Connecting Across Competencies: Leveraging Best Practices for Processing

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Abstract

The AAMI working group ST/WG 93 is finalizing a standard (AAMI ST98) for the cleaning validation of reusable medical devices based on guidance from the technical information report AAMI TIR30:2011/(R)2016. A number of analytical best practices are being considered for this new standard. Test method suitability for processing cleaning validations historically has been established using one positive control and performing an extraction efficiency. The new cleaning validation standard is proposed to require a change from only one replicate test sample to three when performing method suitability. This change will affect manufacturers; therefore, the value of and consideration for performing these additional replicates requires explanation. This article discusses how variation of validation parameters can affect the accuracy and precision during method suitability testing. Multiple replicates are needed to understand the variability of method extraction and impact on cleaning validations of reusable medical devices.

Reusable medical devices, which are intended to be processed for subsequent patient use, rely on the validation of their instructions for use (IFUs) to ensure patient safety. Via the use of objective evidence, validation is a confirmation process through which specified requirements are consistently fulfilled.[1] As the validation of the IFU is important to ensuring patient safety, it is critical that the testing methods associated with the IFU validation undergo a test method validation for each test analyte.

Validation of analytical detection methods used during cleaning validations for reusable medical devices (e.g., protein, TOC [total organic carbon]) should evaluate the following: specificity, linearity, range, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability testing.[2] When working with nonliquid/absorbable products (e.g., reusable medical devices), validating the extraction method of the analyte from the device is equally important. To adequately understand if the extraction method is removing the test analyte, validation elements also should be applied, as well as variables controlled, in order to yield a repeatable experiment.

The extraction method validation is not a unique requirement of cleaning validation studies. In addition to it being required for all analytical techniques where the sample is not dissolved in the extraction eluent, the technique also is used when determining device bioburden (or the population of viable microorganisms on or in a product and/or sterile barrier system[1]). The method suitability (i.e., recovery efficiency) measures the ability of a specified technique to remove, collect, and/or culture microorganisms from a product.[1] Bioburden test results generally do not fit a mathematical distribution model. Therefore, extraction method validations provide the measurement of uncertainty, precision, and bias of the extraction procedure and have a goal of being as high as practical.[3] The following extraction variables in combination can greatly affect the outcome of the recovery validation:

- Amount of analyte
- Extraction volume
- Shaking method (e.g., mechanical, manual, orientation)
- Extraction container configuration (e.g., size, orientation)
- Shaking force (e.g., distance, frequency of shake)
- Device size and mass
- Inoculation/soiling location
- Extraction eluent
- Compatibility of analyte detection method

A combination of these variables to maximize the recovery efficiency should be well established in a method validation before test samples are evaluated.

The technical information report AAMI TIR30:2011/(R)2016, *A compendium of processes, materials, test methods, and acceptance criteria for cleaning reusable medical devices*,[4] provides little guidance regarding the expectations of a recovery method validation. As a result, the industry has accepted as little as one data point, using an exhaustive recovery technique on the positive control, to establish the method recovery validation.

To address this issue, the AAMI working group ST/WG 93 is finalizing a standard (AAMI ST98, *Cleaning validation of health care products—Requirements for development and validation of a cleaning process for medical devices*). Working drafts of ST98 include new language to close this gap and set requirements for extraction method recovery validations.

The new draft language proposes that a minimum of three data points be generated to establish the extraction method recovery efficiency and associated correction factor. The recovery method should be optimized for recovery efficiency, and if needed, modifications to the method should be used to improve the recovery rate. This change in industry guidance will require a shift in the timing for cleaning validations, as best practice for establishing a recovery rate and associated correction factor is to optimize the method of extraction prior to performing testing on test devices in the cleaning validation. To achieve the new requirements in ST98, method development for optimized extraction will need to be prioritized before a full cleaning validation is performed on reusable medical devices, in order to avoid having to repeat the entire validation.

