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Standard Guide for Blood Cleaning Efficiency of Detergents and Washer-Disinfectors[1]

This standard is issued under the fixed designation D7225; 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 guide is based on a standardized test soil correlating to coagulated blood suitable for screening tests and the evaluation of the cleaning efficiency of washer-disinfectors used for reprocessing of surgical instruments. This guide strictly deals with cleaning and does not describe any methods that are related to disinfection. See the Referenced Documents D5343, D4008, D4265, D4488, D2960, D3050, in Section 2 for additional information.

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

1.3 *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 to determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D5343 Guide for Evaluating Cleaning Performance of Ceramic Tile Cleaners

D4008 Test Method for Measuring Anti-Soil Deposition Properties of Laundry Detergents (Not Suitable for Detergent Ranking)

D4265 Guide for Evaluating Stain Removal Performance in Home Laundering

D4488 Guide for Testing Cleaning Performance of Products Intended for Use on Resilient Flooring and Washable Walls (Withdrawn 2009)[3]

D2960 Guide for Controlled Laundering Test Using Naturally Soiled Fabrics and Household Appliances

D3050 Guide for Measuring Soil Removal from Artificially Soiled Fabrics (Not Suitable for Detergent Ranking)

2.2 *AAMI Standards:*[4]

ANSI/AAMI ST79 Comprehensive guide to steam sterilization and sterility assurance in health care facilities

AAMI TIR 12 Designing, testing, and labelling reusable medical devices for reprocessing in health care facilities: a guide for medical device manufacturers

ANSI/AAMI ST81 Sterilization of medical devices – information to be provided by the manufacturer for the processing of resterilizable medical devices

AAMI TIR 30 A compendium of processes, materials, test methods, and acceptance criteria for cleaning reusable medical devices

2.3 *ISO Standards*[5]

ISO 15883–2 Washer-disinfectors, part 2: requirements and tests for washer-disinfectors employing thermal disinfection for surgical instruments, anaesthetic equipment, bowls, dishes, receivers, utensils, glassware, etc.

ISO/TS 15883–5:2005 Washer-disinfectors, part 5: test soils and methods for demonstrating cleaning efficacy of washer-disinfectors

3. Summary of Guide

3.1 The standardized test soil is based on a proteinous matrix containing fibrinogen and thrombin in two separated components. Coagulation and formation of fibrin fibers are induced after mixing the two components.

3.2 The suggested methods are based on the removal of standardized test soil as a result of mechanical or chemical action, or both, of the tested detergents or washer-disinfectors, or both. The screening test provides qualitative results for cleaning efficacy. After testing the practical situation in a washer-disinfector, the end result is visually checked for immediate evaluation. Minor residue is detected by using the

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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] The last approved version of this historical standard is referenced on www.astm.org.

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

[5] Available from International Organization for Standardization (ISO), 1, ch. de la Voie-Creuse, CP 56, CH-1211 Geneva 20, Switzerland, http://www.iso.org.

peroxidase reaction. The quantitative test provides quantitative results for cleaning efficacy by providing measuring the removal of a known amount of protein. After placement of the test coupon within a washer or in a beaker of water, the end result is measured gravimetrically or by spectophotometer detection of protein residue, or both.

4. Significance and Use

4.1 *Significance*—Dried blood represents a significant challenge to cleaning surgical instruments. The water-soluble components of blood are easily rendered insoluble when exposed to heat, chemical solutions, or time at room temperature. The water insoluble component of blood is fibrin built up during coagulation. These proteins bind quite readily to the surfaces of surgical instruments making them difficult to remove even with the aid of chemical cleaning agents. Instruments contaminated with blood residue after reprocessing represent a significant threat for infection to healthcare workers and patients. Healthcare facilities typically employ the use of automated instrument washers. These devices combine mechanical action along with chemical cleaning agents in a staged cleaning cycle designed to thoroughly clean surgical instruments. To function properly, these machines must be performing at targeted mechanical efficiency and deliver the correct chemical cleaning agents at the correct temperature, at the correct dosage for the correct period of time. Manufacturers of automated washers and manufacturers of cleaning detergent need to evaluate the performance of their products utilizing a surrogate for surgical instruments soiled with blood. The results of the performance testing will be used to improve product design and for validation of the performance of their product for various regulatory requirements.

