INTRODUCING CYTOQUANT: 1

FLOW CYTOMETRY AND CLEANING VERIFICATION IN FOOD MANUFACTURING FACILITIES

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Food safety depends in large part on the hygienic conditions in food manufacturing facilities. The presence of high levels of non-pathogenic bacteria can affect the shelf life and quality of foodstuffs for consumers. Equally important is the absence of pathogens (such as salmonella and listeria) in food that could potentially cause illness. Food manufacturers must be diligent in keeping their processing environment clean to prevent the cross-contamination of the final product. But how is this currently being done?

Visual inspection

While visual inspection is a prerequisite, it is in and of itself not sufficient. It is a subjective and imprecise means of verifying proper cleaning. More importantly, even if a surface has no apparent residue, this does not mean it is immaculate. Visual inspection cannot ensure that all food residues from the previous run have been cleaned away or that a sanitiser has effectively reduced the microbial level on the surface.

Microbial enumeration with agar-based methods

These are the traditional methods for monitoring the hygiene of the processing environment. Cultural methods are addressed in ISO 18593, 'Microbiology of the food chain -– Horizontal methods for surface sampling', part of the FDA's Bacteriological Analytical Manual and USDA's Microbiology Laboratory Guidebook. Generally, there are two ways to conduct agar media-based methods: direct or indirect. With direct or contact-based methods, plates or dip-slides are placed on the surface to be sampled and then incubated. Indirect sampling is carried out with swabs, tissues or sponges that are then diluted in a buffer solution before being pipetted into petri dishes and streaked out. The main limitation of these traditional methods of microbial detection is the amount of time it takes to obtain results. Furthermore, most species of bacteria cannot be cultivated on agar, a phenomenon known as the great plate count anomaly.

ATP detection

Adenosine triphosphate (ATP) is a nucleotide that cells use to deliver energy. It can be thought of as the molecular 'unit of currency' for transferring energy within all living cells. Energy is needed for all cellular activities including the synthesis of proteins and membranes, cell movement and cellular division. Energy is transferred when ATP breaks down into adenosine diphosphate and a free phosphate. Hydrolysing the covalent links of the phosphates liberates energy that is used for reactions. Commercial ATP test systems harness the luciferin/luciferase reaction, which is very common in nature, to generate visible light with the energy provided by ATP. The more light is emitted, the more ATP is present, which could indirectly indicate more food residues or more micro-organisms. Yet there is one important caveat: as these systems are widely used for cleaning efficiency validation, disinfectants are also commonly involved in the reaction. These disinfectants can break down the cell walls of micro-organisms but preserve their ATP, meaning that there may not be a real correlation between living organisms present on the surface and the results of the ATP measurement.

ATP methods harbour a further potential disadvantage: they vary in their applicability depending on the food residue to be detected. For example, ATP testing would not lend itself to testing wheat flour as it is a matrix that leaves behind little ATP in its residue. Meat product residue, however, contains high levels of ATP. Although no bacteria can be directly counted with ATP test systems, it is widely used because results are generated within seconds, a time-to-result available in no other widely applied technology until now.

So, why can't we have it all? The holy grail of cleaning verification

What food manufacturers need is a quick method that directly quantifies both bacteria and particles and is not influenced by disinfectants and temperature. Although this seems out of reach, the basic technology for making this happen already exists and is being applied today.

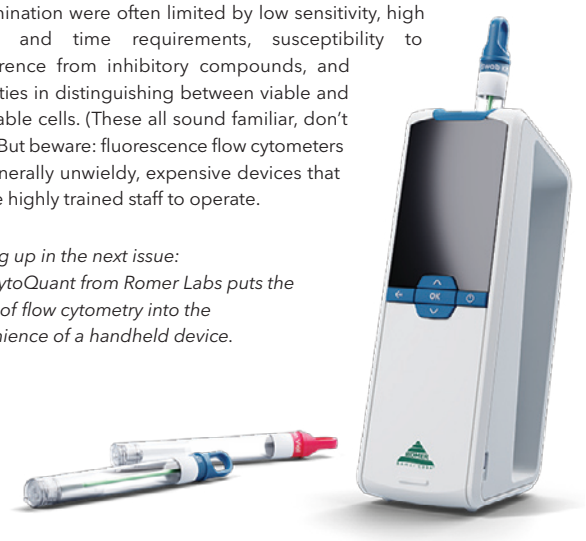
Introducing Flow Cytometry

Flow cytometry (FCM) refers to a group of techniques that use optical or electrical signals to detect and measure certain physical or chemical properties of cells and particles suspended in a fluid. Nearly 300 studies conducted between 2000 and 2018 assessed FCM as a tool to characterise microbial water quality. This research was able to illustrate the value of FCM in water treatment, distribution and reuse. There is now a body of research documenting successful applications of FCM robust enough to suggest that it could reasonably and realistically see widespread adoption as a routine method for water quality assessment.

