Designation: F3438 – 24

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 ST98 Cleaning validation of health care products—Requirements for development and validation of a cleaning process for medical devices

2.3 *ISO Standard:*[4]

ISO 15883-5 Washer-disinfectors—Part 5: Performance requirements and test method criteria 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).

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.

5.4 This guide specifies the validation criteria for analyte detection methods.

6. Analyte Validation

6.1 The sensitivity of the analyte method is specified by the limit of detection (LOD), the lowest amount of analyte that can be detected; and the limit of quantification (LOQ), the lowest amount of an analyte in a sample that can be reliably quantified with acceptable accuracy and precision. To establish these values, perform a robust analytical validation of the method to trust the trueness of the reported value. The validation must contain the following validation elements.

6.1.1 *Linearity/Range*—The linearity is assessed by preparing analyte standards in a concentration curve that spans the range of expected analyte concentration and includes method acceptance criteria. The linearity, measured by the R^2 value of the line, should be greater than 0.9900. For the range, the analyte residual acceptance criteria should be in the portion of the curve that demonstrates the best accuracy and precision with the upper and lower points of the curve being both accurate and precise (**1**).[6]

6.1.2 *Accuracy*—The accuracy is expressed as the closeness of agreement between the reference value and the value found, and is determined by comparing the calculated (actual) concentration from the calibration curve to that of the nominal (theoretical) concentration of the protein standard using the following equation:

$$\text{Accuracy} = 100 - \left(\frac{[\text{Nominal}] - [\text{Calculated}]}{[\text{Nominal}]}\right) * 100 \quad (1)$$

6.1.2.1 To demonstrate accuracy, the calculated protein value should be ±15 % of the nominal value.

6.1.3 *Precision*—Defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same sample, and is quantified using the relative standard deviation (RSD) of each protein calibration point of low and high concentrations across the range.

$$RSD = \frac{S}{\bar{x}} * 100 \quad (2)$$

$$S = \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + \ldots + (x_n - \bar{x})^2}{n - 1}} \quad (3)$$

where:

S = standard deviation,
$x_1, x_2, \ldots x_n$ = a given response value, and
$\bar{x}$ = average response value.

6.1.3.1 When validating with a non-homogeneous sample matrix, like a test soil, precision must be evaluated first with the reference protein to establish the capability of the instrumentation, and then again with the test soils used within the method. This comparison of a homogenous protein to a complex protein matrix used in test soils validates the use of the specified reference protein within the assay. Precision should be evaluated by both intermediate precision (that is, system suitability measurement of a minimum of three concentrations with three replicates each) and robustness (that is, interlaboratory trial). Precision is achieved when the RSD value is ≤15 % for the average response determinations.

7. Analytes and Detection/Quantification Methods

7.1 *General Considerations:*

7.1.1 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 or test method is mostly based on the compatibility of 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.

assay with the samples and the potential interfering substances included in samples that may affect certain methods.

7.1.2 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. Modifications can be made to improve the sensitivity of the method, but a validation using the criteria in Section 6 should be completed.

7.1.3 The standard addition method, addition of 10 µg/mL of protein standard added to unknown sample concentrations, can be used to shift low concentrations into more accurate and precise portions of the calibration curve to increase sensitivity. A spiked blank should be included and subtracted from the unknown value to deliver the final unknown sample result (**2**).

7.1.4 Diluents (that is, extraction fluids) must be validated for use in the method.

7.1.5 Extraction hold times and containers must be validated to ensure results of unknown samples, especially if the unknown samples are not analyzed directly after collection.

7.2 *Protein Detection/Quantification:*

7.2.1 *Bicinchoninic Acid (BCA) Assay* (**3**)*:*

7.2.1.1 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.

7.2.2 *Bradford Assay* (**4**)*:*

7.2.2.1 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.

7.2.3 *Ortho-Phthalaldehyde (OPA) Method* (**5**)*:*

7.2.3.1 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.

7.2.3.2 *Liquid OPA Assay*—There is a published indirect OPA assay that can be used to measure protein soil on device surfaces without the use of extraction. This can help to eliminate any protentional errors inherent in extraction of challenging test soils and has a detection limit of 1.6 µg/cm^2 protein. See Ref (**6**) for more information.

7.3 *Hemoglobin Detection/Quantification:*

7.3.1 *Tetramethylbenzidine (TMB) Assay* (**7**)*:*

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

7.3.1.2 *Liquid TMB Assay*—This method uses test strips to detect hemoglobin in the sample collected. It has shown that it is 500× more sensitive than MicroBCA in a 1 min assay, as well as unaffected by cleaning reagents at 10 ppm. See Ref (**8**) for more information.

7.3.2 *Drabkin's Assay* (**9**)*:*

7.3.2.1 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.

7.3.3 *Copper (II)-Phthalocyanine Complex Assay* (**10**)*:*

7.3.3.1 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.

7.3.4 *Triton/NaOH Assay:*

7.3.4.1 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.

7.4 *Carbohydrate Detection/Quantification:*

7.4.1 *Phenol-Sulfuric Acid Assay* (**11**, **12**)*:*

7.4.1.1 The phenol-sulfuric acid method is a 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.

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

7.5.1 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.

8. Keywords

8.1 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).

A1.1.5.2.4 1 mg/mL BSA protein standard (catalog no. P0914).

A1.1.6 *Responsibility:*

A1.1.6.1 It is the responsibility of the supervising personnel to ensure that the laboratory personnel performing these tasks are trained appropriately.

A1.1.6.2 The laboratory personnel will ensure that these procedures are carried out accurately.

A1.1.6.3 It is the responsibility of laboratory personnel to accurately record all required data and results.

A1.1.6.4 It is the responsibility of laboratory personnel to document any deviation from these procedures and to consult supervising personnel on the matter.

A1.1.7 *Abbreviations:*

A1.1.7.1 sRO water: sterile reverse osmosis water.

A1.1.7.2 BCA: bicinchoninic acid.

A1.1.7.3 BSA: bovine serum albumin.

A1.1.7.4 nm: nanometers.

A1.1.8 *References*—Product information sheet provided with QuantiPro BCA assay kit, Sigma catalog no. QP-BCA.

A1.1.9 *Safety Considerations:*

A1.1.9.1 Follow appropriate safety guidelines for handling and disposing of all chemicals.

A1.1.9.2 Refer to Safety Data Sheets (SDS) for the handling of all reagents provided in the QuantiPro BCA assay kit, including bovine serum albumin.

A1.1.9.3 Wear gloves throughout the entire procedure.

A1.1.10 *Procedure:*

NOTE A1.1—The BCA assay cannot be performed on any samples collected with rayon, calcium alginate, or cotton swabs due to interference with the assay. Only samples collected with Dacron swabs are acceptable to use with this kit.

A1.1.10.1 *Preparation of Standards and Blanks:*

[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.