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Standard Guide for Development of Automated Membrane Microscopy Test Methods for the Counting and Sizing of Particulate Matter Present in Parenteral Pharmaceutical Manufacturing Processes and Final Drug Products¹

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1. Scope

1.1 This guide provides guidance on the development of test methods that apply automated membrane microscopy (AMM) to the measurement of extraneous solid insoluble particulate matter present in parenteral pharmaceutical manufacturing processes and drug products. For the validation requirements for AMM test methods, see Practice E3411.

1.2 In an AMM test method, a test liquid containing suspended particles is filtered through a membrane filter, the particles are retained on the surface of the membrane filter, the membrane filter surface is imaged with an optical microscope and digital camera, and application of image analysis software determines particle count and particle sizes.

1.3 AMM test methods may be applied to the measurement of the commonly defined size categories of subvisible ($<100\ \mu\text{m}$) or visible ($\geq 100\ \mu\text{m}$) or both particulate matter present during any stage of the manufacturing of parenteral pharmaceuticals.

1.4 The test liquid characterized by an AMM test method may be a process fluid, drug substance or drug product, or liquid extracts from the surfaces of processing equipment, drug containers, delivery devices, and components thereof.

1.5 This guide does not apply to the characterization of particles inherent to parenteral suspensions (for example, cells, protein aggregates, or vaccine adjuvants) or the characterization of liquid droplets (for example, silicone oil).

1.6 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *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 appro-*

priate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.

1.8 *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

E1169 Practice for Conducting Ruggedness Tests

E3060 Guide for Subvisible Particle Measurement in Biopharmaceutical Manufacturing Using Dynamic (Flow) Imaging Microscopy

E3230 Practice for Extraction of Particulate Matter from the Surfaces of Single-Use Components and Assemblies Designed for Use in Biopharmaceutical Manufacturing

E3411 Practice for Validation of Automated Membrane Microscopy Test Methods for the Counting and Sizing of Particulate Matter Present in Parenteral Pharmaceutical Manufacturing Processes and Final Drug Products

2.2 ICH Guidelines:³

ICH Q2 Validation of Analytical Procedure—Scientific Guideline

ICH Q9 Quality Risk Management

2.3 ISO Standard:⁴

ISO 8536-4:2019 Infusion Equipment for Medical Use—Part 4: Infusion Sets for Single Use, Gravity Feed

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

³ Available from International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), ICH Secretariat, Route de Pré-Bois, 20, P.O. Box 1894, 1215 Geneva, Switzerland, <https://www.ich.org>.

⁴ Available from International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, <https://www.iso.org>.

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 E55.07 on Single Use Systems.

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ISO 8871-3:2003 Elastomeric Parts for Parenterals and for Devices for Pharmaceutical Use—Part 3: Determination of Released Particle Count

ISO 13322-1:2014 Particle Size Analysis—Image Analysis Methods—Part 1: Static Image Analysis Methods

ISO 16232:2018 Road Vehicles—Cleanliness of Components and Systems

2.4 USP Chapters:⁵

USP <788> Particulate Matter in Injections

USP <1058> Analytical Method Qualification

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

USP <1788> Methods for the Determination of Subvisible Particulate Matter

USP <1788.1> Light Obscuration Method for the Determination of Subvisible Particulate Matter

USP <1788.2> Membrane Microscope Method for the Determination of Subvisible Particulate Matter

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

2.5 VDI Standard:⁶

VDI 2083 Part 21 Cleanliness of Medical Devices in the Manufacturing Process

2.6 Other Standards:

EP 2.9.19 Particulate Contamination: Sub-visible Particles, European Pharmacopoeia⁷

JP 6.07 Insoluble Particulate Matter Test for Injections, Japanese Pharmacopoeia⁸

NIST 8634 Ethylene Tetrafluoroethylene for Particle Size Distribution and Morphology⁹

3. Terminology

3.1 Definitions:

3.1.1 *analysis membrane*, *n*—membrane filter with particles retained on the membrane filter surface as created by a filtration procedure.

3.1.2 *analysis membrane occupancy*, *n*—percentage of the total inspected area of the analysis membrane covered by particles.

3.1.2.1 *Discussion*—Total area covered by all detected particles divided by the inspection area.

3.1.3 *automated membrane microscopy (AMM) instrument*, *n*—instrument consisting of an optical microscope, a motorized scanning stage to hold and allow scanning of an analysis membrane, a digital camera to capture images of the analysis

membrane as it is scanned, and image acquisition and image analysis software for implementing the image acquisition and analysis process.