What does all of this have to do with cleaning validation in food manufacturing facilities?

Methods that were previously common in water quality determination were often limited by low sensitivity, high labour and time requirements, susceptibility to interference from inhibitory compounds, and difficulties in distinguishing between viable and non-viable cells. (These all sound familiar, don't they?) But beware: fluorescence flow cytometers are generally unwieldy, expensive devices that require highly trained staff to operate.

Coming up in the next issue:
How CytoQuant from Romer Labs puts the power of flow cytometry into the convenience of a handheld device.



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INTRODUCING CYTOQUANT: 2

FLOW CYTOMETRY AND CLEANING VERIFICATION IN FOOD MANUFACTURING FACILITIES

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Our series continues with a look at impedance flow cytometry and how, as a portable technology, it stands to revolutionise cleaning verification.

Getting the power of flow cytometry into a handheld device

To make flow cytometry (FCM) a viable solution for cleaning verification in food processing facilities, it needs to come in a portable format that is simple and easy to use, yet accurate enough to provide reliable counts of bacteria and residue particles in environmental samples. This has been made possible by the use of impedance flow cytometry.

Impedance flow cytometry is a specific kind of flow cytometry: instead of optical systems such as laser technology, impedance flow cytometers employ an alternating current at varying frequencies which enable the device to detect and count cells and residue particles separately. While optical-based flow cytometers are only able to count cells labelled with dyes, impedance flow cytometers can perform the same operation without any need for labelling. Compared to other flow cytometric devices, they are compact, portable and battery-powered, enabling them to be used where the sample is taken.

How can impedance flow cytometers distinguish between cells and residue particles?

The electro-magnetic properties of bacteria enable flow cytometers to distinguish them from other particles. The cytoplasm and the cell membrane of a bacterium change the electrical field in unique and identifiable ways. While the electrical current will move through metallic particles mostly unimpeded, non-conductive particles resist the field.

Intact bacteria, however, resemble both non-conductive and conductive particles: the cell membrane prevents low frequencies from penetrating it, causing it to resemble non-conductive particles; at high frequencies, however, the electrical current penetrates the membrane.

The microelectrodes in the impedance flow cytometer generate these electrical fields and enable the device to quantify the changes in conductivity and resistance in terms of separate measurements of intact cells and particles.

Application of impedance flow cytometry to food safety: introducing CytoQuant

The CytoQuant impedance flow cytometer is designed for use at critical control points where hygiene is an overriding concern, including food production facilities and clean rooms. Impedance flow cytometry brings considerable advantages to food producers looking to verify their food safety and cleaning programmes: the fast and separate quantification of bacteria and residue particles (which can serve as an indicator for the cleaning efficacy), the sensitivity of the method, and the robustness of the swabbing kit and the cytometer itself.

The CytoQuant system is easy to use, as the device handles all the work except swabbing. A test run begins by swabbing a predefined area (for example, 20 x 20cm or 8 x 8 inches) of the surface to be tested.

The swab is placed in a tube containing a proprietary, conductive solution. After shaking the swab kit to suspend the bacteria, the user inserts it into the CytoQuant. Two needles penetrate the bottom of the tube, connecting the liquid to the flow system in the device. Then, after the solution is introduced to the flow system, it is passed by the electrodes in the flow cell. After 30 seconds, the device registers separate results for bacteria and particles and displays them on the screen.

Revolution or evolution?

The CytoQuant mobile flow cytometer enables the immediate, on-site verification of cleaning and disinfection procedures in food production facilities or other areas where hygiene is crucial. By directly quantifying bacteria and residue particles on surfaces without the negative influence of disinfectants or temperature, it provides substantial advantages over ATP devices, while the 30 second time-to-result makes it a perfect enhancement to hygiene programmes that already use cultural methods.

Considering the huge potential of impedance flow cytometry, it may at some point come to be regarded as equal to or even replace cultural methods as the standard in cleaning verification. This would amount to a true revolution in the field.