Automated Surface Swab Sampling: A Statistical Comparison of a Novel Approach to Existing Methods.

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Abstract

Analytical testing, coupled with an appropriate sampling method is instrumental to confirm equipment surfaces have been properly cleaned during cleaning validation or verification activities. The sampling method, preferably with a high percentage recovery, is critical to generating accurate results. Regulatory Health Authorities (RHA's) recommend manufacturers employ rinse, surface swab, or a combination of the two, with one favoring swab sampling methods. Until recently, surface swab sampling could only be performed manually by directly holding the swab (hand swabbing) or attaching the swab to the end of an extension pole (remote swabbing). Remote swabbing is commonly used for cases where the equipment surfaces to be sampled are not readily accessible due to access or the need for confined space entry. Both forms of manual sampling are prone to variability, but the remote swabbing method has greater variability as it is not only technique dependent [7], but also more difficult to control given the increased moment arm associated with the length and mass of the extension pole. To solve this problem Swabbot, Inc. has developed an automated swabbing device using readily available and configurable microcontrollers, microelectronics, and electromechanical components.

Swabbot designed and built a robot to automate the swabbing process for many surfaces exhibiting a wide range of accessibility. Automated swab sampling of pharmaceutical manufacturing equipment offers several advantages over hand swabbing or remote swabbing including decreased variability, the necessity for swab qualification of operators, increased accuracy versus remote swabbing, and decreased risk to personnel. For this case study, Swabbot teamed with Hyde Engineering + Consulting to perform a comparative study of hand, remote, and automated sampling using multiple replicates, concentrations, representative soils and controls to gauge the relative performance of hand, remote, and automated swab methods. The Swabbot achieved comparable recoveries to hand swabbing but with lower variability. Remote swab results exhibited higher variability, lower recovery, and was statistically dissimilar to both hand and automated sampling.

Keywords:

Cleaning, Cleaning Validation, TOC, Swabbing, Direct Surface Sampling, Remote Swabbing, Confined Space Entry Alternatives

Introduction

In the pharmaceutical and biopharmaceutical industry, sampling product contact surfaces is an integral part of cleaning process qualification and ongoing monitoring. This process has not changed dramatically in the past two decades. Sampling is generally accomplished by either indirect or direct sampling methods. Rinse sampling, an indirect method, and the first and simplest of the two sampling methods, is employed to sample large surface areas and equipment surfaces that are otherwise inaccessible. Surface swab sampling, a direct method, is the most commonly employed method employed for assessing surface cleanliness, and that most preferred by regulatory health authorities. This method, however, is more complex from both safety and qualification perspectives especially when considering sample locations that require confined space entry or the use of extension poles and remote swabbing techniques. Accordingly, in this paper we discuss the regulatory drivers for direct sampling, review the advantages and disadvantages, approach specific challenges of the surface swab sampling techniques, review the methods and materials used to compare performance, and present the

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results and analysis of the empirical data acquired to evaluate a novel automated surface swab sampling methodology.

While Regulatory Health Authorities (RHA's) such as the FDA, EMA, Health Canada, and the World Health Organization discuss both indirect and direct sampling methods, the FDA indicates a clear preference for surface swab sampling [1,2,3]. The primary reason for this is that accessible surfaces can be sampled and recover not only soluble residues, but dried or adherent soils can be transferred through mechanical action of the sampling method. Further, since there is greater precision with the direct sampling method, a level of soil per unit area may be established. There are, however, clear challenges and disadvantages of direct sampling that must be addressed to ensure that it is a viable technique.

While methods vary somewhat between manufacturers, PDA Technical Report 49 notes that "…swab sampling is not unlike manual cleaning processes, in that it is highly dependent on a person for consistency, consideration should be given to retraining and/or requalifying swab sampler on an established basis," [5]. Swabbing effectiveness is dependent on such technique and equipment variables as swab pressure, contact time, stroke consistency, swabbed surface area, swab head size, swab material, wetting diluent, wetting technique, and number of swabs. In general, it is important to ensure that sampling technicians are familiar with and can consistently reproduce the same exerted pressure on the sampled surface, which is typically accomplished through training, practice, and qualification. As the swab is drawn across the surface, technicians must also use an unhurried, smooth consistent stroke to ensure that there is adequate time for the dissolution and absorption of soils on the equipment surface. This also requires that the technicians not only use parallel overlapping strokes until the appropriate surface area has been sampled, but to properly gauge and reproduce a defined surface area. A typical pattern employed, and the one used for this study, is shown in Figure 1.

