Choosing the correct luminometer

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Few people in the food industry today doubt the benefits of environmental surface testing. The contamination of food products from unclean surfaces is considered a major issue in food processing.

The cleanliness of food contact surfaces and the processing environment, in addition to being a legal requirement, is also required within various third party standards.

For example the British Retail Consortium (BRC) Global Standard for Food Safety 2008 (section 4.9) requires the effectiveness of cleaning to be verified and recorded although different methods exist for assessing surface cleanliness (Fig. 1).

An integrated process

Visual assessment is unreliable as the sole means of assessing surface cleanliness, although it is important as part of an integrated process (see Fig. 2). There is no single, ideal. method for assessing surface cleanliness and the use of ATP (adenosine triphosphate) detection, as part of an integrated protocol, is currently the optimum approach and provides rapid and reliable results. If on an initial visual assessment the surface is dirty there is no point in any further testing. The second stage of an integrated protocol should assess any invisible residual organic soil (cleaning is defined as the removal of soil).

A number of methods can be used to assess a variety of soil types, however ATP can be detected at low levels and is a widely used chemical marker, being found in food debris and microbial cells. Surfaces are swabbed to remove the ATP which can be detected rapidly by light emission by means of a luciferin-luciferase reaction. This technique provides results within seconds, unlike microbiological testing which normally requires days, is more repeatable and reproducible and is used by most of the world's leading food companies in over 40 different countries.

Standards for 'what is clean' have been proposed to help in managing the cleaning process although these relate to specific instrument and swab combinations and are not transferable between manufacturers.

ATP detection should then, if necessary, be followed by appropriate microbiological testing which is more a measure of surface disinfection.

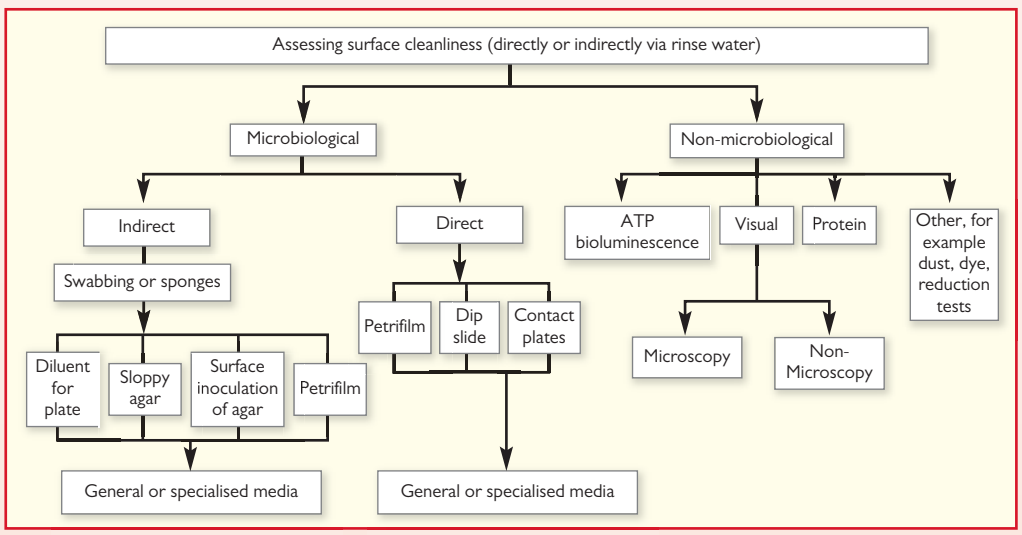
Not all ATP or microbial test methods are as effective, repeatable or sensitive as others. For microbial surface testing ISO 18593 has been proposed although this still allows considerable variation on what is already a highly variable and sometimes unreliable process.

ATP bioluminescence detects residual surface ATP (higher on poorly cleaned surfaces) which can be detected rapidly by light emission by means of an enzyme based reagent system, with the light detected and measured by an instrument known as a luminometer

However, not all luminometers or the swab reagent systems used with them are the same and developments in reagents and instruments occur on a regular basis. These systems can vary considerably in their limits of detection, repeatability and reproducibility. All of these attributes are important. Test combinations lacking sensitivity (used here to mean the limit of detection) can, by failing to detect residual soil, erroneously pass dirty surfaces as clean (possibly leading to product contamination).

The ability to detect marginal or low levels of contamination provides an early warning of failings in the cleaning regime and/or the early development of biofilms. Ones that lack repeatability provide inconsistent results which can be difficult to interpret. A range of factors interact to affect both of these attributes – some relate to the instrument characteristics and some to the reagent chemistry and/or device format.

Fig. 1. Assessing surface cleanliness.



