NSW GOVERNMENT | Food Authority

Environmental swabbing

A guide to method selection and consistent technique

NSW/FA/FI170/1303

Contents

Summary

Environmental sampling can provide useful information to food business operators and food regulators. Food premises swabbing is often used in the investigation of foodborne illness and the verification of cleaning and sanitation.

There are many methods of environmental sampling. Often the choice of method will be determined by operator familiarity, availability of supplies or limitations of the local laboratory. However, not all methods are suitable for use in all situations, so an appropriate method should be chosen.

Some important requirements for environmental sampling are:

- use an appropriate method,
- use good aseptic technique and do not contaminate samples,
- control inhibitory effects of residual sanitisers or disinfectants,
- follow instructions for use of commercial sampling kits and, after sampling,
- clean the swabbed area with an alcohol wipe.

Introduction

The microbiological testing of surfaces and utensils is a useful tool for food safety professionals. The two main uses are in the investigation of foodborne illness outbreaks and the verification of cleaning and sanitation. Some of the methods used for environmental swabbing also make good visual aids for training food handlers.

The isolation of a pathogen that is identical to the type that caused an outbreak of foodborne illness from an implicated food premises is a significant strand of evidence. In this situation the investigator wants to find the pathogen if it is present. The count of organisms is not relevant. Using a large cloth or sponge to sample an extensive area is most likely to be successful.

For cleaning verification, a quantitative method is needed. Even though the results of swab tests are only semi-quantitative, an estimate of the bacterial count per 100cm^2 can be used as an indication of cleanliness, to gauge improvement by comparing bacterial levels over time, or to compare standards in similar businesses. Swab sticks or sponges, often supplied as a proprietary kit, could be used.

Contact plates or dipslides can also be used for cleaning verification. Dipslides, in particular, require little equipment and can be processed in the most rudimentary laboratories. They are easy to use, easy to understand and—with the right choice of microbiological growth media—can demonstrate in a visually interesting way that surfaces are contaminated (see Figure 1).

Figure 1: Dipslides with selective media

Where results are required immediately, ATP swabs or protein staining methods are appropriate. The instant feedback can be useful in an inspection. These tests are often used for cleaning validation (prior to start up) by food businesses.

The International Standard that specifies surface sampling techniques using contact plates and swabs—ISO 18593:2004(E)—allows a lot of choice in method selection. However, samplers often opt for the method used previously. That might make perfect sense.

If it is the 'house method' and supplies are on hand, or the method you know, and familiarity with a method is very important, it would be unusual to try something new. However, if another method has special benefits in a certain situation, then its use should be considered should the situation arise.

Issues common to most methods[1]

The presence of residual sanitisers can interfere with microbiological testing. Surfaces should not be sampled until the specified contact time for the sanitiser has elapsed. The contact time should be specified in the cleaning procedure or the directions for use of the sanitiser. Contact times in the range 5 to 15 minutes are common.

When sampling dry surfaces, swabs, sponges and cloths must be moist, either supplied pre-moistened or moistened with a suitable diluent prior to use. The diluent typically contains neutralisers to counteract the effects of any residual sanitiser. Sampling kits include prepared diluents which contain neutralisers such as Tween 80, lecithin, sodium thiosulphate or catalase. The directions for the sampling kit should include information on the applicability of the diluent. Handle swabs carefully to avoid contaminating the sample.

Microbiological media for contact plates and dipslides will, where possible, include neutralisers. Dipslides usually have two sides with the second side often being a selective agar that might not include neutralisers. Even so, the dipslide can be enlightening if used on rinsed surfaces well after the sanitiser contact time has elapsed. However, if you are searching for a pathogen such as *Salmonella* or *Listeria*, contact plates and dipslides do not provide conclusive results and should not be used.

Keep inoculated swabs cool (at 1 to 4°C) and, if possible, transport them to the laboratory within four hours. Examine in the laboratory as soon as possible, and not later than 24 hours after the swab was taken.

Transport contact plates or dipslides to the laboratory within four hours if possible. Protect the plates and slides from contamination.

Cloths and sponges[2]

Cloths and sponges can be purchased moist or dry. Some sponges have handles and others are gripped with sterile forceps, a gloved hand or a plastic bag used as a glove. Cloths and large sponges are ideal for sampling large surface areas. A good example would be a search for pathogenic bacteria in a kitchen which has been linked to an outbreak of foodborne illness. A sample area of 1000cm^2 is reasonable and you should swab a wide range of areas where contamination might be found, including under or behind equipment.

Always refer to the manufacturer's instructions.

To use:

- Label the sample bag with sufficient detail.
- Moisten the cloth or sponge, without using excess fluid, using suitable diluent if required.
- Remove the cloth or sponge from the plastic bag by using the handle or the gripping method of choice where there is no handle.
- Sample the chosen surface in two perpendicular directions, changing the face of the cloth or sponge.
- Return the cloth or sponge to the plastic bag.

---

[1] Information is from ISO 18593:2004(E). Instructions for some proprietary kits might differ.
[2] Guidance is general in nature, always refer to the specific instructions.

