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Standard Guide for Selecting Test Soils for Validation of Cleaning Methods for Reusable Medical Devices[1]

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1. Scope

1.1 This guide describes methods for selecting test soils for cleaning validations based upon the characteristics of the soil, the physical characteristics of the device, and the clinical use of the device.

1.2 This guide describes the preparation and use of some test soils for the validation of cleaning instructions for reusable medical devices.

1.3 Reusable medical devices such as endoscopes, arthroscopic shavers, surgical instruments, and suction tubes are exposed to biological soils during clinical use. Preparation of these devices for reuse requires cleaning and disinfection and/or sterilization as applicable. Adequate cleaning is the first step in a process intended to prevent contaminant transfer to the next patient and medical practitioner. The soils, if inadequately removed, can interfere with disinfection and sterilization processes, as well as performance of the device. Acceptance criteria are based either on a visual assessment or quantitatively specified marker(s) endpoint(s) of the soil or both (ISO/TS 15883-5, Section 1). Endpoints after cleaning should be based upon possible interference with disinfection/sterilization, risk to the patient or health care worker from the contaminant during further handling, and endpoints for cleaning established in the scientific literature.

1.4 The test soils are designed to simulate the contaminants that medical devices are likely to come in contact with during clinical use. The test soils discussed in this guide are a mixture of constituents that simulate what is commonly found in human secretions, blood, tissue, and bone fragments/shavings as well as non-patient derived soil (e.g., bone cement, lubricants, and dyes) during clinical procedures. The test soils also simulate the physical parameters (e.g., viscosity, adhesion) of clinical material to which the medical devices will be exposed.

1.5 *Exclusion:*

1.5.1 This guide does not include methods to validate cleaning processes to remove residues from manufacturing

1.5.2 This guide does not describe the soil/inoculum used for validation of disinfection or sterilization instructions. Disinfection or sterilization validation requires separate testing that is independent of cleaning validation studies.

1.5.3 Test soils described are not intended for use by health care facilities to verify the effectiveness of their cleaning process.

1.5.4 The test soil recipes are not intended to encompass every biological residue with which a medical device is likely to come into contact.

1.6 Test soil formulations not described in this guide may be clinically relevant and may be more appropriate for simulated-use testing depending upon the clinical use of the medical device. The burden is upon the medical device manufacturer to determine and justify scientifically the selection of test soil(s).

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

1.8 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D445 Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity)

D1193 Specification for Reagent Water

D3330/D3330M Test Method for Peel Adhesion of Pressure-Sensitive Tape

D3359 Test Methods for Measuring Adhesion by Tape Test

D4212 Test Method for Viscosity by Dip-Type Viscosity Cups

D4287 Test Method for High-Shear Viscosity Using a Cone/Plate Viscometer

[1] This test method 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.

D7042 Test Method for Dynamic Viscosity and Density of Liquids by Stabinger Viscometer (and the Calculation of Kinematic Viscosity)

D7225 Guide for Blood Cleaning Efficiency of Detergents and Washer-Disinfectors

D7867 Test Methods for Measurement of the Rotational Viscosity of Paints, Inks and Related Liquid Materials as a Function of Temperature

F2809 Terminology Relating to Medical and Surgical Materials and Devices

2.2 *AAMI Standards:*[3]

TIR12:2010 Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities: A guide for medical device manufacturers

TIR30:2011 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

2.4 *FDA Standard:*[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*—Unless provided otherwise in the following definitions, terminology shall be in conformance with Terminology F2809.

3.1.1 *cleaning, n*—removal of contamination from a medical device to the extent necessary for further processing or for intended use.

3.1.2 *cleaning marker, n*—that which is being detected/measured to determine soil removal/retention.

3.1.3 *contamination, n*—procedure of applying simulated test soil onto a medical device for determination of process capability (that is, cleaning efficacy and extraction yields).

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

3.1.5 *validation, n*—documented procedure for obtaining, recording, and interpreting the results required to establish that a process will consistently yield results complying with predetermined specifications.

3.1.5.1 *Discussion*—Under U.S. FDA guidelines, validation of the instructions for cleaning is the responsibility of the medical device manufacturer.

4. Summary of Guide

4.1 This guide provides information on the selection of test soil formulation(s) based upon clinical use and physical characteristics of clinically occurring soiling of the device.