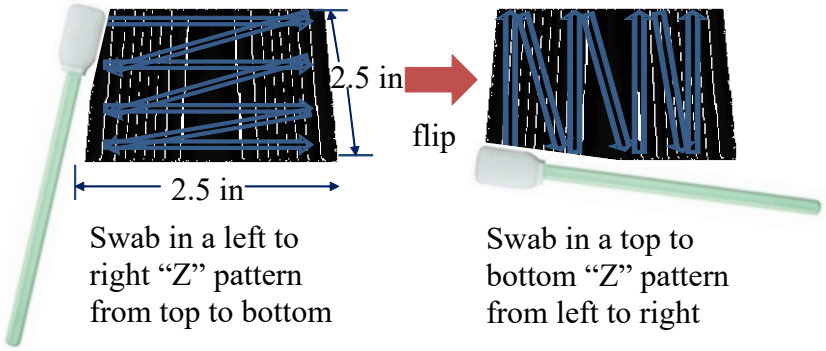

Figure 1. Swabbing pattern for hand swabbing and that performed by Swabbot

A qualified swab method defines the swab diluent, swab material, swabbing pattern, and swab extraction solution. Generally, the swab diluent and extraction solution are the same, although there are rare instances where they will differ. Once the surface is swabbed, the head of the swab is broken off in a vial containing the swab extraction solution and submitted to the quality control (QC) laboratory for testing. In the laboratory, additional operations may be performed on the contents of the vial per the qualified method and the residue extracted through agitation. The prepared sample is then tested using an analytical method such as High-Performance Liquid Chromatography (HPLC) or

Total Organic Carbon (TOC). There are, of course, additional concerns and considerations when sampling in the field and for swabbing less accessible sample sites.

Sampling process equipment in manufacturing areas is either conducted manually for directly accessible surfaces, or in less preferable situations, through confined space entry for those not immediately accessible, or with a remote extension pole that allows for sampling without personnel entry into confined spaces.

Confined space entry requires additional safety measures to mitigate job hazards to technicians performing the swabbing such as air monitoring, fall protection and rescue and extraction procedures. Failure to do so can have catastrophic results up to and including fatalities [6]. Swabbing within a confined space typically entails entry into process vessels in which an individual wearing a harness and fall protection is physically lowered through a manway into the equipment to perform the sampling. Specific risks include falls on slippery surfaces and asphyxiation. For companies that have deemed this risk too significant, alternative remote sampling strategies such as swab extension poles or automated sampling equipment constitute favorable alternatives.

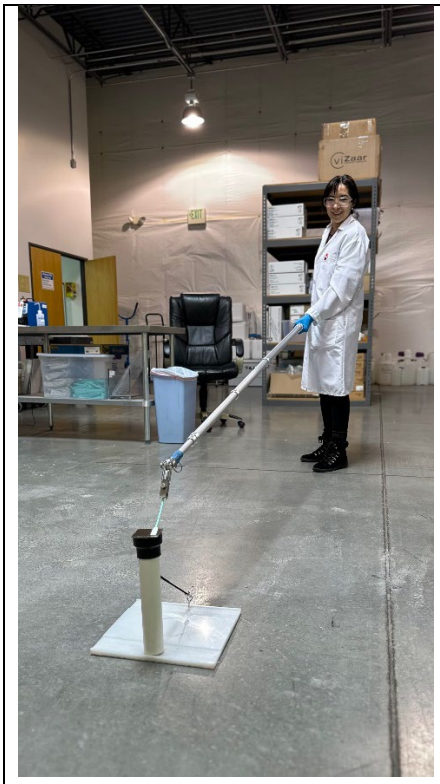

Figure 2:Remote Swab Sampling

Until recently, limited alternatives were available to confined space entry, the most commonly employed of which is remote surface swab sampling using an extension pole. While frequently used, sampling with an extension pole is not without challenges and drawbacks. Remote swabbing can be difficult to perform, time intensive, requires increased training and qualification, often produces lower recoveries than direct swab methods, requires either the use of a template to control the sampling surface area or estimating the sampling area, and has higher variability than direct manual sampling methods.

An automated sampling device, depicted in Figure 3, that can be deployed to remote and relatively inaccessible locations while ensuring a repeatable and controlled sampling method provides an additional alternative that can address many of the previously mentioned challenges. Accordingly, Swabbot collaborated with Hyde Engineering and Consulting to rigorously investigate the comparability of an automated method relative to existing methodologies.