- Add a known volume of diluent so that the cloth or sponge will be still moist at the time of analysis, and close the bag in a manner that will ensure no leakage.
- Clean the sampled area with an alcohol wipe.

Swabs

Swab sticks and related products can provide an indication of the numbers of bacteria present on a surface of a known size. ISO 18593 cautions that results are not quantitatively reliable or reproducible. A single bad result is of limited value. Multiple results can be used to compare similar businesses or to indicate performance over time. A number of agencies have drafted guideline values for assessment of cleanliness. Average counts or trends over time can be compared to guideline values in Table 1.

A wide variety of swab stick tips are available: cotton, alginate, Dacron, Rayon, sponge, viscose and some novel proprietary products.

Always refer to the manufacturer's instructions.

To use:

- Label the swab tube with sufficient detail.
- Remove the swab stick from the sterile wrapping.
- Moisten the tip, if required, by immersing it in a tube containing dilution liquid.
- Press the tip of the tube against the inside wall of the tube to remove excess fluid.
- Select the area to be swabbed and apply a suitably sized (often 100cm^2) sterile template.
- Press the tip of the swab onto the surface and streak in two directions at right angles within the template whilst rotating the swab stick between thumb and forefinger.
- Put the swab back in the tube with the dilution material and aseptically break or cut off the stick.
- Clean the sampled area and the templates with alcohol wipes.

Contact plates and dipslides

These products, like swab sticks, also generate indicative bacterial counts. However, they sample a surface area of less than 100cm^2, which is commonly sampled with swab sticks. Contact plates (55mm diameter) sample about 24cm^2 and dipslides sample about 10cm^2 per side. These products could be a viable option in regions where laboratory facilities are limited. A low cost incubator and a method of sanitising the plates or slides are minimal requirements.

Contact plates are available with microbiological media containing neutralisers. After incubation the result is an approximation of the total count of bacteria on the surface sampled. Contact plates can be provided with selective media for the detection of specific organisms such as *Enterobacteriaceae*. ISO 18593 does not endorse the use of contact methods for the specific detection of pathogenic organisms.

Dipslides (see Figure 1) are usually double-sided, with different microbiological media on either side. For surface testing work dipslides usually have a bendable handle that allows the media to contact the surface at a constant pressure. Some of the media available for dipslides contain neutralisers.

Always refer to the manufacturer's instructions.

To use:

- Remove the contact plate or dipslide from the transport container.
- Label the contact plates or dipslides with sufficient detail.
- Open the plate or dipslide and press the agar surface (or surfaces) firmly against the test surface without any lateral movement.
    - For contact plates, optimal results have been obtained with a contact time of 10 seconds and a pressure obtained with a mass of 500g.
- Close the contact plates or dipslides and return them to the transport container.
- Clean the sampled area with an alcohol wipe.

ATP methods

ATP is a chemical found in the cells of animals, plants and bacteria. Effectively cleaned food contact surfaces will have low levels of ATP. The detection of higher levels of ATP on a surface implies that food or bacteria remain on the surface. ATP methods are used to measure cleanliness. Results are generally thought to be site specific and they are best interpreted by comparing results over time. Single results would not usually be used for regulatory purposes. A number of ATP instruments are available, each with their own specific instructions for use and guideline values.

Protein swabs also detect residues of food. They are simple, cheap and useful where protein based foods are processed.

Always refer to the manufacturer's instructions.

Interpretation of indicative counts

The European Community (2001) has established limits for hygiene surface samples from meat or poultry abattoirs or cutting rooms. The standards apply to surfaces which have been cleaned and sanitised and are dry and smooth. Samples are taken before production starts.

Table 1: Mean values for the number of colonies (cfu) for testing of surfaces

	Acceptable	**Unacceptable**
Total viable counts	0 – 10/cm^2	>10/cm^2
Enterobacteriaceae	0 – 1/cm^2	>1/cm^2

Other agencies have established guidelines for total viable counts of <2.5 cfu/cm^2 or <5 cfu/cm^2 for cleaned and sanitised food contact surfaces (Dancer, 2004). The European Community ranges provide a suitable point of reference for average and trend results of samples from cleaned and sanitised surfaces in food service businesses.

The Food Safety Authority of Ireland (FSAI) surveyed the microbiological status of in-use food preparation surfaces. Their classification of results was based on UK Public Health Laboratory Service (PHLS) guidelines (see FSAI 2006).