4.2 This guide provides the sample preparation technique for some test soils that simulate the soils found on medical devices as a result of clinical use.

4.3 An important aspect of the cleaning validation is determining the appropriate test soil(s) used to contaminate the device.

4.3.1 The manufacturer of the medical device or the reprocessing equipment shall justify why the specific soil(s) was chosen and is appropriate for all cleaning markers/assays to be measured.

4.3.2 The determination and selection of soil(s) shall be based upon the intended clinical use of the medical device. The manufacturer needs to determine what the device will come in contact with (e.g., blood, mucus, cerebrospinal fluid, neurological tissue, etc.) during clinical procedures how (e.g., duration, complete immersion).

4.3.3 The manufacturer should select a test soil(s) composed of a formulation that includes or accurately represents materials that the device would likely be subjected to during clinical use and would create the worst-case challenge to the cleaning process

4.3.4 Ideally, the formulation of the test soil should be composed of well-defined chemical/biochemical ingredients and readily reproduced by any laboratory globally.

5. Significance and Use

5.1 This guide provides information on how to select the test soil(s) that best simulates clinical use for devices. The test soil(s) selected for the validation should be clinically relevant and simulate what the device/component will come into contact with during the clinical procedure.

5.2 This guide will help standardize the test soils used by medical device manufacturers when validating the cleaning procedures of reusable medical devices and reprocessing equipment

5.3 For devices that come into contact with blood, the simulated test soils are blood-based soils, such as those described under 7.1.1 – 7.1.5.

5.4 For devices that come into contact with mucus, the simulated test soils are those described under 7.1.6.

5.5 For devices that come in contact with soils of a source other than the patient (e.g., bone cement), the simulated test soils should be similar to those described in 7.2. These can be used alone or in combination with 7.1.

5.6 A combination of test soils may be used (e.g., blood with mucus) to simulate clinical soiling. For example, flexible endoscopes may come in contact with a different combination of sources of soiling (e.g., gastrointestinal (GI) tract, vasculature for biopsies) during clinical use.

5.7 Any simulated test soil(s) or formulations can be used for simulated use testing but shall be scientifically justified by the medical device manufacturer.

[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 International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

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

6. Soil Selection Criteria

6.1 The test soils mentioned in this guide may be used to validate cleaning procedures as long as they can be scientifically justified as simulating clinical soiling.

6.2 Other formulations or modifications to the test soil(s) can be made but shall be scientifically justified by the manufacturer as simulating clinical soiling.

6.3 Mixtures of different soils can be made depending on how the device would be contaminated during a clinical procedure.

6.3.1 When selecting a combination of soils, consideration shall be given to the clinical use of the device and the ratio of the soil(s) in the combined test soil that best simulates clinically occurring soiling reflecting worst-case conditions.

6.4 Contamination by blood during clinical use is a common occurrence. There are multiple choices in the kind of blood soil selected for use.

6.4.1 In clinical procedures in which the predominant soil is blood, and other agents (e.g., water for irrigation) that will not interfere with the coagulation cascade, blood that can be chemically induced to coagulate is used. The coagulation process is induced before soiling the device.

6.4.2 In clinical procedures in which blood is not the predominant soil or other agents are present in sufficient volume to prevent coagulation, defibrinated blood is used.

NOTE 1—If whole blood is extracted without defibrination or without adding an anticoagulant, it forms clots and clumps. As a result, it is not easily used in test soil formulations.

6.4.3 *Types of Blood*

6.4.3.1 *Defibrinated Blood*—Whole blood that has been treated to denature fibrinogen without causing cell lysis.

6.4.3.2 *Anticoagulated Whole Blood*—Anticoagulated whole blood is selected in instances in which coagulation is to be induced at the time of application to the device.

(1) Citrated Blood—Blood treated with sodium citrate to prevent coagulation. Coagulation is induced with calcium chloride.

(2) Heparinized Blood—Blood rendered incoagulable by addition of heparin. Coagulation is induced with protamine sulfate.

7. Test Soils

7.1 *Test Soils Formulated to Simulate Patient-Derived Soils*—During clinical use, the key contaminants a medical device comes in contact with are from the patient. The soils described in this section are formulations that are intended to simulate the characteristics of these soils as they represent a challenge to cleaning.