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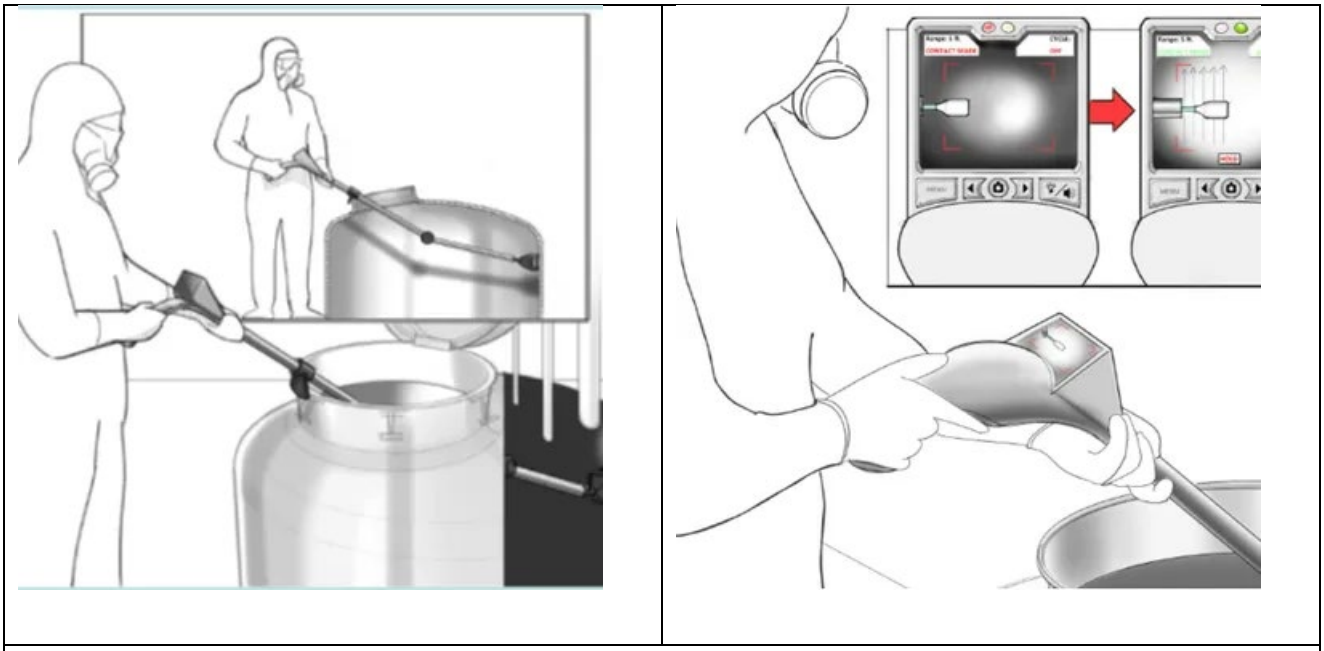

Figure 3: Conceptual Rendering of Automated Sampling Device

Materials/Methods:

Two soils were used to compare the Swabbot sampling method to the traditional hand swabbing and remote swabbing techniques. Sucrose (ACS grade) and BSA (1 mg/mL) were selected for their similarities to media and proteinaceous biological soils commonly found in pharmaceutical equipment and were spiked on 316L stainless steel. 316L stainless steel was chosen due to its prevalence as material of construction for pharmaceutical equipment. Ultrapure water was used as diluent. Texwipe TX714K Large Alpha Snap Swabs, and 40mL Low TOC glass vials (certified ≤ 50 ppb TOC) were used.

Total Organic Carbon (TOC) was used as the analytical method. Samples we analyzed using a Sievers M9 analyzer with an Acid Flowrate of 1.0 µL/min and Oxidizer Flowrate of 1.0 µL/min. Negative controls and blanks were run with an Acid Flowrate of 0.3 µL/min and Oxidizer Flowrate of 0.0 µL/min. Sample sequences were bracketed with a 5-ppm carbon sucrose standard followed by an ultrapure water suitability blank. Negative controls, diluent and coupon blanks, were run before positive controls and samples.

The representative soils were spiked at multiple surface concentrations, 0.5 µg/cm^2, 1 µg/cm^2, and 5 µg/cm^2, with 4 replicates per concentration, and allowed to dry overnight before swabbing. Positive controls were made by spiking the corresponding volume directly to the TOC vial.