3.1.4 *automated membrane microscopy (AMM) test method*, *n*—application of an AMM instrument to determine the count and sizes of particles on an analysis membrane via a test liquid preparation procedure, a filtration procedure, an image acquisition process, and an image analysis process.

3.1.5 *blank count*, *n*—particle count obtained upon executing a blank test.

3.1.6 *blank liquid*, *n*—nominally particle-free liquid applied in the blank test.

3.1.7 *blank test*, *n*—determination of the blank count by application of all steps of an AMM test method using a blank liquid.

3.1.8 *bright-field microscopy*, *n*—a form of optical microscopy in which the sample is illuminated by a light beam that passes through the sample, and the microscope objective detects both the transmitted beam as well as light scattered from the sample.

3.1.9 *dark-field microscopy*, *n*—a form of optical microscopy in which the sample is illuminated by a transmitted beam, using an annulus of light at an angle large enough so that unscattered light of the incident beam will not be detected by the microscope objective.

3.1.10 *equivalent circular diameter*, *n*—diameter of a circle that has a total area equal to the projected area of a particle found after image segmentation.

3.1.10.1 *Discussion*—The equivalent circular diameter is the output of a USP <788> test method and may differ significantly from the maximum Feret diameter (see Fig. 1). In the segmentation process, if holes in a particle are filled, the equivalent circular diameter will increase.

3.1.11 *Feret diameter*, *n*—measure of particle size along a specified direction defined as the distance between two parallel tangents to the outer contour of the particle along the specified direction (analogous to a caliper or slide gauge measurement).

3.1.12 *filtration procedure*, *n*—filtration of a test liquid through a membrane filter to create an analysis membrane.

3.1.13 *image acquisition process*, *n*—process by which the analysis membrane is brought into focus, moved by a motorized scanning stage, scanned by the camera field of view, and each field of view imaged by the digital camera.

3.1.14 *image acquisition software*, *n*—software program applied in the image acquisition process.

3.1.15 *image analysis process*, *n*—implementation of image analysis software to perform the stitching and the segmentation processes and analyze the resulting segmented image to determine particle count and particle sizes.

3.1.16 *image analysis software*, *n*—software program applied in the image analysis process.

3.1.17 *imaging system*, *n*—part of the AMM instrument consisting of the microscope optics, digital camera, and illumination system.

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

⁶ Available from Verein Deutsche Ingenieure (VDI), e.V. VDI-Platz 1 40468 Düsseldorf, Germany, <https://www.vdi.de>.

⁷ Available from the European Directorate for the Quality of Medicines & Healthcare (EDQM), Council of Europe, Avenue de l'Europe F-67075 Strasbourg Cedex, France, <https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-11th-edition>.

⁸ Available from the Pharmaceuticals and Medical Devices Agency (PMDA), Shin-Kasumigaseki Building, 3-3-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-0013 Japan, <https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0029.html>.

⁹ Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, <http://www.nist.gov>.

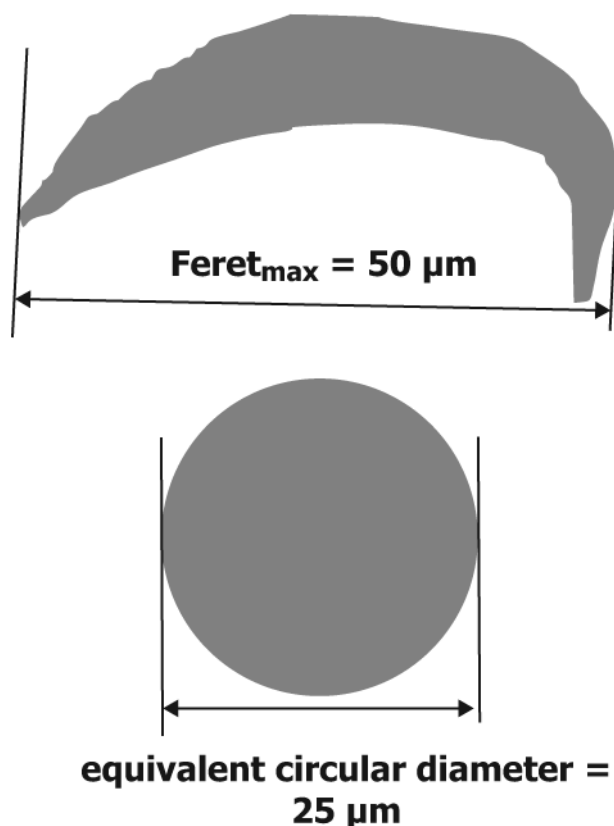


FIG. 1 (Top) Example of an Irregularly Shaped Particle with a Maximum Feret Diameter of 50 μm and (Bottom) Based on the Total Area of the Irregularly Shaped Particle, the Equivalent Circular Diameter (ECD) for the Irregularly Shaped Particle is 25 μm

3.1.18 *liquid extract*, *n*—test liquid obtained by liquid extraction (rinsing, washing, and flushing) of the surfaces of a material.