7.1.1 *Coagulating Blood-Based Soils*—Blood is often the only or predominant patient derived soil that medical devices come into contact with during clinical use. When this is the case, the blood is likely to coagulate prior to the initiation of cleaning steps, particularly in a worst-case scenario. Coagulated blood proteins are highly water insoluble and can significantly adhere to the medical device. The test soils in this section are intended to simulate the characteristics of coagulating blood.

7.1.2 *Two Component Blood Test Soil*

7.1.2.1 *Description*—A test soil correlating to coagulated blood is based on a proteinaceous matrix containing fibrinogen and thrombin in two separated components. Coagulation of the soil is induced after mixing these two components. Guide D7225 and Pfeifer (**1**)[6] describe this soil in further detail.

7.1.2.2 *Constituents*—The blood test soil includes the following:

(1) Component A

Albumin, bovine, protease free: 400 mg;
Hemoglobin, bovine, lyophilized: 400 mg;
Fibrinogen, bovine, lyophilized: 60 mg; and
Solvent A, 5.0 mL 0.4 % NaCl solution (reagent grade NaCl dissolved in sterile water).

(2) Component B

Albumin, bovine, protease free: 400 mg;
Hemoglobin, bovine, lyophilized: 400 mg;
Thrombin, reagent grade from bovine plasma: 12.5 NIH units; and
Solvent B, 5.0 mL 0.4 % NaCl solution (reagent grade NaCl dissolved in sterile water) + 8.0 mmol/L $CaCl_2$.

7.1.2.3 *Preparation*

(1) The soil is prepared by separately dissolving Components A and B in their respective solvents by shaking for 1 h at room temperature.

(2) Immediately before use, Components A and B are mixed together in a 1:1 ratio.

7.1.2.4 *Markers*—Suggested (not exclusive) cleaning markers for residual analysis: protein, hemoglobin, and total organic carbon (TOC).

7.1.3 *Blood Test Soil*

7.1.3.1 *Constituents*—Components of test soil include the following:.

(1) Whole sheep blood, citrated: 100 mL;

(2) Bovine calf serum 50 mL;

(3) Physiological saline solution (PHSS): 50 mL, 0.9 % NaCl; and

(4) 0.01 mL of 2*M* $CaCl_2$.

7.1.3.2 *Preparation*—The soil is prepared by mixing all the whole blood with blood serum and the saline solution together thoroughly. Then add the 0.01 mL of 2*M* $CaCl_2$ calcium chloride immediately before use to activate the coagulation.

NOTE 2—Because of different composition of citrated sheep's blood commercially available, it may be necessary to adjust the amount of calcium chloride used to ensure adequate coagulation.

7.1.3.3 *Markers*—Suggested (not exclusive) cleaning markers for residual analysis: protein, TOC, hemoglobin, and carbohydrates.

7.1.4 *Non-Coagulating Blood-Based Soils*—Depending upon the area of the body, or the nature of the clinical procedure, blood may be present, but coagulation of the blood does not occur. For example, while blood may be present for procedures in the GI tract, other contaminants are also found in such significant proportions as to inhibit the coagulation of blood. In other procedures, such as many laparoscopic

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

procedures, copious water flushing is used during the procedure, thus inhibiting the coagulation of the blood. The soils described in this section are meant to simulate clinical contaminants of this kind.

7.1.4.1 *Artificial Test Soil (ATS)* (2)

(1) Constituents

Rosewell Park Memorial Institute (RPMI) 1640: 10.4 g;
Heat inactivated Bovine Calf Serum: 100 mL;
(optional) Bovine Oxgall: 20 g;
Sterile defibrinated sheep blood: 20 % by total volume of final solution;
Sodium bicarbonate: 2 g;
100 mM sodium pyruvate: 10 mL;
L-Glutamine: 10 mL of 200 mM; and
Total volume 1 L with distilled water.

(2) Preparation—Add Add the Bovine Oxgall, RPMI and sodium bicarbonate to a beaker. Add 700 ml of distilled water and mix well. Then add sodium pyruvate and L-glutamine and again mix well. Pour this mixture into a graduated cylinder. Add distilled water as needed to reach a total volume in the graduated cylinder of 1 L. Filter the entire volume through a 0.22 µL membrane filter into a sterile bottle. Using sterile technique add the sterile sheep blood and calf serum and mix well.