Although cleaning validations certainly share similar extraction variables that may affect the method performance, concern has been raised about the reproducibility within the extraction technique as a realistic expectation when using test soil and measuring against a test analyte. This experiment was designed to test the null hypothesis that if testing variables are well controlled within an extraction recovery experiment, the data reproducibility using the standard deviation of the test set should fall within the normal accuracy range for analytical testing methods of ±20% of the expected value.[2]

Materials and Methods

Given the variety of variables within a cleaning validation, the extraction recovery validation must be uniquely established for the validation testing in which the resulting correction factor will be applied. (This is elucidated in the forthcoming ST98 standard.) Within this experiment, specific extraction variables were selected to be constant for each experiment, while a select few variables were challenged to demonstrate how small changes to variable combinations can cause variations in the recovery rate data.

Controlled Variables

Test coupons. To eliminate the variable of device design and/or construction from the experiment, testing was performed using a rectangular precleaned stainless steel test coupon with a surface area of 34 cm^2 for the Miles and Miles modified test soils. The Lysozyme soil was performed using a butterfly coupon with a surface area of 25 cm^2.

Soil volume and application method. Using a micro-pipettor, 0.5 mL soil was applied to one side of the coupon. Soil was applied at a consistent location on the coupons and spread in an effort to achieve a uniform coat thickness (Figure 1).

Extraction vessel, eluent, and volume. Coupons were extracted using 40 mL ACS Reagent Grade water (Ricca Chemical, Arlington, TX) in a sterile 50-mL conical bottom polypropylene tube. Coupons were completely immersed in the water in the extraction vessel.

Extraction. Extraction was performed by agitation. This was done by rotating the tube to a horizonal orientation to ensure the coupon received the greatest mechanism of the extract against the surface and shaking the tube vigorously by hand (moving the extraction vessel in a horizontal 12-in/30.5-cm path at a frequency of 150 bpm as measured by a metronome) for 5 minutes.

Aliquot preparation. After the coupon was removed from the extraction vessel, the conical tube was vortexed until all remaining soil particulates were dissolved into the solution. An aliquot of 3 mL was then removed for testing.

Analyte testing. Analyte testing was performed using a bicinchoninic acid (BCA) protein residual assay using the Micro BCA Protein Assay Kit (ref. no. 23235; Thermo Fisher Scientific, Waltham, MA). The kit instructions were followed for the microplate procedure, with the only change being the volume of working reagent per well. The volume was increased 25% (from 150.0 to 187.5 µl).

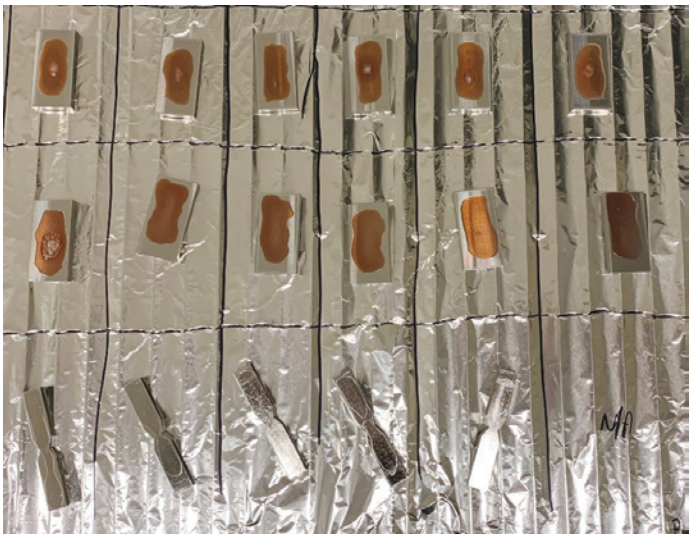

Figure 1. Soiled coupons.

Challenged Variables

Test soil. Soil composition will affect how the test soil adheres to the device and therefore affect how efficiently it can be removed. If the residual soil cannot be extracted, then the measurement of cleanliness is meaningless. Therefore, in addition to being clinically relevant, the components in the test soil formulation ideally should be able to be extracted for measurement in cleaning validations. If some of the components in the test soil are not extractable (e.g., cement, simethicone), a scientific justification for not including them in the measurement of extraction is documented. In this experiment, three test soils were selected to challenge the stainless-steel test coupon (Table 1).