3.1.19 *magnification calibration standard*, *n*—stage micrometer with an International System of units (SI) traceable size scale applied in primary calibration of the AMM instrument to determine pixel size in the microscope images.

3.1.20 *manual visual check*, *n*—visual comparison of the images of individual particles obtained after the image acquisition process with the corresponding segmented images of the particles obtained after application of the image analysis process.

3.1.21 *maximum Feret diameter*, *n*—largest diameter of the particle found when measuring the Feret diameter in all directions.

3.1.22 *membrane filter*, *n*—membrane of specified type and pore size rating that retains particles upon filtration.

3.1.22.1 *Discussion*—Membrane filter type is specified by material, pore size rating, color, and diameter.

3.1.23 *particle, particulate, and particulate matter*, *n*—portion, piece, or fragment of mobile solid and insoluble matter.

3.1.24 *particle count*, *n*—number of particles in a chosen particle size range.

3.1.25 *particle detection effectiveness*, *n*—qualitative or quantitative assessment of the capability of the AMM instrument

and test method to detect and segment particles and determine particle count and particle sizes properly.

3.1.26 *particle size*, *n*—measure of the size of a particle, commonly described by the maximum Feret diameter.

3.1.27 *reference analysis membrane*, *n*—analysis membrane created from particles of interest or reference particles for the purpose of serving as a particle size or count reference standard or both.

3.1.28 *reference particles*, *n*—particles of known particle type, size, and/or number concentration.

3.1.28.1 *Discussion*—Examples are polystyrene spheres or NIST reference material 8634 (abraded ethylene tetrafluoroethylene (ETFE)).

3.1.29 *segmentation process*, *n*—part of the image analysis process that determines which pixels are associated with individual particles and which pixels are associated with the membrane filter background resulting in a segmented image.

3.1.30 *segmented image*, *n*—binary image of pixels associated with individual particles and pixels associated with the membrane filter background.

3.1.31 *stitching process*, *n*—part of the image analysis process that combines the multiple images of the analysis membrane surface created in the image acquisition process into one image and fuses any particle images lying at the border between two adjacent camera frames.

3.1.32 *system suitability standard, n*—substrate with a stable reference pattern that is measured to confirm size calibration and AMM instrument alignment before measurements of analysis membranes.

3.1.33 *test liquid, n*—liquid to be tested in the AMM test method.

3.1.33.1 *Discussion*—May be a process fluid, liquid extract, drug substance, drug product, or reconstituted powder.

3.1.34 *test liquid preparation procedure, n*—procedure by which a test liquid is obtained, either by direct sampling of process fluids, drug substance or drug product, or the extraction of material surfaces.

4. Significance and Use

4.1 The presence of extraneous solid and insoluble particulate matter in a final parenteral drug product is a potential hazard that may cause harm to the patient receiving the drug product in addition to potentially reducing drug product quality, efficacy, and availability.

4.2 This guide provides guidance on the development of AMM test methods designed to count and size particulate matter present in the manufacturing of parenteral pharmaceuticals. An AMM test method consists of four steps: a test liquid preparation procedure, a filtration procedure, an image acquisition process, and an image analysis process.

4.3 Test liquids such as process fluids, drug substances, or drug products sampled from pharmaceutical manufacturing processes may be directly filtered through a membrane filter to create an analysis membrane. Test liquids of interest may also include liquid extracts obtained by extraction (rinsing, washing, and flushing) of the surfaces of process equipment, drug containers, delivery devices, and components thereof. Particles dispersed over sometimes large surface areas are collected in the liquid extract and, upon filtering through a membrane filter, become concentrated onto the relatively small surface area of an analysis membrane.

4.4 In the image acquisition process, the analysis membrane is scanned by an optical microscope and digital camera creating multiple images of the analysis membrane surface. The image analysis process stitches together the multiple images and determines which pixels are associated with individual particles and which pixels are associated with the membrane filter background. Analysis of the resulting segmented image determines the particle count and particle sizes of the particles present on the surface of the analysis membrane.

