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The Importance of Correct Sampling and Swabbing in ICM Programs

By Dr. Jay Glasel

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TAGGED: ATP, ICM, MEASUREMENT, SAMPLING, SWAB

The swabs used for sampling surfaces for microbial contamination are examples of devices that are simple in design and construction, but are difficult to use without discipline and training. This article discusses why this is so.

At present, swabs are widely used in the cleaning industry to perform Integrated Cleaning and Measurement (ICM). Samples collected from surfaces by specialized swabs may be introduced directly into instruments for indirect detection of food and microbial contamination via ATP bioluminescence measurement. In other cases surface sample-containing swabs may be placed into solutions to elute microbials from the swabs so that the microbials can be cultured for counting or identification.

In considering the determination of amounts of contamination of all kinds on surfaces, we need to distinguish between the taking of samples and the measurement of the materials collected in the samples. The contemporary methods and devices used for making contamination measurements are very accurate (see below for a discussion of the term "accurate"). For example, a well-calibrated and properly used ATP luminometer will almost invariably produce a number that accurately reflects the number of ATP molecules that have been released from the ATP-containing sample placed in the luminometer. The question is: what do these numbers mean?

What we are concerned with in this article is whether the surface contamination picked up and released by the swab sampler is meaningfully related to the amount of contamination on the surface. The theme of the article is: A meaningful relationship between what is sampled and what is there can exist, but that thought and training is necessary for it to exist.

The reason the meaning of contamination measurement data obtained from swab samples needs to be discussed is that if cleaning efficacy based on the data is charted on a day-to-day basis, and the numbers being charted have no constant interpretation, the "M" in ICM stands for "meaningless".

Sampling for Microbial Contamination Using Swabs

Using swabs to sample surfaces for microbial contamination has a long history dating back to 1917 when swabbing was first used in the food industry to detect the efficiency of cleaning dishes in a time when dishes in restaurants were washed by hand.

The swabs that are commonly used today for testing for microbial cleanliness are equivalent to sterile "Q-Tips"® that were originally a twisted cotton tab-on-a-stick device that was invented in 1923 to clean out babies' ears.

In the years since their first use in testing surfaces for microbial contamination, swabs and swabbing techniques have been repeatedly and thoroughly investigated by microbiologists who have been interested in the question "how accurately and consistently does what a swab picks up from a surface represent the contamination on the surface?" There have been hundreds of scientific papers devoted to this seemingly simple question.

On the basis of a large amount of data that has been obtained as a result of these investigations of the details of swabs and swabbing, it is easy to summarize the answer to the question posed in the previous paragraph: Both the accuracy and consistency of estimating the amount of microbial contamination are highly dependent on the material nature of the swab, its moisture content, the physical features of the surface being sampled, the swabbing technique used by the human sampler, and even the species of microbial present on the surface (because different microbials adhere differently on surfaces).

Swab sampling is therefore a method with a very large number of variables—many of them unknown to the

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person doing the sampling—that can affect the meaning of the microbial contamination data derived from swab samples.

Before we go further in this discussion, we need to understand the difference between consistency and accuracy because these two concepts are often confused and an understanding of the difference between them is important to understanding how sampling for surface cleanliness must be done.

Consistency and Accuracy

Consistency refers to how closely grouped together the results from any repeated action are. In the case of sampling microbials on a surface, sampling is consistent if it results in samples picking up and releasing the same number of microbials found on a uniformly contaminated surface every time the sampling is done.

Accuracy refers to how closely the results of any repeated action are to what is desired from the action. In the case of sampling microbials on a surface, sampling is accurate if what is taken up and released from the samples reflects the true value of whatever the numbers of microbials on the surface actually are every time the sampling is done.

For example, microbial counting measurements of surface contamination derived from swab samples can be consistent without being accurate if the swab pickup and release of microbials from surfaces are not perfectly efficient (i.e., the swab either doesn't pick up all surface contamination, or doesn't release all it picks up, or has both of these imperfect characteristics) provided that the swab pickup and release is equally inefficient for all samples taken.

Ideally, in order to make a meaningful analysis of microbial contamination data for surfaces we would like our estimations of the amounts of contamination to be both consistent and accurate. For reasons explained in the rest of this article, the best we can hope for is good consistency in data taken resulting from swab samples.

Poor accuracy with good consistency isn't really a problem when our interest is focused on the efficiency of surface cleaning: For ICM, we're really interested in comparing microbial contamination before and after cleaning. Consequently, if the consistency of the sampling before-and-after cleaning is good, and the counting measurement following samplings is accurate, our comparisons of cleaning efficiencies will be valid even though we won't know with great accuracy the actual numbers of microbials on the surfaces before-and-after cleaning.

The Foundation of Swabbing Protocols: Sample the Same Areas

The ideal swab and swabbing protocol would pick up all contamination from a defined surface area and release it completely onto (or into) whatever device is going to be used to score the amount of contamination. As mentioned previously in this article, swab samples could be eluted into a solution that is going to be applied to an agar surface of a petri dish or eluted into a solution containing the reaction mixture for producing ATP-induced bioluminescence.

Since modern swabs used for contamination sampling don't differ very much in structure from the Q-Tips that were originally used for this purpose, it's easy to see that it is going to be almost impossible to sample the whole of any surface completely with devices this narrow. Consequently, the basis of all swabbing protocols must be to sample the surface to be analyzed using a constant rubbing pattern of the swab within defined areas of the surface. After picking up the contamination, the release of each acquired sample must be consistent.