(3) The test soil may also be purchased in a lyophilized state.

(4) Markers—Suggested (not exclusive) cleaning markers for residual analysis: protein, TOC, hemoglobin, lipids and carbohydrates.

7.1.4.2 *Blood Test Soil (BTS)*

(1) Constituents

Bovine albumin: 0.5 g;
Bovine hemoglobin: 1.0 g;
Sodium alginate: 8.3 mg;
27 mL of sterile water for irrigation; and
Calcium chloride solution: 15 mM $CaCl_2$.

(2) Preparation—Mix the albumin, hemoglobin and sodium alginate in a sterile container. Add the 27 mL of sterile water for irrigation and calcium chloride and vortex to homogenize the soil. Sodium alginate and calcium chloride cross link to form alginate hydrogels. These three-dimensional (3-D) protein hydrogels simulate the fibrin hydrogels in clotted blood.

(3) The blood test soil may also be purchased in a lyophilized state.

(4) Markers—Suggested (not exclusive) cleaning markers for residual analysis: protein, TOC, and hemoglobin.

7.1.4.3 *Defibrinated Blood Soil*

(1) Constituents—The test soil shall have the following composition:

Fresh egg yolk: 100 mL;
Defibrinated blood: 100 mL sheep blood; and
Dehydrated hog mucin: 2g.

(2) Preparation—Mix all the ingredients together thoroughly in a blender until a liquid uniform mixture is achieved.

(3) Markers—Suggested (not exclusive) cleaning markers for residual analysis: protein, TOC, hemoglobin, and carbohydrates.

7.1.4.4 *Simulated Ophthalmic Test Soil* (3)

(1) Constituents—The test soil shall have the following composition:

Dried egg yolk: 0.8 g;
Defibrinated blood: 10 mL (horse or sheep); and
Dehydrated hog mucin: 01 g.

(2) Preparation—Mix the components to give a liquid of uniform consistency.

(3) Markers—Suggested (not exclusive) cleaning markers for residual analysis: protein, TOC, hemoglobin, and carbohydrates.

NOTE 3—This test soil is not recommended to evaluate the cleaning of contact lenses.

7.1.5 *Simulated Mucus Test Soils*

7.1.5.1 *British Standard Soil*

(1) Constituents—The test soil shall have the following composition:

Bovine serum: 10 mL;
Dry milk powder: 6 g; and
Saline (PHSS): 10 mL.

(2) Preparation—Mix all the ingredients together thoroughly in an appropriate container using a hot plate (temperature between 30 and 35°C) and a magnetic stirrer until a liquid uniform mixture is achieved. Heat to dissolve; do not boil.

(3) Markers—Suggested (not exclusive) cleaning markers for residual analysis: protein, TOC, hemoglobin, and carbohydrates.

7.1.5.2 *Miles Test Soil*

(1) Constituents—The test soil shall have the following composition:

Fetal bovine serum: 10 mL;
Saline physiological saline solution (PHSS): 1 mL;
Dry milk powder: 6 g; and
Rabbit blood in citrate: 1 mL.

(2) Preparation—Mix the fetal bovine serum, saline, and dry milk powder together thoroughly using a hot plate (temperature between 30 and 35°C) and a magnetic stirrer until a uniform liquid mixture is achieved. When the soil cools down to 20-25°C , add the rabbit blood to the prepared soil and mix thoroughly.

(3) Markers—Suggested (not exclusive) cleaning markers for residual analysis: protein, TOC, hemoglobin, and carbohydrates.

7.1.5.3 *Artificial Mucus Soil* (4)

(1) Description—The artificial mucus soil uses a modified artificial cystic fibrosis mucus formulation.

(2) Constituents—Components of test soil:

Mucin from pig mucosa: 1000 mg;
Casein hydrolysate: 500 mg;
NaCl: 500 mg;
Diethylene triamine pentaacetic acid (DTPA): 0.59 mg;
ASTM Water Type 1 (Specification D1193) water: 80 mL;
KCl: 500 mg;
Salmon sperm DNA: 140 mg;
Freeze dried egg yolk emulsion: 232 mg; and
Phosphate buffered saline (PBS) without Ca and Mg: 8.5 mL.