and vaccines. In some cases the cells themselves, after cryopreservation and thaw, constitute the patient therapy, and in other cases the cells are propagated or otherwise manipulated ex vivo in order to generate the product (e.g., a culture-expanded cellular therapy, a therapeutic protein, a monoclonal antibody, or a vaccine). In all cases, proper cryopreservation is essential for retention of required cellular properties and, ultimately, for application toward the advancement of patient therapies.

PRINCIPLES OF CRYOPRESERVATION

Overview

Understanding the role of water and the need to adequately remove it from cells or abrogate its ability to form ice crystals, which damage the cell membrane, is critical to successful cryopreservation. When cells are frozen in aqueous suspension, often they are destroyed. However, in the 1940s Polge and others discovered the cryoprotective properties of glycerol. Since then several chemicals, generically called *cryoprotectant agents* (CPAs), have been identified. The mechanism of action of CPAs is complex and is not fully understood. However, according to the commonly accepted theory of *colligative* action, CPAs increase solute concentration both within the cell and extracellularly, thereby suppressing ice formation. For this purpose, the so-called penetrating (or intracellular) CPAs [e.g., dimethylsulfoxide (DMSO), glycerol, propanediol, and methanol] must be able to cross the cell membrane readily and penetrate the cell without significant toxicity. There also is a group of nonpenetrating (or extracellular) CPAs (e.g., sucrose and trehalose) whose mechanism of action is thought to be related at least in part to their stabilizing interaction with cell membranes. This property also may explain the cryoprotective activities of certain large molecular weight compounds such as hydroxyethyl starch and polyvinylpropylene. Theoretical models of cryoprotection typically evoke the colligative theory, but full explanation of CPA action is yet to be established.

An alternative form of cell preservation, commonly called *vitrification,* whereby the cell suspension is loaded with high levels of penetrating CPAs (often several in combination), induces a glass-like state in which cellular and extracellular water cannot readily form ice crystals. When cell suspensions prepared in this way then are cooled very rapidly (cooling rates of 100°–1000°/min or more) the extreme viscosity prevents osmosis, and the water molecules are unable to form ice. This procedure has been widely used for complex structures including a variety of human, plant, and animal tissues and may help preserve those cell preparations that have variable degrees of cellular permeability or when standard cryoprotection cannot deliver the range of conditions required to optimally preserve viability in all the tissues' component cell types.

CPAs have biological activities beyond their cryoprotective properties. Some, like DMSO, can affect the cell membrane, cytoskeleton, and gene expression and may be toxic to cells following prolonged exposure. Therefore, during development of