That is, for a given type of surface, the exact area that is swab-sampled needs to be defined and constant for all similar surfaces. And whatever is done with the swab samples to release the contents must be defined and consistently done.

The most convenient method for swabbing a defined area on a surface is to use a thin template with a hole with a defined area cut out of it. For example, templates may be cut out of thin plastic that can be sanitized after use and reused. Templates with different sized holes are needed for different surfaces: large areas for flat surfaces, smaller areas for curved surfaces.

Depending upon the material the swab tab is made of, the tab moisture content, the nature of the surface being sampled, and the operator's technique, the efficiency of microbial contamination pickup and deposit onto a testing device or solution has been measured to be anywhere from 20% to 80%.

What is important is that, whatever the swab is made from, its moisture content, etc. the method of use should be the same so that the efficiency remains at whatever it is for that type of swab. In other words, the efficiency of pickup and deposit needs to be kept as constant as possible—so that even though the microbial count may not be accurate, good consistency may be obtained. That is, in determining cleaning effectiveness, as long as the consistency is constant for each swab sample, we can obtain consistent meaningful data.

On the other hand, if the swab sampling pickup and deposit efficiencies vary greatly from sample to sample the microbial count data becomes meaningless no matter how many samples we obtain even though the samples are scored using a perfectly accurate instrument—for example, an ATP luminometer. This is another example of "garbage in, garbage out".

Effects of Swab Materials, Swab Wetness, Different Surfaces

Over the course of the many years that swabs have been used to analyze microbial contamination of surfaces, many materials have been used to form the pickup/release tabs of swabs. Among the materials tested are: cotton, rayon, polyester, microfiber, macrofoam, and flocked nylon. The pickup and release efficiencies of many of these materials have been the subject of many studies. Further studies have been reported on the effect of pickup efficiencies of some of these tab materials from surfaces made from different materials, and other studies have been reported on the effects of tab wetness on pickup efficiencies. In addition, there have been reports of release efficiencies into solutions of microbials that have been picked up by swabs whose tabs are made of different materials.

Having read in detail many of these reports, I have come to the firm conclusion that, in practical situations, it is basically impossible to predict the accuracy with which a swab measurement of any kind will reflect the actual numbers of microbials that existed on a sampled surface, either before cleaning or after.

So what can be done to get meaningful data from contamination measurements based on swab samples? The answer is to perform the most consistent sampling possible.

Effective Sampling

The requirements for obtaining consistency in swab sampling may be summarized as:

1. Swabbing protocols for each type of surface (flat, curved, irregular shapes) must be established, written down, and understood by all persons performing the swab sampling.

- Surfaces of different shapes or made of different materials will require different protocols.
- Hands-on training in the use of the protocols must be a featured part of any effective cleaning effectiveness program.

2. The path pattern and total lengths of travel for the swab tab over each type of surface must be the same.

- For example, on flat surfaces the same total areas must be sampled and the resulting contamination data can only be meaningfully compared between surfaces of the same material (flat ceramic tile to flat ceramic tile, stainless steel to stainless steel, etc.)
- The persons doing the sampling must be trained to rotate the swabs constantly while following the swabbing pattern established by protocol for each type of surface.
- The most serious variable in swabbing is the pressure the swabber exerts on the swab while rubbing over a surface. Controlling the swabbing pressures exerted by two different individuals is impossible. Therefore, individual swabbers need to be trained to consistently exert whatever pressures they use while swabbing.

3. Surfaces where food particle contamination is possibly mixed with microbial contamination cannot be expected to give meaningful raw data on microbial contamination separated from food contamination.

- For example, an area free of food contamination but seriously contaminated microbially may give the same contamination concentrations if the measurement of the contamination is done via the ATP bioluminescence method.
- The only practical way the amount of microbial contamination may be estimated in the presence of food particle contamination is by conventional microbiological methods (ie, obtaining an aliquot from the swab sample and incubating it in growth medium or by using a RODAC plate to sample a surface near the one that has been swabbed).

4. For estimating cleaning efficiencies, day-to-day data comparisons of contamination measured from swabbed samples are only meaningful if the comparisons are between samples obtained when:

- The swabbing was over the same surface areas.
- The swabbed surfaces were made of similar materials.
- The same swabbing protocols were used for similar surfaces.
- The same operator did the before-and-after swabbing.

This article was made possible by an educational grant from Kaivac.

The Importance of Correct Sampling and Swabbing in ICM Programs: Created on May 3rd, 2010. Last Modified on May 3rd, 2010

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About Dr. Jay Glasel

Dr. Jay Glasel is the Managing Member and Founder of Global Scientific Consulting, LLC. He is a Professor Emeritus in the Department of Microbial, Molecular and Structural Biology at the University of Connecticut Medical/Dental School in Farmington, Connecticut. He has lectured and done research in many countries in Europe and Asia. Dr. Glasel's scientific research has been in the fields of structural biochemistry, molecular immunology, pharmacology, and cell biology. Major portions of the research involved the structure and properties of water and aqueous solutions and on the structural chemistry and molecular biology of opiates and opiate peptides. He pioneered the uses of anti-morphine monoclonal antibodies and anti-opiate receptor anti-idiotypic antibodies in research on the cellular effects and actions of narcotics.

Dr. Glasel is co-editor and an author for the Academic Press textbook "Introduction to Biophysical Methods for Protein and Nucleic Acid Research" and many other contributed book chapters and original scientific research articles.

Dr. Glasel obtained a B.S. in chemistry and physics from Caltech. His Ph.D. from the University of Chicago was in chemical physics for work on chemical reactions on comets. He has served on active duty in the U.S. Air Force as a nuclear research officer.

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