Methods of Hygiene Monitoring

INTRODUCTION

It is essential in the food industry that all equipment is kept hygienically clean. Food production sites must also be able to show evidence of the effectiveness of their hygiene procedures be this CIP (cleaning in place), equipment washers or manual cleaning methods. There are a variety of methods available to the food manufacturer to do this. It is important that the correct method is utilised to be both cost effective and to ensure food safety. A mixture of these methods can be used to balance the cost and effectiveness whilst ensuring the requirement of validation and verification of the cleaning methods are met. This document has been written with the aim of helping the food manufacturer understand the various methods available to them and to make an informed choice for their business. Also, as there are a considerable number of commercially available monitoring kits, one commercial example of each of the most used techniques is described to enable the reader to have a better understanding of the different methods.

Why Monitor Hygiene

Hygiene procedures are designed to visibly remove debris from the previous production run prior to starting the next run. At the same time, the cleaning should also reduce the invisible microbiological and allergen debris to an acceptable level. Further to this, the chemical agents used should not leave a residue.

It can therefore be seen that hygiene practices are of importance within the HACCP system. They help control physical, biological and chemical contaminants. As part of the due diligence defence, all parts of the HACCP system should be validated and verified to a set frequency.
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Clearly, there is a simple method that can be used to validate and verify the removal of debris - visual inspection. A programme can easily be created for regular verification of key inspection points on any production line with more in depth audits being used to validate the cleaning. It is not as obvious which methods to use for the ‘invisible threats’ described earlier.

Methods for Validation

Laboratory methods remain the usual manner to validate a cleaning procedure in relation to microorganisms and allergenic proteins.

Microbiology

All methods rely on the incubation of a sample - for example swab, rinse liquid or contact plate - to estimate the level of microorganisms remaining post cleaning. These methods therefore require specialist equipment, time and expertise.

*An example of a laboratory method for the isolation of a microorganism would be as follows:*

- **Swab of surface taken and placed in transport media**
- **On arrival in lab, swab vortex mixed to force cells into solution**
- **Sample inoculated onto specific media and spread prior to incubation**
- **Post incubation at a set time and temperature, colony forming units (cfu) are counted**
- **0.5ml sample extracted**
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In this way, organisms can be used to assess the efficacy of the hygiene procedure. The organism of choice should be selected based on the risks associated with the food matrix being produced. In general however, a generic count and faecal indicator organism count is used rather than specific pathogens. These tests are quicker (24-48 hours) and do not have to have a confirmation step which adds time and expense.

Allergenic Proteins

More recently as allergic reactions to various food stuffs become more prevalent, it has become the food manufacturer’s responsibility to prevent cross contamination within the manufacturing area. Whilst alibi labelling can be used, it is not the best solution for the end consumer.

For validation of cleaning methods in relation to allergenic proteins, both product samples and swabs are analysed. For product samples, the ‘last down – first down’ sampling technique is used where a sample of the last unit of the allergen containing food product is taken along with the first unit of the non allergen containing product post cleaning. In all cases, the usual testing method is an ELISA (enzyme-linked-immunosorbent serologic assay)
Hygiene Chemical Residues
In this case, validation is carried out by taking a sample of the final rinse liquid and carrying out a titration against a suitable chemical with an indicator.

This method has been simplified by many chemical suppliers who provide test kits for their chemicals. The methods are also used to show that the correct strengths of chemicals are being used to avoid bacterial static effects. While these test kits have removed the need for laboratory expertise, they should not be used in the production areas to reduce the risk of cross contamination. Due to the simple, rapid methods that have been developed, these tests can also be used for verification of the removal of chemical residues through hygiene methods.

Methods for Verification
In all cases, it can be seen that the methods used for validation of hygiene procedures would not be suitable for verification techniques. Methods for verification must give results that allow the user to react in a timely fashion to prevent and control any food safety issues that may arise on a day to day basis. These methods must be simple, rapid and robust.
Microbiology

All living organisms contain an energy transfer system that relies on the movement of chemicals through a chain of molecules within the cell. One of these molecules is adenine triphosphate or ATP. Rapid methods for verifying the removal of living organisms rely on the existence of this molecule. The systems developed analyse samples for the presence of these molecules through a chemical reaction:

Levels of ‘RLU’ are specific to each system and so the pass / fail limits must be set in accordance with guidelines from the manufacturer of the system. This method is not a replacement for all microbiological testing as it does not discriminate between live and dead cells. Both environmental swabs and rinse liquid samples can be tested using this method which makes it a flexible solution.
Allergenic Proteins
Both specific and non specific tests are available for the rapid detection of proteins. The non specific tests rely on patented technology where copper ions are reduced when they form a complex with peptide bonds. Bicinchoninic acid reacts with the reduced copper to form a purple complex. Therefore any protein residues left behind create a purple colour in the test.

\[
\text{Protein + Cu}^{2+} \rightarrow \text{protein-Cu}^+ \rightarrow \text{Purple complex}
\]

Tests for a specific allergen are carried out using test sticks with specific antibodies.