Intrinsically instruments based upon a photo multiplier tube rather than a less sensitive photodiode offer greater detectability but are more expensive.

However, the chemistry of the reagents is also important. ATP has to be extracted from the organic residues that may be left behind after inadequate cleaning. It is relatively easy to extract ATP from somatic cells (food) but more difficult to extract it from bacterial cells.

Current protocols

The current test combinations detect total ATP (microbial and non microbial), but the ratio between these two types of ATP will depend upon the nature of the soil. Typically for hand contact surfaces approximately 30% of the ATP will be microbial, however it can be much higher or lower depending on the type of food debris. UHT milk will be mostly non microbial ATP, whereas soil from raw meat areas will have a higher microbial ATP content and may further vary in the presence of biofilms. Therefore, in providing a reliable result the effectiveness of the microbial extractants used in the swab as well as the configuration of the reagents and the relationship between signal and background levels will be critical. It is, therefore, desirable for any potential purchaser to evaluate carefully the system they are thinking of purchasing.

Two types of approaches can be taken in such evaluations – each having advantages and disadvantages.

Laboratory trials allow the direct comparison of the repeatability, reproducibility and sensitivity test of different test combinations under controlled conditions.

Field or 'in use factory trials' allow the convenience and usability of the systems to be assessed but by their very nature factory trials are more difficult to control due to the varying degrees of soiling that may be present and are not suitable for sensitivity or reproducibility evaluations. In practice, any potential purchaser should use both types of approaches

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in assessing an ATP test system. Not all methods of comparison testing are suitable especially as each instrument provides a reading on a scale unique to that make of instrument

The methodology presented here is based on one previously published in a number of scientific journals. A sufficiently large but typical area should be marked out, with 10cm squares being frequently used.

Normally a minimum of 30, depending on the number of instruments and dilutions being compared will be needed. The surface used for testing should reflect the nature of the surfaces in use within the food plant with food grade stainless steel often used. The surface needs to be thoroughly cleared and disinfected prior to testing. A standardised thorough cleaning protocol is required as depending on the test kit used, the assay can be very sensitive.

The squares can then be inoculated with 0.1ml of increasingly dilute samples of food debris, made using an ATP free diluent (for example sterile deionized water), to represent increased levels of cleanliness.

Sample testing

After inoculation the samples should be evenly spread across the test surface using a sterile disposable 'hockey stick', left to air dry and then tested using one or more of the luminometer/swab combinations.

Inocula can initially be tenfold dilutions but may need to be reduced to five or two fold near the limits of detection and should be typical of the organisms and the debris likely to be contaminating surfaces in that particular type of food plant.

For liquid foods simple dilutions of the original sample can be prepared. For solid foods (for example raw chicken) 10g can placed into a stomacher bag and homogenised with 90ml of sterile deionised water, the supernatant being used as an initial one in ten dilution. Various combinations and dilutions of foods, for example chicken juice, milk, carrot extract from a one in 10 to a one in 1,000 detection with and without added micro-organisms can be used to test the repeatability (results obtained from repeated testing by the same person) and sensitivity of different instruments and swabs.

To represent samples with higher bacterial counts either the supernatants can be left at room temperature or 37°C for several hours to allow the micro-organisms present chance to increase in numbers or supplemented by inoculation with an overnight culture of a test organism, for example Staphylococcus aureus.

Although primarily for detecting total ATP, in some circumstances ATP testing can be a better indicator of microbial contamination than traditional microbial swabbing and this additional experiment is useful to screen out test systems which do not have such effective microbial ATP extractants.

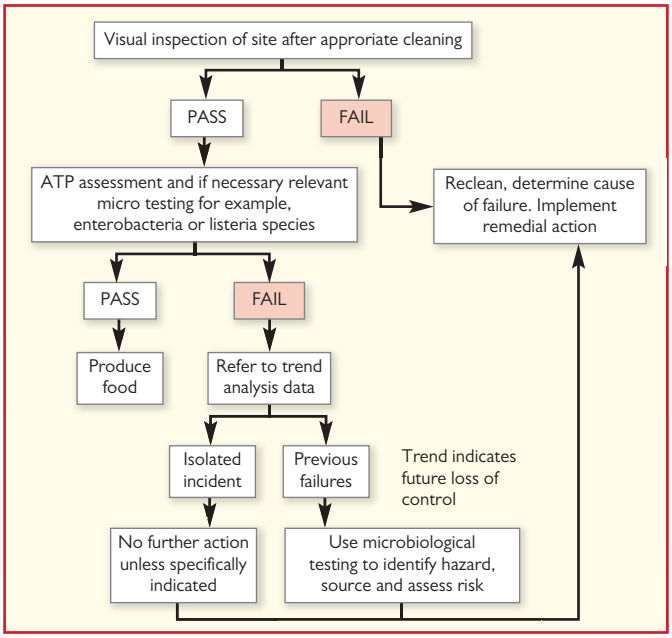

Fig. 2. An integrated protocol.

