Designation: F3438 – 21

Standard Guide for Detection and Quantification of Cleaning Markers (Analytes) for the Validation of Cleaning Methods for Reusable Medical Devices[1]

This standard is issued under the fixed designation F3438; 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 This standard guide provides methods and considerations for detecting and quantifying test soil(s) from reusable medical device(s) that result from simulated-use testing of medical devices during validation of the cleaning procedures as described in the instructions for use (IFU) provided by the medical device manufacturer.

1.2 The methods described are for detecting and measuring markers (analytes) that are components within the most common test soils and are relevant to the clinical use of the device. Appropriate test soils without protein, carbon, or carbohydrates (for example, bone) will require other methods.

1.3 This is a part of a series of ASTM standard guides for validating cleaning instructions. The scope of the first guide in the series is selecting appropriate test soils (Guide F3208). The second in the series (Guide F3293) describes methods for inoculating medical devices with test soil. The third in the series (Guide F3321) describes methods for extracting soils for measuring residual soil on medical devices after the performance of cleaning process. This is the fourth guide in the series and describes the methods of detecting and quantifying residual analytes on the device.

1.4 *Units*—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]

D7573 Test Method for Total Carbon and Organic Carbon in Water by High Temperature Catalytic Combustion and Infrared Detection

E1097 Guide for Determination of Various Elements by Direct Current Plasma Atomic Emission Spectrometry

E2520 Practice for Measuring and Scoring Performance of Trace Explosive Chemical Detectors

F3127 Guide for Validating Cleaning Processes Used During the Manufacture of Medical Devices

F3208 Guide for Selecting Test Soils for Validation of Cleaning Methods for Reusable Medical Devices

F3293 Guide for Application of Test Soils for the Validation of Cleaning Methods for Reusable Medical Devices

F3321 Guide for Methods of Extraction of Test Soils for the Validation of Cleaning Methods for Reusable Medical Devices

2.2 *AAMI Documents:*[3]

AAMI TIR12 Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities: A guide for medical device manufacturers

AAMI TIR30 A compendium of processes, materials, test methods, and acceptance criteria for cleaning reusable medical devices

2.3 *ISO Standard:*[4]

ISO/TS 15883-5 Washer-disinfectors—Part 5: Test soils and methods for demonstrating cleaning efficacy

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.15 on Material Test Methods.

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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 Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

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

2.4 *FDA Guidance Document:*[5]

Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling, Guidance for Industry and Food and Drug Administration Staff

3. Terminology

3.1 *Definitions:*

3.1.1 *limit of detection (LOD), n*—the limit of detection is the lowest quantity of a substance that can be distinguished from the absence of that substance within a stated confidence limit (Practice E2520). LOD is also generally defined as three times the standard deviation of the blank (Guide F3127).

3.1.2 *limit of quantification (LOQ), n*—the limit of quantification is the lowest concentration at which the instrument can measure reliably with a defined error and confidence level (Guide E1097). LOQ is also generally defined as ten times the standard deviation of the blank (Guide F3127).

3.1.3 *test soil, n*—a single substance or a mixture of substances that reflect the contaminants likely to be encountered during the use of the device for its intended clinical procedure (Guide F3208).

4. Summary of Guide

4.1 This standard guide describes methods for detecting and quantifying cleaning markers (analytes) extracted from soiled medical devices during validation testing of the instructions for medical device reprocessing by a healthcare facility.

5. Significance and Use

5.1 This standard guide may be used by medical device manufacturers as part of their design plan and implementation of the validation of the cleaning instructions of their reusable medical devices.

5.2 This guide helps medical device manufacturers to identify the appropriate method(s) for detecting and quantifying markers for the simulated-use test soil (see Guide F3208), thereby evaluating whether the medical device can be adequately cleaned.

5.3 This guide describes various test methods for the different analytes.

6. Analytes and Detection/Quantification Methods

6.1 *General Considerations:*

6.1.1 Accurate measurement of analyte concentration is critical in reprocessing validations. This section covers the assay methods that are most frequently employed in cleaning validations. There is no one method that is considered as the best for a particular analyte. Each method has its advantages and disadvantages. The decision on selecting the appropriate assay is mostly based on the compatibility of the assay with the samples and the potential interfering substances included in samples that may affect certain methods.