Drying conditions. Time and temperature can have an effect on the binding properties of proteins within test soils, influencing soil adhesion and extraction recovery.[5] Two drying conditions were challenged within this experiment: (1) 18 hours under ambient conditions (e.g., ~25°C) in a drying cabinet without forced air flow and (2) 40 minutes at 82°C with ambient relative humidity in an oven.

Experiment

Test coupons were wiped with an isopropyl alcohol–wetted, lint-free cloth and air dried before being inoculated with test soil to remove any residual protein remaining on the coupon. Test soil was applied to the coupon surface with a pipette and distributed using the pipette tip. The coupons were laid flat to dry under the specified drying conditions. Then, they were placed in the extraction vessel with eluent and extracted. The coupons then were moved to subsequent extraction vessels until a total of four extractions had been performed for each coupon.

Extractions were prepared for protein detection using the BCA method for analysis. Protein values for extractions that fell outside of the calibration curve (e.g., first extraction) were diluted for more precise measurement. Testing was performed immediately after extraction. Testing for residual protein was performed and the exhaustive extraction efficiency calculated for each coupon using the following formula[3]:

$$\% \text{ Extraction efficiency} = \left(\frac{\text{First extraction}}{\sum \text{All extractions}}\right) \times 100$$

Results

Replicate extraction efficiency calculations were found to be consistent within the challenged variable combination, with the exception of Miles test soil at 18 hours drying under ambient conditions. The Miles test soil at 18 hours resulted in efficiencies between 44% and 40% in five of the six extractions and one result outside the range at 69% (Table 2).

Of the two variables challenged, soil composition had a demonstrable effect on recovery efficiency. This was evident because the only difference was a lower concentration of dry milk power in the modified Miles compared with the Miles test soil recipe. The study showed a significant increase ($P = 0.00$) in difficulty of removal (>40%) when one ingredient was changed in the test soil.

The comparison of two drying parameters did not seem to affect the range of recovery efficiency data to a great degree, with the exception of the Miles soil (Figure 2). The average recovery efficiency at the target specification and the standard deviation for each of these test scenarios supported the acceptance of the null hypothesis for data reproducibility.

Discussion

As demonstrated in this experiment, changes in the composition of the test soil can have a substantial impact on recovery rate. Because it is not well understood which test parameters will have the greatest effect on recovery efficiency, it is critical to evaluate the extraction method and optimize performance before using the extraction method to evaluate test samples. To truly have an optimized extraction method, various test parameters should be taken into

Test Soil	Recipe
Miles test soil[6]	Mix fetal bovine serum (10 mL), physiological saline solution (1 mL), dry milk powder (6 g), and rabbit blood (1 mL) thoroughly using a hot plate (temperature between 30°C and 35°C) and a magnetic stirrer until a uniform liquid mixture is achieved. When the soil cools to 20–25°C, add the rabbit blood to the prepared soil and mix thoroughly.
Modified Miles test soil	Mix fetal bovine serum (10 mL), physiological saline solution (1 mL), dry milk powder (3 g), and rabbit blood (1 mL) thoroughly using a hot plate (temperature between 30°C and 35°C) and a magnetic stirrer until a uniform liquid mixture is achieved. When the soil cools to 20–25°C, add the rabbit blood to the prepared soil and mix thoroughly.
Lysozyme	In a 500-mL volumetric flask, add lysozyme (1 g) and fill to line with reagent-grade water. If needed, use a magnetic stirrer until the lysozyme is thoroughly dissolved.

Table 1. Test soil formulations.

consideration during the experimental design and associated testing should be performed to demonstrate effectiveness. Determining the extraction efficiency using only the positive controls used in the cleaning validation test system is not best practice, as no opportunity exists for optimizing the method.