4.5 Development of test methods applying AMM to the counting and sizing of the wide variety of particulate matter potentially present in parenteral pharmaceutical manufacturing presents significant challenges. Particles composed of different materials, varying in size and morphology, may be found in the various stages of parenteral pharmaceutical manufacturing. Although definitions vary, “intrinsic” typically describes a class of particles originating from formulation ingredients and the materials of construction of processing equipment, final containers, and drug delivery devices. “Extrinsic” typically

describes a class of particles originating from human operators and the environment surrounding the manufacturing process. Solid and insoluble particulate matter may include, but is not limited to, textile fibers, hair fibers, paper fibers, plastic and elastomeric particles, metal and ceramic particles, skin flakes, dust, insect parts, and other organic matter. Semi-solid particles (for example, protein aggregates) or liquid droplets (for example, silicone oils) may partially or completely penetrate membrane filters and, thus, are not usually measurable by an AMM test method. An AMM test method may be useful for the measurement of particulate matter inherent to drug products, vaccine adjuvants, and cells in cell and gene therapies. However, the development of AMM test methods for inherent particles is out of the scope of this guide.

4.6 This guide is applicable to the development of AMM test methods for measurement of particles in both the commonly defined size categories of subvisible (<100 µm) and visible (≥100 µm) particles as described in the pharmacopoeias. This guide recommends use of the maximum Feret diameter as a characteristic particle size parameter. Determination of other particle morphology parameters is out of the scope of this guide.

5. Background Information

5.1 From a quality risk management perspective (ICH Q9), minimization of the probability of occurrence and maximization of the detectability of particulate matter decreases the risk of potential harm from particulate matter.

5.2 Minimization of the probability of occurrence of particulate matter in a drug product depends upon conformance to current good manufacturing practices (CGMPs) along the entire pharmaceutical manufacturing process from starting ingredients to final drug product. Particulate matter may be carried into the final drug product from formulation ingredients and processing equipment. Conventional multi-use processing equipment (usually composed of stainless-steel components) requires cleaning before implementation and benefits from test methods that validate cleanliness with respect to particulate matter. Single-use processing equipment (usually composed of plastic components), often manufactured in cleanrooms and delivered sealed and sterilized to pharmaceutical manufacturers, is typically not cleaned before implementation. Single-use systems benefit from test methods that validate cleanliness with respect to particulate matter, especially when applied in critical applications without downstream filtration (for example, final filling, and aseptic processes for cell and gene therapies).

5.3 Particulate matter may also be carried into the final drug product via final containers and drug delivery devices along with components thereof (for example, glass containers, elastomeric closures, syringe assemblies, and infusion bags).

5.4 Good detectability of particulate matter in parenteral drug products results from the required combination of 100 % visual inspection along with sampling and destructive testing of final drug products for subvisible

matter as described in USP <788> and related USP and equivalent harmonized European (EP 2.9.19) and Japanese (JP 6.07) monographs.

5.5 In USP <788>, EP 2.9.19, and JP 6.07, two test methods for counting and sizing subvisible particulate matter in injections (parenteral pharmaceutical drug products) are described. In the Method 1 light obscuration particle count test, a particle suspended in liquid is drawn into a tube, passes through a light beam detector, and the particle is counted when the light beam is attenuated because of scattering or absorption or both of light by the particle. The light attenuated by the particle is related to a particle size via comparison with a calibration obtained using spherical particle size standards. Thus, the output of USP <788> Method 1 is an equivalent circular diameter. Method 1 is automated and, thus, a relatively quick and labor-saving method. However, since the optical properties of the typical particle size standards (spherical polystyrene particles) often differ significantly from that of the particulate matter of interest (often non-spherical and of indeterminate refractive index), achieving accuracy in the determination of particle sizes is challenging, especially for particles with a refractive index close to that of the suspending fluid. However, achieving good precision and reproducibility is possible. In addition to solid and insoluble particles, bubbles and liquid droplets are also detected by light obscuration instruments. Light obscuration methods are generally considered most effective at counting particles less than approximately 100 μm in size. Depending upon particle shape and density, some particles larger than approximately 100 μm may not be reliably drawn into the tube and flow through the light beam detector of the light obscuration instrument or may not be fully illuminated by the light beam in the sensor head.