6.1.2 The limit of detection depends on the assay or reagent kit that is being used. It also depends on the standard curve used, the sensitivity and stability of the instrumentation, any minor modifications in reagents used, and background/interference effects from the sample extract.

6.2 *Protein Detection/Quantification:*

6.2.1 *Bicinchoninic Acid (BCA) Assay* (**1**):[6]

6.2.1.1 BCA assay is a biochemical assay for determining the total concentration of protein in a solution. The assay is based on protein-copper chelation and secondary detection of the reduced copper. The assay relies on two reactions. First, the peptide bonds in the protein sample reduce Cu^{2+} ions, in a temperature-dependent reaction, from the copper solution to Cu^{+}. The amount of Cu^{2+} reduced is proportional to the amount of protein present in the solution. Next, two molecules of BCA chelate with each Cu^{+} ion, forming a purple-colored product. The total protein concentration is exhibited by a color change of the sample solution from green to purple in proportion to protein concentration. The amount of protein present in a solution can be quantified by measuring the absorption spectra and comparing with protein solutions with known concentrations. The advantage of the BCA assay is that it is compatible with a wide range of ionic and non-ionic detergents and denaturing agents. A limitation of the method is the presence of reducing agents and copper chelating agents, which can affect the accuracy of the results.

6.2.2 *Bradford Assay* (**2**):

6.2.2.1 The Bradford assay is used to measure the concentration of total protein in a sample. The assay is based on the binding of protein molecules to Coomassie dye under acidic conditions. When mixed with a protein solution, the acidic Coomassie dye changes color from brown to blue in proportion with the amount of protein initially present in the sample. The assay is done at room temperature and the resultant blue color is measured spectrophotometrically. Protein determinations are made by comparison to the color response of protein assay standards. Among the advantages of the Bradford assay is the compatibility with reducing agents used to stabilize proteins in solution. The main limitation of the Bradford assay is its incompatibility with most detergents routinely used to solubilize membrane proteins.

6.2.3 *Ortho-Phthalaldehyde (OPA) Method* (**3**):

6.2.3.1 The OPA method is based on quantitative detection of primary amines in amino acids, peptides, and proteins. OPA reacts with primary amines in the presence of mercaptoethanol to yield a blue-colored fluorescent product. The reaction can be monitored by absorbance and by fluorescence. The inherent sensitivity and speed of OPA, along with its broad linear range, make it a useful protein and peptide assay reagent. The OPA assay functions well in the presence of lipids and detergents.

6.3 *Hemoglobin Detection/Quantification:*

6.3.1 *Tetramethylbenzidine (TMB) Assay* (**4**):

6.3.1.1 The TMB assay is a catalytic test that is based on the peroxidase-like activity of hemoglobin. With its peroxidase-like activity, hemoglobin catalyzes the oxidation of TMB in the

[5] Available from U.S. Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993, http://www.fda.gov.

[6] The boldface numbers in parentheses refer to a list of references at the end of this standard.

presence of hydrogen peroxide. TMB produces a colored end product that can be read spectrophotometrically.

6.3.2 *Drabkin's Assay* (**5**)*:*

6.3.2.1 Drabkin's assay is used for the quantitative, colorimetric determination of hemoglobin concentration in a solution. This assay is based on the oxidation of hemoglobin (except sulfhemoglobin, which normally occurs in only minute concentrations in blood) to methemoglobin in the presence of alkaline potassium ferricyanide. Methemoglobin then reacts with potassium cyanide to form cyanmethemoglobin. The color intensity measured spectrophotometrically is proportional to the total hemoglobin concentration.

6.3.3 *Copper (II)-Phthalocyanine Complex Assay* (**6**)*:*

6.3.3.1 This assay uses copper (II)-phthalocyanine complex to determine the concentration of hemoglobin in the test samples. It is a quantitative, highly sensitive, colorimetric method that relies on the oxidative decomposition of copper (II)-phthalocyanine complex in the presence of hemoglobin and a peroxomonosulfate. The samples are mixed with a liquid copper phthalocyanine reagent and read on a spectrophotometer, then quantified using a calibration curve. The color of test sample changes from blue to clear in the presence of hemoglobin.