New industry guidance will leverage learning from microbiological methods and suggest that extraction method validations be performed using a minimum sample size of three. As has been demonstrated in this experiment with the Miles test soil, an increased sample number might be required to achieve a higher confidence in the efficiency of

Soil	Drying Condition	Coupon No.	Total Mass of Protein (µg)	Protein per Surface Area (µg/cm²)	Extraction Efficiency (%)
Miles	18 h, ambient	1	197,727.92	5,815.53	44.64
		2	203,625.78	5,988.99	46.80
		3	202,930.46	5,968.54	46.34
		4	200,978.19	5,911.12	48.76
		5	170,747.61	5,021.99	69.15
		6	205,250.39	6,036.78	49.90
	40 min, 82°C	1	188,775.11	5,552.21	50.57
		2	176,561.63	5,192.99	58.53
		3	180,535.19	5,309.86	50.76
		4	188,315.02	5,538.68	56.56
		5	183,980.15	5,411.18	53.22
		6	192,078.15	5,649.36	52.88
Modified Miles	18 h, ambient	1	117,217.71	3,447.58	99.01
		2	115,247.62	3,389.64	99.12
		3	112,872.51	3,319.78	99.34
		4	117,505.08	3,456.03	99.01
		5	115,331.03	3,392.09	98.97
		6	116,399.05	3,423.50	98.91
	40 min, 82°C	1	117,611.23	3,459.15	98.07
		2	114,660.05	3,372.35	99.30
		3	116,384.01	3,423.06	99.28
		4	117,207.11	3,447.27	97.77
		5	118,540.71	3,486.49	98.79
		6	116,414.16	3,423.95	97.62
Lysozyme	18 h, ambient	1	1,685.77	67.43	87.02
		2	1,665.73	66.63	90.21
		3	1,724.22	68.97	88.17
		4	1,694.38	67.78	88.31
		5	1,666.40	66.66	90.34
	40 min, 82°C	1	1,693.75	67.75	89.89
		2	1,661.73	66.47	89.28
		3	1,664.61	66.58	90.39
		4	1,707.78	68.31	90.44
		5	1,704.03	68.16	90.00

Table 2. Extraction efficiency results for the test variable combinations.

the test method. The following questions should be considered to determine if a sample size of greater than three is appropriate:

- Is the soiling method reproducible (e.g., soil recipe, application method)?
- Is the sample set able to deliver the minimum desired requirement (e.g., is the recovery efficiency as high as practical)?
- Is there an allowable variation of results from sample to sample (e.g., is the analyte variation expected)?
- Do the results achieve a hypothetical 90% confidence interval of the mean, which predicts if the average of all recovered analyte will be greater than any minimum target requirement?

The extraction efficiency, which is expressed as percent recovery, is a mathematical number that should not be viewed as an absolute number or considered more precise than the analytical method from which it was derived. Using a correction factor with inappropriate significant figures is one example where precision of the method may affect the results of the test samples. The correction factor calculated using the extraction efficiency should be applied to all sample results individually, including limit-of-detection values, before the test sample is evaluated for patient safety and significant figures are carried from the analytical measurement significant figures.

The extraction efficiency is a measure of the extraction method bias.[3] When evaluating devices for patient safety, if the individual extraction efficiency numbers in a set vary widely, using the worst-case individual number is recommended as a conservative measure. For example, in the case of the Miles test soil (18 h, ambient conditions), it would not be appropriate to use the average extraction efficiency value of 50% when the lowest value obtained was 44%. Using the average would bias the correction factor due to a high value within the data set. A small sample set (i.e., n = 3) does not provide enough data to categorize one result as an outlier and exclude it from the average calculation; therefore, the most conservative value should be used for the correction factor. Although consistency within a data set for recovery efficiency is important, with this example, the extraction method should be modified to increase the extraction efficiency rate.

A low extraction recovery rate may indicate a need to reevaluate the extraction method or the soil inoculation method. Devices should be appropriately challenged for worst-case use conditions. Overchallenging each test variable may result in the inability to remove the test soil. The extraction method is critical to the success of a cleaning validation for a reusable medical device.