For maximum repeatability the swabbing of the inoculated squares should be as consistent as possible.

A recommended protocol is to use approximately 20 zig-zag strokes over the surface at a constant pressure rotating the swab. This should then be repeated by turning the swab through 90° to the first swabbing. A quicker way of assessing repeatability on its own can be to inoculate the swab test system with an ATP control provided by the manufacturer and then read in the luminometer. However, this should not be used to assess sensitivity as the effectiveness of the ATP extractant is not tested.

For reproducibility (spread of results obtained when more than one person uses the luminometer/swab combination) more than one person is required to repeat the swabbing part of the testing using identical sources of ATP/test solutions. An attempt can be made to apply consistent pressure by trying out the swabbing technique on an appropriate top pan balance.

For statistical analysis a minimum of five identical replicates, although ideally 10 of each test dilution of each food type for each instrument and person, should be inoculated onto separate squares. Controls of diluent only inoculated onto the squares (again 5 or 10 replicates) should be performed at the same time.

Repeated sampling of identically inoculated squares can indicate the precision of each test system (the closer together the values are for identical replicates the better).

The most sensitive instrument/test combination is the one able to detect the highest dilution (least concentrated) sample (dilution of food). This is assessed by identifying and comparing the readings from the most diluted sample which are still statistically different from the results obtained from the control.

Any statistical tests used will depend upon the data obtained and the type of information needed. Due to the use of different scales and values comparing the standard deviations on their own is meaningless and for comparing repeatability and reproducibility the coefficient of variation (CV) can be used (CV = standard deviation times by 100 divided by the mean), although care should be taken in interpretation if the instrument uses a logarithmic scale.

Statistical analysis

Repeatability values should be obtained from a dilution between the mid to lower range of the instrument's readings. Sensitivity determinations are based on the lowest dilution capable of providing 5/5 positive replicate tests compared to the controls. More powerful statistical tests can be used depending on the information needed and the type of data obtained.

If this is normally distributed then analysis of variance (ANOVA) can be used with significance set at a p value <0.05 followed by, if required, Tukey's modified comparison test. If the data is not normally distributed the less powerful Mann-Whitney test can be used.

Field trials should not be used for reproducibility and sensitivity testing but provide valuable but different types of information including ease and practicality of use which could include ease of swab activation, instrument battery life, size and weight of instrument and robustness.

Additional information on instrument back up, reliability and technical support is best obtained from other users of the instrument in conjunction with manufacturers guarantees. This type of information can have a considerable impact on a purchasing decision. Effective technical support will help to ensure that operatives are fully and effectively trained to both operate and understand the value of the results. This is especially important in setting and then improving on benchmark clean values. This, in conjunction with trend analysis, contributes to an improved cleaning culture. Trend analysis of cleaning results (if there is good repeatability/reproducibility) is recognized as increasingly important and is required both by the EU Micro Criteria Regulations and by third party audit standards. Good trend analysis software provided with the luminometer will make the capture and subsequent reporting of this much easier.

Testing cleaning effectiveness is increasingly important especially with increased concerns over cross contamination with allergen residues. All ATP instruments are not the same and ideally an instrument should be sensitive (detect low dilution levels) and reproducible.

It has been claimed that instruments can be too sensitive – although this is unlikely with the present technology. Greater sensitivity provides confidence in cleaning programmes. Poor sensitivity will allow dirty surfaces to be passed as clean leading to a greater potential for product contamination with the ultimate possibilities of product recalls, shorter shelf life or even food poisoning. Good reproducibility/repeatability allows continuous incremental improvement in reducing threshold values of pass fail. Good reproducibility (a low coefficient of variation) allows greater use and benefits from trend analysis of the data and informs the user about gradual loss of cleaning control or cleaning inconsistency.

ATP in conjunction with visual and microbiological testing is important in the validation, monitoring and verification of cleaning but, unlike microbiological testing, provides results within seconds. However, in assessing a company's cleaning programmes auditors may, in addition to seeking data on a company's cleaning results, also ask to see data on the validation of the ATP instrument test combination and its efficacy. Auditors are increasingly being asked to concentrate on the validation and calibration of monitoring equipment and failure to consider this may result in money being wasted on valueless/meaningless testing, which provides an illusion, rather than a reality, of cleanliness. n

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References are available from the author on request