4.2 *Use*—The regular, periodic use of the blood soil test is a systemic challenge to the functioning of an automated washer. To properly challenge the cleaning device, the test must be analogous to the dried blood soil, to the stainless steel substrate, and to the physical barriers presented by surgical instruments. These physical barriers include the box lock, or pivot joint of a hinged instrument, the serrated tips, and crevices of surgical instruments. On the test coupon, the components of blood are similar to the state of dried blood on instruments. By utilizing a grooved stainless steel coupon, the substrate is similar to that of stainless steel instruments. By mounting the soiled coupon in a plastic holder the physical barriers represented by cracks and crevices of instruments (for example, box locks) are represented. Users are provided with an interpretation guide that aids them in interpreting results that are less than optimal. For instance, failure to remove the fibrin layer of blood soil (which is water insoluble) indicates a problem with the chemical cleaning agent(s). Failure to evenly remove a hemoglobin soil indicates a mechanical failure. Failure to remove any soil indicates either a catastrophic mechanical failure, or inappropriate settings for the initial rinse stage. As a standardized challenge, the test provides a reproducible means for the washer manufacturer and the detergent manufacturer to compare new designs and formulations to those existing within their own product line as well as those of others in the market. For the purpose of submitting their instructions for use, the test provides a means to validate the performance of their product with a device that is a surrogate for the devices their products will be used to clean in the practical setting. This validation testing can be used as part of any necessary documentation for regulatory filings and records.

5. Reagents and Materials

5.1 *Standardized Test Soil*

5.1.1 *Composition:*

5.1.1.1 *Component A:*

400-mg albumine, bovine, protease free;
400-mg hemoglobin, bovine, lyophilized; and
60-mg fibrinogen, bovine, lyophilized.

5.1.1.2 *Component B:*

400-mg albumine, bovine, protease free;
400-mg hemoglobin, bovine, lyophilized; and
12.5-NIH units thrombin, reagent grade from bovine plasma.

5.1.1.3 *Solvent A*—5.0-mL 0.4 % NaCl solution (Reagent grade NaCl dissolved in sterile water).

5.1.1.4 *Solvent B*—5.0-mL 0.4 % NaCl solution + 8.0-mmol/L $CaCl_2$. (Reagent grade NaCl dissolved in sterile water).

5.2 *Preparation*—Components A and B are dissolved in their corresponding Solvents A and B by shaking for 1 h at room temperature (from 20 to 37°C) in two sealed 10-mL glass vials. Shaking by hand is acceptable and the most easy/practical solution and is tested to give reliable results. A laboratory quality shaker could be used for a more reproducible process.

6. Sampling, Test Specimens, and Test Units

6.1 *Preparation of Test Coupons*

6.1.1 *Coupons for Screening Test*

6.1.1.1 Stainless steel plates (70 × 20 × 1 mm, material: 1.4301) are used for the screening test to evaluate the chemical efficiency of detergents. The steel plates must be precleaned and free of any residue. They should be marked with a waterproof marker. A blind control of the finished test should always be conducted: Immersion test of a test object in demineralized water at room temperature (58 to 77°F).

6.1.1.2 Fifty microlitres of the dissolved Component A and 50 µL of the dissolved Component B are dosed onto the surface of the coupons, mixed, and spread to cover approximately 4 cm^2. Both components are dosed on the same spot of the same side of the plate, mixed (with pipette) and spread onto the surface.

6.1.1.3 The test coupons are then dried at room temperature (68 to 77°F) and 40 to 60 % humidity for 24 h (convection may be used to speed the drying process). Coupons needs to be stored out of reach of any chemicals or fumes (for example, disinfectants, detergents, or solvents).