5.6 In the USP <788> Method 2 microscopic particle count test, particles suspended in liquid are filtered onto a membrane filter, and under controlled illumination conditions, the particles collected on the membrane filter are manually counted and size classified under a microscope using a reticule consisting of a defined inspection field and reference circles (graticules) of 10 μm and 25 μm diameter. The number of particles found $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$ are reported. Method 2 is more labor intensive and susceptible to human error than Method 1, since the particle suspension shall first be filtered onto the membrane filter, and each particle is individually size classified and counted manually by the human analyst. However, one advantage of microscopic analysis (Method 2) over light obscuration (Method 1) is that, in a microscopic analysis, particles are directly visualized. Thus, particles larger than 100 μm and particles of complex morphology (such as fibers) are detected and sized with greater accuracy than possible from light obscuration, which only measures an equivalent circular diameter. In addition, the particles collected on the membrane filter are available for further analysis with physical and chemical identification techniques such as polarized light microscopy, infrared (IR) or Raman micro-spectroscopy, or scanning electron microscopy.

5.7 USP <1788>, <1788.1> (light obscuration), and <1788.2> (membrane microscopy) give extensive guidance on development of USP <788> test methods. In addition, USP <1788>, USP <1788.3>, and Guide E3060 give guidance on developing methods for direct imaging of particles suspended in test liquids using flow imaging. USP <1787> also gives guidance on additional techniques appropriate for the measurement of subvisible particles present in therapeutic protein injections.

5.8 Since the original publication of USP <788> and related monographs, significant advances in digital cameras, image analysis software, and microscope automation allow what is referred to in this guide as AMM, whereby particles collected on a membrane filter are scanned with an optical microscope under controlled illumination conditions, multiple images are captured with a digital camera, and, via an image analysis process, the particles on the membrane filter are counted and sized in a rapid and automated way. In USP <1788.2>, in Section 6, guidance on “automated approaches” in implementation of a USP <788> microscopic particle count test is given. This guide provides additional in-depth guidance on best practices for development of AMM test methods.

6. Guidance on the Development of an AMM Test Method

6.1 General Principles:

6.1.1 In an AMM test method, a test liquid containing suspended particles is filtered through a membrane filter, the particles are retained on the surface of the membrane filter, the membrane filter surface is imaged with an optical microscope and digital camera, and application of image analysis software determines particle count and particle sizes. Thus, an AMM test method should specify these four essential steps: a test liquid preparation procedure, a filtration procedure, an image acquisition process, and an image analysis process.

6.1.2 Guidance is given on obtaining the test liquid, preparing the analysis membrane, development and optimization of the image acquisition process and image analysis process, definition of the blank test and system suitability test, along with guidance on data reporting. Most importantly, guidance is given on approaches for determination of the quality of the output of an AMM test method. Finally, guidance is also given on the alignment of an AMM test method with a USP <788> Method 2 microscopic particle count test. For the validation requirements for AMM test methods, see Practice E3411.

6.2 *Definition of the Intended Purpose and Scope of an AMM Test Method*—The purpose of an AMM test method is to count and size particulate matter, and the scope is defined by the test liquids to be tested (for example, process fluids, drug products, and liquid extracts) and what test outputs are required.

6.2.1 A possible output of the AMM test method is a full determination of the particle size distribution (particle count per particle size bin: a differential particle size distribution) for the particle size range of interest.

6.2.2 Another possible output could be the particle count for particles greater than or equal to a specified particle size. For example, in USP <788>, determination of the particle count or particle number concentration for particles $\geq 10\ \mu\text{m}$ and $\geq 25\ \mu\text{m}$ (a cumulative particle size distribution) is required. In another example, an AMM test method could focus only on measurement of the particle count for potentially visible particles ($\geq 100\ \mu\text{m}$ in particle size).

6.3 *Test Liquid Preparation Procedure*—The test liquid preparation procedure results in a suspension of particles in liquid suitable for filtration through a membrane filter. In the test liquid preparation procedure, a test liquid is either obtained by directly sampling process fluids, drug substance, or drug product along the parenteral pharmaceutical manufacturing process or obtaining liquid extracts from material surfaces contacted in the manufacturing of the parenteral pharmaceuticals.

6.3.1 Given the wide range of possible test liquids obtainable from a parenteral pharmaceutical manufacturing process, in this guide, it cannot be specified how a test liquid should be obtained and prepared for filtration. Process fluids, drug substance, and drug product obtained along the parenteral pharmaceutical manufacturing process may, in many cases, be directly filtered with a membrane filter to create an analysis membrane. In the case of a viscous test liquid, dilution may be required to facilitate filtration. In PDA Technical Report No. 79,¹⁰ methods are described for destructive testing of difficult to inspect drug products with the goal of detecting particulate matter in, for example, turbid suspensions or lyophilized solids.