Samplers were previously qualified to perform both hand and remote swab methodologies. A common sampling pattern was utilized for both the Swabbot and hand swabbing where the swab is moved back and forth across the coupon surface from top to bottom. Then the swab head is flipped and turned 90 degrees. Then the swab is moved up and down the coupon surface from side to side. A slightly

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modified sampling pattern was utilized for the remote swabbing to more accurately simulate the swab pattern utilized in the manufacturing/ cleaning validation environment. The remote swabbing pattern starts similarly to the hand swabbing method where the swab is moved back and forth across the coupon surface down the face of the coupon, however, the swab typically cannot be turned 90 degrees within a tank as the swab is held at an angle by the attachment at the end of the pole. In order for the swab to be flipped, the swab must be removed from the end of the pole or the angle of the attachment changed. In addition, to reduce the risk of contaminating the swab head by taking the swab in and out of the vessel, the swab is often not flipped over prior to performing a top to bottom swabbing. Remote swabbing was set at a distance of 10 feet from the sampling surface. Further, the remote swabbing test employed for this study was conducted in the horizontal plane (see Figure 2) rather than vertically in a process vessel, so the variability and results may vary from what many readers have experienced in the field. The swab patterns are depicted in Figure 1 and Figure 2.

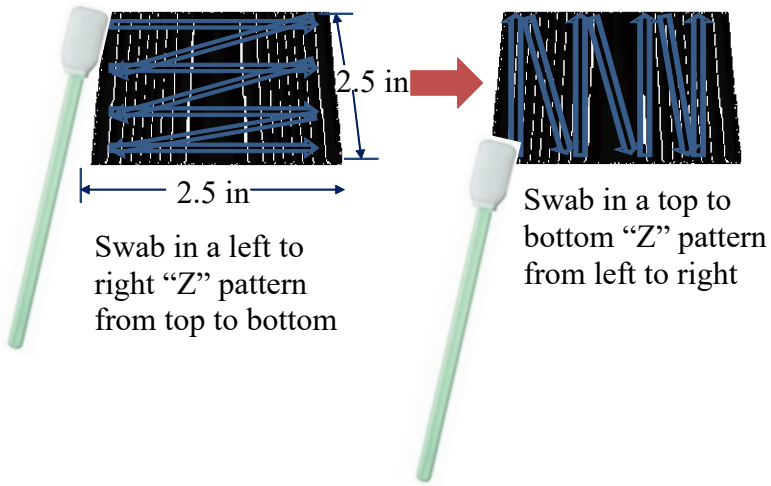

Figure 3. Swabbing pattern for remote swabbing

The swab was wetted with ultra-pure water and then the coupon swabbed with the corresponding method. Once swabbing was completed, the swab head was broken off inside the TOC vial for extraction. Residue was extracted by placing the sample vials in an ultrasonic bath for 15 minutes. The recovered material was determined using a Sievers M9 TOC analyzer. The results were then analyzed using Microsoft Excel for Microsoft 365 MSO (Version 2212, Build 16.0.15928.20278), and Minitab 21.2.

Results & Discussion:

A percentage recovery was calculated for each concentration as a ratio of the recovered material to that of the positive control samples as shown in Equation 1. The recovered TOC values and positive controls were both corrected for background TOC levels.

$$Recovery = \frac{(Recovered\ TOC - Coupon\ Blank)}{(Positive\ Control - Diluent\ Blank)} \times 100 \qquad \textit{Equation 1}$$

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From these values, standard deviations were calculated for each concentration and swab method to quantify variability. For comparison to automated swabbing, a percentage differences between the methods were also calculated. These results are shown in Table 1. In addition, a One-Way ANOVA (analysis of variance) was conducted for each recovered material to compare the means of the three swab methods for both sucrose and bovine serum albumin.

Table 1: Sucrose and BSA Recovery Data

Swab Sampling Results Summary (316L Stainless Steel)							
Expected Concentration (ppm C)	Method	Recovery (%)		Recovery % SD		% Difference from Swabbot	
		Sucrose	BSA	Sucrose	BSA	Sucrose	BSA
0.5	Hand	100	107	1	3	1	6
1	Hand	95	102	7	6	3	3
5	Hand	96	101	7	2	3	5
0.5	Remote	89	90	3	4	11	11
1	Remote	90	86	5	10	8	14
5	Remote	92	82	5	8	7	16
0.5	Swabbot	99	101	1	3		
1	Swabbot	98	99	1	4		
5	Swabbot	99	96	1	6		