6.3.4 *Triton/NaOH Assay:*

6.3.4.1 This assay is based on the improved Triton/NaOH method in which hemoglobin is converted to a colorimetric product measured at 400 nm. The intensity of color is directly proportional to the hemoglobin concentration.

6.4 *Carbohydrate Detection/Quantification:*

6.4.1 *Phenol-Sulfuric Acid Assay* (**7**, **8**)*:*

6.4.1.1 The phenol-sulfuric acid method is a widely used colorimetric method to determine total carbohydrates in a sample. The method detects all classes of carbohydrates. The basic principle of this method is that carbohydrates react with concentrated sulfuric acid to produce furfural derivatives, which further react with phenol to develop a detectable color. Light absorption is recorded on a spectrophotometer. The absorbance is proportional to the carbohydrate concentration initially present in the sample.

6.5 *Total Organic Carbon Detection/Quantification*—(United States Pharmacopeia <643> Total Organic Carbon, Test Method D7573):

6.5.1 Total organic carbon (TOC) is the amount of carbon found in a water-soluble organic compound. TOC is an accurate, nonspecific test for quantifying cleaning marker residues and detergent residuals and is often used as an indirect indicator for water quality or cleanliness of devices after reprocessing. The main stages of TOC analysis are sampling, oxidation, detection, and quantification. A typical analysis for TOC is the differential method, which first separately measures the total carbon and the inorganic carbon in the sample. The inorganic carbon is then subtracted from the total carbon to yield TOC. This method is suitable for samples in which the inorganic carbon is less than or of similar amount as TOC. Another common method of TOC analysis is the direct method, which involves removing the inorganic carbon from the sample first and then measuring the leftover carbon.

7. Keywords

7.1 analyte; carbohydrates; hemoglobin; protein; total organic carbon (TOC)

ANNEX

(Mandatory Information)

A1. SAMPLE STANDARD OPERATING PROCEDURES (SOP) FOR ANALYTE QUANTIFICATION

A1.1 QuantiPro BCA Protein Assay

A1.1.1 *Purpose*—To provide instructions on performing a QuantiPro[7] BCA protein assay using a commercial kit.

A1.1.2 *Scope*—This protocol includes the materials and steps necessary to perform a QuantiPro BCA protein assay.

A1.1.3 *Materials:*

A1.1.3.1 Sterile 1.5-mL Eppendorf tubes.

A1.1.3.2 Gloves.

A1.1.3.3 Sterile pipettes (of appropriate size).

A1.1.3.4 Sterile pipette tips, regular unplugged (of appropriate size).

A1.1.3.5 Rack to hold tubes.

A1.1.3.6 Beaker or container to prepare QuantiPro working reagent.

A1.1.4 *Equipment:*

A1.1.4.1 Vortex (variable speed preferred).

A1.1.4.2 Mechanical pipettor.

A1.1.4.3 Multi-channel pipettor.

A1.1.4.4 Spectrophotometer.

A1.1.4.5 Incubator capable of achieving a temperature of 55 °C to 60 °C (optional).

A1.1.5 *Reagents:*

A1.1.5.1 Sterile reverse osmosis water.

A1.1.5.2 QuantiPro BCA assay kit (Sigma, catalog no. QP-BCA):

A1.1.5.2.1 QuantiPro buffer QA (catalog no. M3810).

A1.1.5.2.2 QuantiPro buffer QB (catalog no. M3685).

A1.1.5.2.3 Copper (II) sulfate pentahydrate 4 % (w/v) solution (catalog no. C2284).

[7] The sole source of supply of the QuantiPro BCA assay kit is Sigma-Aldrich Corp., St. Louis, MO, USA. The QuantiPro kit was used as an example for the purpose of illustrating a sample SOP in the annex. Any suitable BCA kit can be used for protein analysis.