6.3.2 In other cases, the test liquid is obtained by extracting (rinsing, washing) particles from material surfaces with a liquid. Surfaces of interest range from those associated with process equipment, final containers, drug delivery devices, and components thereof. In ISO 8871-3, a method is described for extraction of particulate matter from the surfaces of the elastomeric components applied in final drug containers. In ISO 8536-4, a procedure is described for rinsing particulate matter from the interior surfaces of infusion sets and collecting the particles on a membrane filter for microscopic analysis. In Practice E3230, development and validation of methods are described for the extraction of particulate matter from the surfaces of single-use systems applied in the manufacturing of biopharmaceuticals. In PDA Technical Report No. 85,¹¹ enhanced methods are described for measurement of particulate matter on the surfaces of elastomeric closures and glass containers. In VDI 2083 Part 21, detailed guidance is given on how to develop and validate methods for the extraction and measurement of particulate matter on the surfaces of medical devices.

6.4 *Filtration Procedure*—In a filtration procedure, a test liquid is filtered through a membrane filter, and the particles in

the test liquid larger than the pore size rating of the membrane filter pore sizes are retained on the surface of the membrane filter creating an analysis membrane suitable for membrane microscopy.

6.4.1 *Choosing a Suitable Membrane Filter:*

6.4.1.1 Criteria for choosing a membrane filter type are: (1) chemical resistance to any solvent(s) in the test liquid, (2) ability to retain the particles of interest (appropriate pore size rating), (3) sufficient surface smoothness so that particles remain within the optical depth of field of the microscope upon analysis, (4) homogeneous surface optical properties (color, reflectivity) to facilitate the image analysis process, and (5) adequate cleanliness.

6.4.1.2 Typically, membrane filters composed of materials such as cellulose acetate/nitrate, polyamide (nylon), or track-etched membrane filters will meet smoothness and optical requirements. Depending on the particle sizes of interest, mesh filters (polyester, polyamide) may not present a smooth enough surface. For good particle retention, the pore size rating of the filter is recommended to be not more than one fifth of the size of the smallest particles of interest. Note that semi-solid particles may not always be retained in a membrane filtration and effective retention may depend sensitively on filtration procedure conditions. Colored membrane filters (gray, black, yellow, and so forth) in some cases may improve optical contrast between certain types of particles and the membrane filter background facilitating the image analysis process. Specialized membrane filters may be useful for subsequent particle characterization. Care should be taken to store and handle the membrane filters such that contamination or damage is avoided. Note that some types of membrane filters are smoother on one side than the other.

6.4.2 *Preparation of Filters*—In some cases, membrane filters may need to be rinsed prior to use to remove extraneous particles and to reduce blank counts to acceptable levels. The membrane filters may be held by forceps and rinsed on both sides with running filtered water, or by holding with forceps, rinsing one side, and then transferring the membrane filter to a clean glass slide and rinsing the second side with a more forceful water stream. Particles may also be removed with a sharp needle while observing the membrane filter through a microscope.

6.4.3 *Homogeneously Dispersing Particles on the Surface of the Analysis Membrane:*

6.4.3.1 In a filtration procedure, a membrane filter is placed into a filter holder. Typically, the filter holder consists of a support for the membrane filter and a funnel above the filter to hold the test liquid. The test liquid is poured into the funnel and over the membrane filter, and a vacuum is applied to pull the liquid through the membrane filter. In other cases, the membrane filter may be held (clamped) in an in-line membrane filter holder.

6.4.3.2 The goal of the filtration procedure is to prepare an analysis membrane with singly dispersed particles uniformly distributed across the surface of the membrane filter. Multiple particles that touch or overlap will usually be analyzed incorrectly as a single particle in the image analysis process. Achieving ideal dispersion of particles on the membrane filter

¹⁰ PDA Technical Report No. 85, Enhanced Test Methods for Visible Particle Detection and Enumeration on Elastomeric Closures and Glass Containers, Parenteral Drug Association, Bethesda, MD, <https://www.pda.org>, 2021.

¹¹ PDA Technical Report No. 79, Particulate Matter Control in Difficult to Inspect Parenterals, Parenteral Drug Association, Bethesda, MD, <https://www.pda.org>, 2018.