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Standard Guide for Subvisible Particle Measurement in Biopharmaceutical Manufacturing Using Dynamic (Flow) Imaging Microscopy[1]

This standard is issued under the fixed designation E3060; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 Biotherapeutic drugs and vaccines are susceptible to inherent protein aggregate formation which may change over the product shelf life. Intrinsic particles, including excipients, silicone oil, and other particles from the process, container/closures, equipment or delivery devices, and extrinsic particles which originate from sources outside of the contained process, may also be present. Monitoring and identifying the source of the subvisible particles throughout the product life cycle (from initial characterization and formulation through finished product expiry) can optimize product development, process design, improve process control, improve the manufacturing process, and ensure lot-to-lot consistency.

1.2 Understanding the nature of particles and their source is a key to the ability to take actions to adjust the manufacturing process to ensure final product quality. Dynamic imaging microscopy (also known as flow imaging or flow microscopy) is a useful technique for particle analysis and characterization (proteinaceous and other types) during product development, in-process and commercial release with a sensitive detection and characterization of subvisible particles at ≥2 µm and ≤100 µm (although smaller and larger particles may also be reported if data are available). In this technique brightfield illumination is used to capture images either directly in a process stream, or as a continuous sample stream passes through a flow cell positioned in the field of view of an imaging system. An algorithm performs a particle detection routine. This process is a key step during dynamic imaging. The digital particle images in the sample are processed by image morphology analysis software that quantifies the particles in size, count, image intensity, and morphological parameters. Dynamic imaging particle analyzers can produce direct determinations of the particle count per unit volume (that is, particle concentration), as a function of particle size by dividing the particle count by the volume of imaged fluid (see Appendix X1).

1.3 This guide will describe best practices and considerations in applying dynamic imaging to identification of potential sources and causes of particles during biomanufacturing. These results can be used to monitor these particles and where possible, to adjust the manufacturing process to avoid their formation. This guide will also address the fundamental principles of dynamic imaging analysis including image analysis methods, sample preparation, instrument calibration and verification and data reporting.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E2589 Terminology Relating to Nonsieving Methods of Powder Characterization

2.2 *ISO Standards:*[3]

ISO 3951-1 Sampling Procedures for Inspection by Variables

ISO 8871 Elastomeric Parts for Parenterals and for Devices for Pharmaceutical Use

ISO 9276-6 Representation of Results of Particle Size Analysis Part 6: Descriptive and Quantitative Representation of Particle Shape and Morphology

[1] This guide is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical and Biopharmaceutical Products and is the direct responsibility of Subcommittee E55.03 on General Pharmaceutical Standards.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

2.3 *Other Standards:*

ANSI/ASQ Z1.9-2003 Sampling Procedures and Tables for Inspection by Variables for Percent Nonconforming[3]

ASME BPE-2022 Bioprocessing Equipment[4]

USP <787> Subvisible Particulate Matter in Therapeutic Protein Injections[5]

USP <788> Particulate Matter in Injections[5]

USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems[5]

USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging Delivery Systems[5]

USP <1787> Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections[5]

USP <1788> Methods for Determination of Subvisible Particulate Matter[5]

USP <1788.3> Flow Imaging Method for the Determination of Subvisible Particulate Matter[5]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology E2589.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *aspect ratio, n*—ratio of particle width to particle length.

3.2.1.1 *Discussion*—The definition of particle width and length depends on the image analysis software being used, and reported aspect ratio may not be interchangeable between different software packages.

3.2.2 *binary image, n*—a transformation of a camera image into pixels identified as particles and pixels identified as background.

3.2.3 *brightfield illumination, n*—a method of providing light into a measurement space whereby the illuminated objects are located between the light source and the viewing receiver.

3.2.4 *circularity, n*—degree to which a particle (or its projection area) is similar to a circle, mathematically expressed as $4\pi A/P^2$, where A is particle area and P is particle perimeter.

3.2.5 *cumulative particle size distribution, n*—a representation, as a table, graph, or mathematical function, that gives the total fraction or concentration of particles greater than or less than a set of specified size values.

3.2.5.1 *Discussion*—Cumulative particle size distributions may be expressed as either mass, volume, area, number, or concentration values.

3.2.6 *depth of field, n*—the distance along the optical axis between the nearest and farthest objects that are in acceptably sharp focus in an image.

3.2.7 *dynamic imaging, n*—particle size and shape analysis using computer image analysis techniques on instantaneously captured still frame projected images of particles in motion (also referred to as *flow imaging*, *flow microscopy*, *direct imaging*).

3.2.8 *equivalent diameter, n*—the diameter of a sphere or circle with the same particle volume or area measured by a particle sizing instrument.

3.2.8.1 *Discussion*—For dynamic imaging, the equivalent diameter is calculated from the projected area of a measured particle.

3.2.8.2 *Discussion*—Depending on the choice of software and software settings, the projected area may have any holes in the image filled or left unfilled.

3.2.9 *extrinsic particle, n*—an unexpected particle introduced from sources that are foreign or external to the manufacturing process.

3.2.10 *Feret diameter, F, n*—apparent diameter of an object determined from the distance between two parallel tangents on opposite sides of a binary object.

3.2.10.1 *Discussion*—There are an infinite number of Feret diameters; the maximum and the minimum Feret find most use within imaging.

3.2.11 *field of view, n*—the two dimensional, lateral extent of the imaged area.

3.2.12 *frequency distribution, n*—a representation, as a table, graph, or mathematical function, that gives the frequency or count of values within a set of specified intervals.

3.2.13 *inherent particle, n*—a particle made entirely of components of the formulated drug product or its manufacturing intermediate, arising from the product itself.

3.2.14 *intrinsic particle, n*—a particle composed of materials that the product or intermediate contacts or is mixed with during the manufacturing process or during storage in primary packaging components.

3.2.15 *particle, n*—mobile, self-contained, and undissolved objects.

3.2.15.1 *Discussion*—In the context of particle counting instruments, the term particle may be used to designate any self contained object that is optically distinguishable from the background image, including a liquid droplet or gas-phase bubble.

3.2.16 *particle size distribution (PSD), n*—a frequency or volume distribution of the concentration of particles versus particle size.

3.2.16.1 *Discussion*—Dynamic imaging particle analyzers of use to the biopharmaceutical industry report the PSD as the concentration of particles per unit volume within specified size ranges, where the size is most commonly the equivalent diameter but may be another morphological size attribute. See Appendix X1.

3.2.17 *subvisible particle, n*—a particle with a measured equivalent diameter within the approximate range 1 µm to 100 µm.

NOTE 1—When it is necessary to specify an exact size range, the range should be defined explicitly rather than by such adjectives as subvisible.

3.2.17.1 *Discussion*—The 100 µm upper limit is based on

[4] Available from American Society of Mechanical Engineers (ASME), ASME International Headquarters, Two Park Ave., New York, NY 10016-5990, http://www.asme.org.

[5] Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.