7. Procedure

7.1 *Peroxidase Reaction for the Detection of Residue*

7.1.1 *Test Solution:*

7.1.1.1 *Solution 1*—0.1 % tetramethylbenzidine (TMB) in 5 % acetic acid.

7.1.1.2 *Solution 2*—3 % hydrogen peroxide solution.

7.1.2 *Detection of Residues:*

7.1.2.1 A swab is used to sample nontransparent lumens or surfaces. Only use a clean cotton swab that does not react with the test solution. If surfaces are dry, a swab is moistened with a drop of water. If the surface is wet, the swab need not be pre-moistened.

7.1.2.2 Activate 1 mL of Solution 1 with 100 µL of Solution 2 and drop the swab in the liquid. Blood residue will be indicated by a blue color reaction. Visible color change will occur at 0.1 µg or greater.

7.2 *Screening Test for Enzymatic Detergents:*

7.2.1 *Method*—Prepare enzymatic detergent solution according to manufacturer's recommendations. Enzymatic detergents are normally used at 0.5 % (v/v) solutions. Dissolve 500 µL of enzymatic detergent concentrates in 100-mL flasks and fill up to volume with water of known quality (cleaning efficiency should be compared in demineralized water and water of standardized hardness). Transfer the solution into 100-mL beakers and warm up in a waterbath until the recommended temperature has been reached. Usually enzymatic detergents show best blood-cleaning efficiency at 45°C. Insert vertically three test coupons into the detergent solution so that the test soil and coupon are completely submerged. No stirring, shaking or any other means of mechanical action should be applied. Remove the coupons after 15, 30, and 60 min, let dry, and visually inspect for remaining residues (fibrin fibers).

7.3 *Test for Cleaning Efficiency of Washer-Disinfectors*

7.3.1 *Method:*

7.3.1.1 Contaminate surgical instruments by pipette with test soil prepared in 5.1 and let coagulate and dry. Take care that critical parts of the instruments are contaminated, for example, joints or lumen. Specially designed test coupons might be used instead of real instruments.

7.3.1.2 Place contaminated instruments or test coupons on instrument trays and reprocess in the washer-disinfector with the recommended program for decontamination of contaminated surgical instruments. Ensure that cannulated instruments are connected to the channel irrigation system of the washer.

7.3.2 *Detection of Residue:*

7.3.2.1 *Peroxidase Method*—Inspect instruments or test coupons for any visible residues and use the peroxidase reaction described in 7.1 for detecting blood residues inside invisible places (for example, lumens or joints)

7.3.2.2 *Gravimetric Method*—Measure the weight of the test coupons with a balance capable of measuring 0.1 mg. For this purpose, the test coupons need to be marked (see 6.1) and also measured before the application of the test soil:

$$\text{cleaning efficiency} = 100 - [(m2 - m0)/(m1 - m0)] \times 100\ \% \quad (1)$$

where

m0 = weight of the empty test coupon, mg,
m1 = weight of the test coupon with dried test soil, mg, and
m2 = weight of the test coupon after the dipping test, mg.

7.3.2.3 *Chemical Detection Method*—Elute residual organic material from the surface of the test:

(1) Immerse coupon in a sterile beaker containing 3 mL of Bradford's reagent.

(2) Soak for 30 min at room temperature.

(3) Mix solution.

(4) Transfer 20 µL aliquots to wells of 96 well microtitre tray.

(5) Test for absorbance at 595 nm for protein.

8. Interpretation of Results

8.1 A visual clean surface without detectable traces of residues is the optimum result for checking the cleaning efficacy of processes. Additional information regarding the activity of a detergent or cleaning process can be achieved by the amount of time needed to completely remove all protein. Visible fibrin (colorless protein) indicates a lack of chemical cleaning efficacy while visible hemoglobin (red protein residue) indicates a lack of water/detergent coverage in a washer.

NOTE 1—Pure water will give a cleaning efficiency of approximately 95 %, leaving only the fibrin fibers behind. An active detergent needs to clean the test coupons completely, indicating a pass/fail result.

9. Keywords

9.1 blood cleaning efficiency; detergents; washer-disinfectors