Equipment Available for Rapid Hygiene Monitoring
There are a number of systems available for hygiene monitoring; the following is a description of some of these.

ATP SYSTEMS

3M™ Clean-Trace™ Hygiene Monitoring System
This system uses ATP technology to provide the ability to monitor, control and improve hygiene management processes. The system includes Clean-Trace surface ATP swabs, Clean-Trace water swabs, a Clean-Trace NG luminometer and Clean-Trace data trending software to produce testing plans and to monitor and trend results data. Each swab is a self contained test that contains all the reagents required to carry out the test. The luminometer produces rapid results based on photomultiplier tube technology and is extremely portable. Quantitative results are produced in less than 30 seconds which allows for real time release of the production environment. The
luminometer can store up to 2000 test points which are set up using the software provided. Once results are downloaded to the PC software, trending can be carried out and priority areas can easily be identified. All retests are identified against the original result which helps the user prove that remedial action has been taken following a failure. Sampling plans with specific test points are created by the user and transferred to the luminometer.

This system is produced by 3M, contact details can be found at the end of the document.

**Lightning MVP®**

The Lightning MVP measures and records ATP levels of surfaces and CIP (clean-in-place) rinse water systems to validate the sanitation program, as well as HACCP parameters such as pH, temperature, and sanitizer concentration. The highly accurate and reproducible platform allows for on-site calibration of all parameters, supporting SSOP and HACCP requirements and ensuring effective and documented hygiene and QA programs. By offering an expandable platform, a company can avoid investing in multiple instruments or struggling to consolidate results for reporting.

The Lightning MVP Surface and Liquid Sampling Devices are room-temperature stable and deliver ATP results in just 10 seconds. After collecting a sample the user activates the sample collection device and the reagents flow through the swab shaft rinsing the ATP from the swab tip, producing in a uniform solution of reagents and ATP. Because inherent surface conditions and sampling technique issues result in an uneven distribution of the ATP on the swab, this unique design provides greater...
accuracy and reproducibility when compared to systems attempting to read only a portion of the swab’s surface. The Lightning MVP utilizes a highly sensitive photomultiplier tube to read the photons of emitted light and provides results in “Zones of Cleanliness” as well as a simple “Pass”, “Warn”, or “Fail” value making it easy for technicians to understand, and to take immediate corrective action where appropriate.

![Zones of Cleanliness](image)

Results for ATP, temperature, pH, conductivity and sanitizer concentration (ppm) are all easily uploaded into the accompanying Lightning MVP TRAX data analysis software allowing for sorting, trending and reporting. TRAX offers a series of helpful pre-programmed reports for monitoring the effectiveness of sanitation programs and the option to create customized reports for further analysis.

The system is produced by BioControl®, contact details can be found at the end of the document.

**PROTEIN DETECTION SYSTEMS**

**3M™ Clean-Trace™ Surface Protein Plus**

This system detects levels of proteins, sugars and other compounds associated with food contamination, it does not test for specific allergenic proteins but is a useful indicator test. It is a simple test which does not require an instrument – the colour change occurs in the swab sheath chamber and is directly related to the level of contamination.
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Check the colour in the tube against the colour chart on the swab label

- **Green** - Clean
- **Grey** - Caution (low level contamination)
- **Purple** - Contaminated
- **Dark purple** - highly contaminated.

Surfaces producing a purple colour should be re-cleaned and re-tested

This system is designed for low risk food processing areas and is extremely useful within this application due to the ease of use and cost.

**Flash™**

Flash is a visually interpreted test for the detection of residual proteins. Results are available in just 5 seconds and no instrumentation is required. The system consists of a simple sample collection device and a proprietary wetting solution and a positive control. Flash utilizes a patent-pending technology that immobilizes organic dye molecules on the device membrane. When even trace amounts of protein are present, the dye molecules form a complex with the protein that results in a colour change from yellow to green/blue.

Flash can be used in many food environments, from food processors to retailers, to instantly measure the effectiveness of sanitation programs and procedures. It can be used routinely to test surfaces after cleaning and before sanitizing. If a surface tests positive, it should be cleaned again and retested until a negative result is obtained. After verifying that the surface is clean it can be effectively sanitized.
Reveal® 3-D Test Kits

These kits test for specific allergen proteins and the range currently includes:

- Almond
- Caesin
- Egg
- Gluten
- Hazelnut
- Peanut
- Shellfish
- Soya

The test kits include the test devices, extraction buffers, sample tubes and swabs. Both food matrices and environmental swab samples can be tested. Each test kit describes the limits of detection for the test and also includes instructions for producing a positive control test which is required as part of the testing regime. The test device has a three line readout including a control line which confirms that the method has been performed correctly and further lines to differentiate low and high levels of detection.

This system is produced by Neogen® Europe Ltd. and contact details can be found at the end of the document. This company is also able to help with allergen validation testing as described earlier in the document.
**WHICH VERIFICATION TEST IS RIGHT FOR YOU?**

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<td>Allergen Removal Monitoring</td>
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**CONTACT DETAILS**

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| www.neogen.com | Reveal® 3-D Test Kits |

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