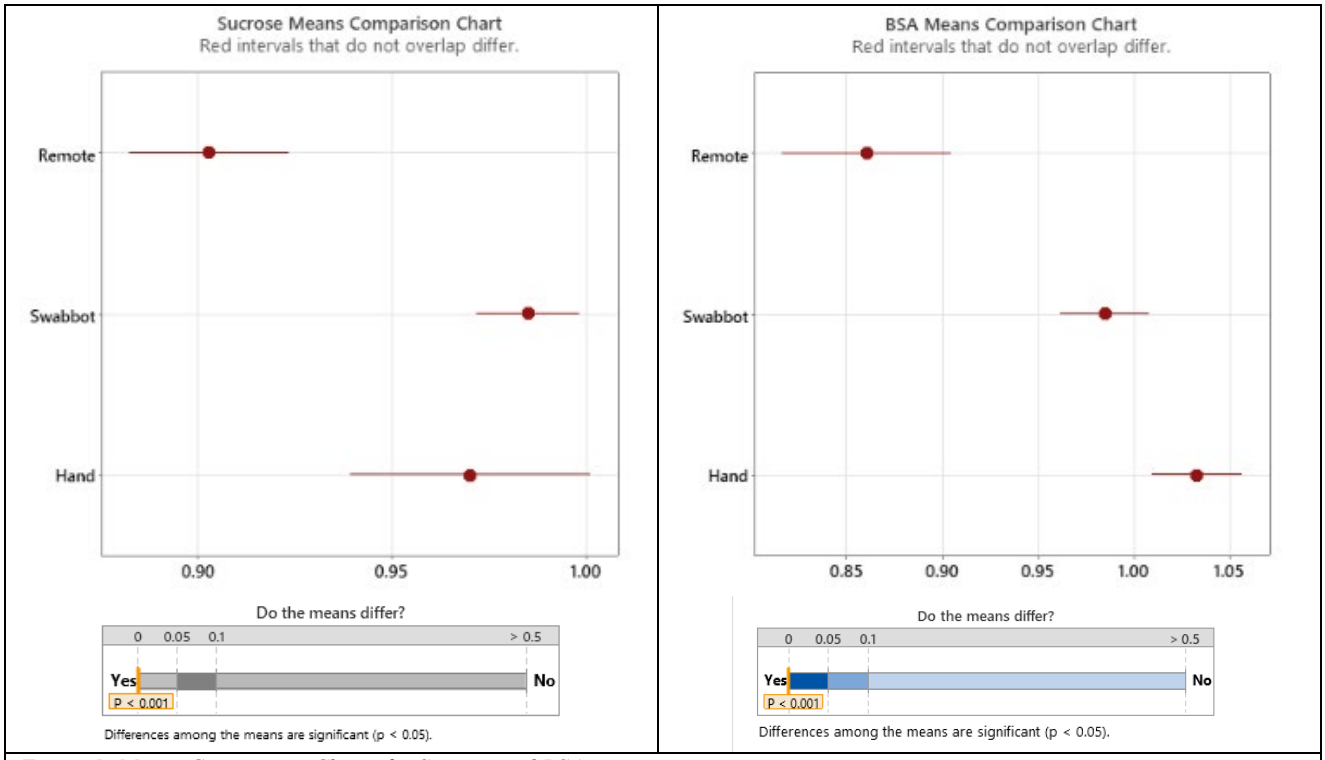

Figure 5: Means Comparison Charts for Sucrose and BSA

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The means comparison charts provide a visual illustration of the differences in the sampling methods. Red comparison intervals that do not overlap indicate means that differ from each other, whereas those that overlap can indicate that there is no statistically significant difference between the intervals. The comparative sizes of the intervals can also be important in determining whether the differences have practical applications.

For sucrose, we can conclude that the remote swab recovery means are lower and differ significantly from both hand and Swabbot data. Given the overlap of the comparison intervals for manual and Swabbot data, it cannot be concluded that the means differ from each other significantly. It is apparent, however, that the Swabbot data has a tighter distribution and less variability than the manual swabbing method with respect to sucrose.

For BSA we can conclude that there are differences between the means at the 95% confidence level for each method. It is apparent that the recovery for the manual swab method, while still precise, exceeded 100%, whereas only the lowest concentration for the swabbot recovery was in excess of unity. During the execution of the study, a slight adjustment of the equipment retaining the coupon for the high BSA concentration led to a slight shift in the swabbed area which slightly shifted the overall recovery distribution for swabbot. While the pattern and method were consistent, the swab pattern was slightly offset from the spiked area of the coupon.

Conclusions

The data indicates that the Swabbot sampling robot performed accurately and repeatably for both soils tested. When compared to more traditional swabbing methods, the Swabbot returned responses that were closer to 100% recovery than either the hand or remote swabbing. In addition, the Swabbot returned tighter datasets than the manual methods tested. The data was analyzed using a one-way ANOVA to determine if the datasets were statistically different from one another. This analysis indicated that the Swabbot swabbing samples are different than remote swabbing for both soils, and different from the hand swabbing for BSA.

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