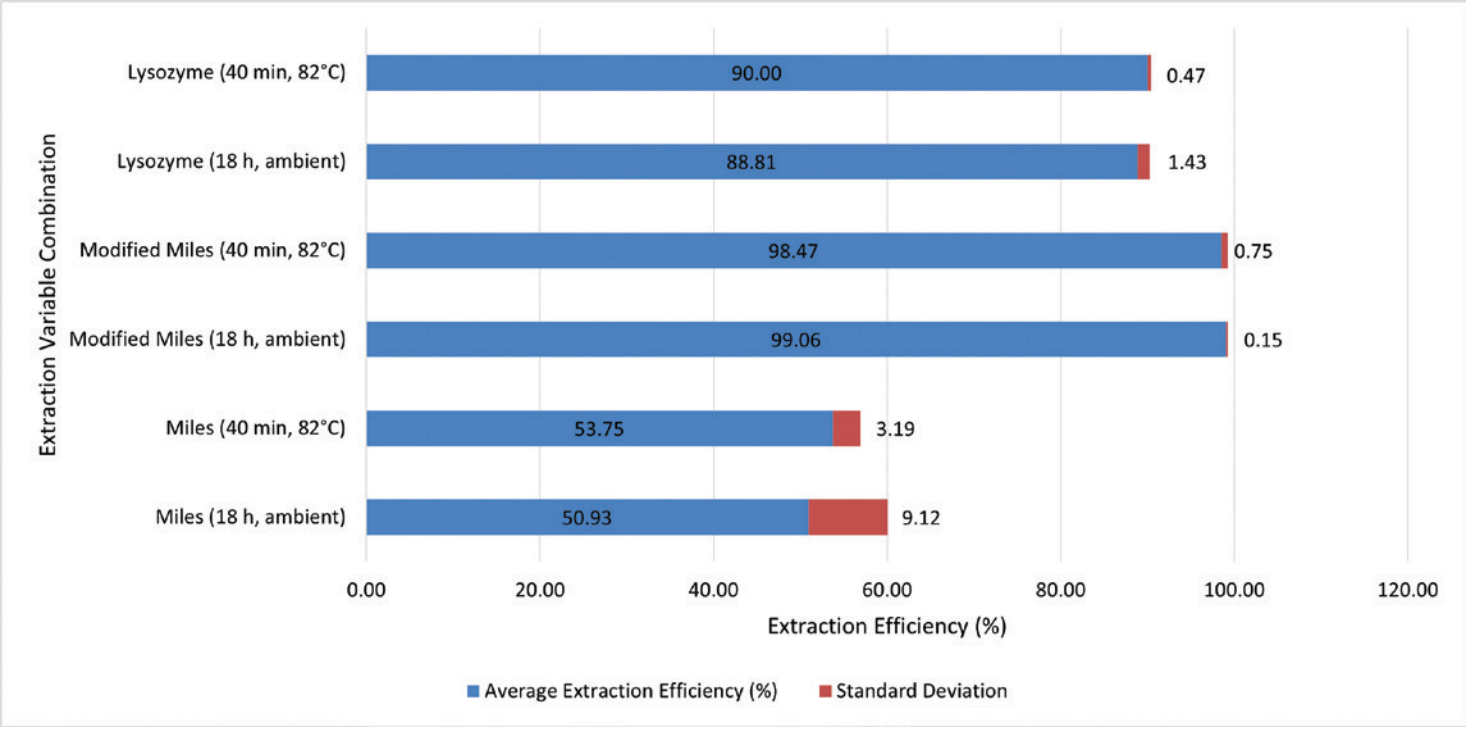

Figure 2. Extraction efficiency results per test variable combination. Data shown are percentage of average recovery efficiency comparison with standard deviation.

Conclusion

Controlling various test parameters in a validation study is an important role in test method development. Any change in a test parameter has the potential to affect the outcome of the validation result. Thus, as a part of method validation, it is important to consider the appropriate test parameters for the target device and develop a test method to facilitate the accuracy and precision of the validation results. The change to multiple replicates in the forthcoming AAMI ST98 standard supports the need to understand the variability of method extraction and impact on cleaning validations of reusable medical devices.

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