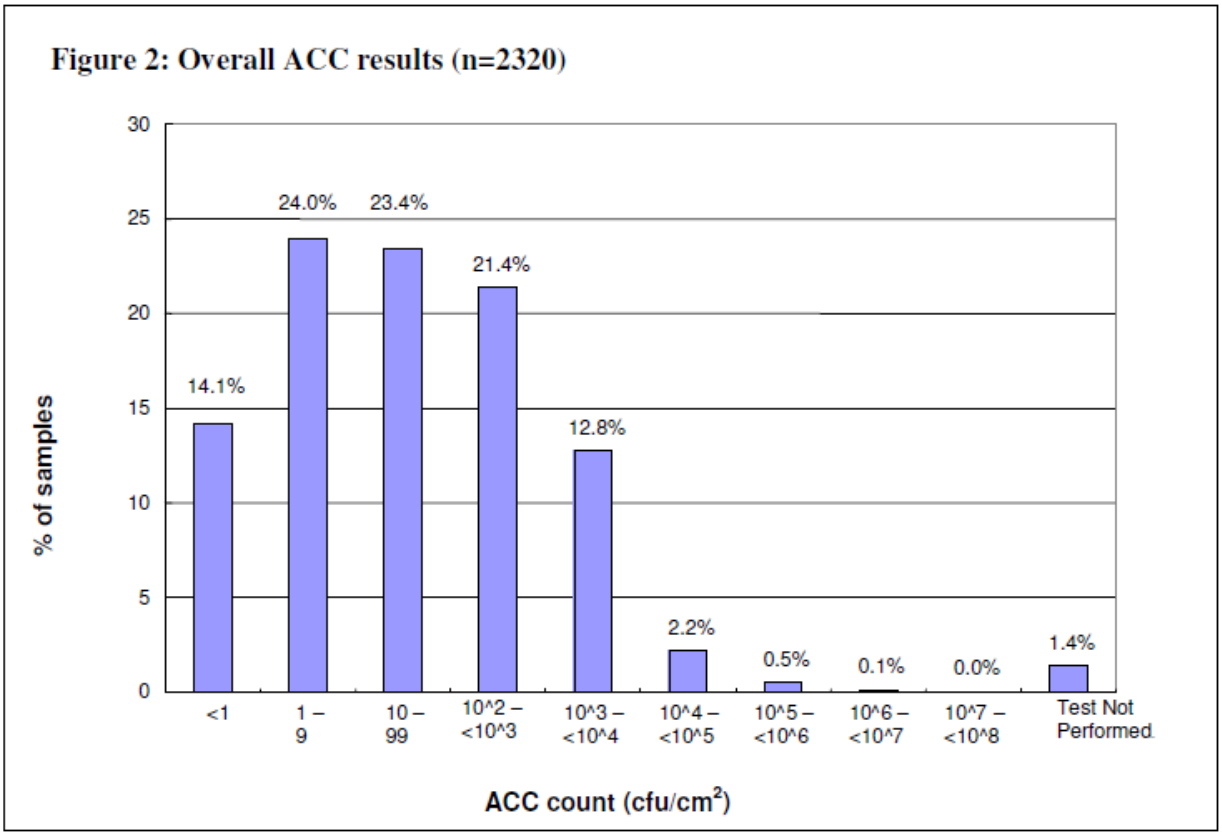
Table 2: Guideline values for surfaces cleaned and put into use

	Satisfactory	Borderline	Unsatisfactory
Total viable counts	<80 cfu/cm^2	80 – 10^3 cfu/cm^2	>10^3 cfu/cm^2

Subsequent to preparation of the PHLS guidelines another UK study (Sagoo et al 2002) found that surface total viable counts >10^3 cfu/cm^2 correlated with businesses that did not have adequate food hygiene training, hazard analysis, cleaning schedules or cleaning records in place.

Figure 2 presents data from the FSAI study. The guideline level of surface total viable counts they used as the 'unsatisfactory' benchmark (>10^3 cfu/cm^2) throughout the report appears to be reasonable. However, the benchmark could be misleading in some circumstances, such as where fermented foods (cheese, salami, olives or sauerkraut), aged meat or pre-cut salads are handled. The guideline should be interpreted with care. Samples taken prior to start up might help clarify any potential problem.

Figure 2: Distribution of total viable counts[3] in FSIA survey of food preparation surfaces



Higher counts were more often from samples of chopping boards than worktops.

[3] Aerobic colony count (ACC) and total viable count (TVC) are synonyms in this instance.

Conclusion

Many surface sampling methods are recognized by ISO 18593. However, not all methods are suitable in all situations.

- Contact plates and dip slides are suitable for semi-quantitative sampling but not for specific pathogen detection in environmental samples.
- Swab sticks are suitable for semi-quantitative sampling and for the detection (presence or absence tests) of specific pathogens but not for foodborne illness investigations where more powerful detection methods are required.
- Cloths and large swabs are ideal for large area sampling and have the high level of sensitivity required for foodborne illness investigations.

References

European Community (2001). Commission Decision 2001/471/EC 2001. Retrieved 7 November 2012 from http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:165:0048:0053:EN:PDF

Dancer, S. J. (2004). How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals. *Journal of Hospital Infection, 56*(1), 10-15.

FSAI (2006) 3rd Trimester National Microbiological Survey 2006 (06NS3): Examination of the Microbiological Status of Food Preparation Surfaces. Retrieved 7 November 2012 from http://www.fsai.ie/uploadedFiles/Monitoring_and_Enforcement/Monitoring/Surveillance/food_prep_surfaces.pdf

Sagoo, S. K. Little, C. L. Griffith, C. J. and Mitchell R. T. (2002) LACOTS/ PHLS Co-ordinated Food Liaison Group Studies: A Study of Cleaning Standards and Practices in Food Premises[4]. Retrieved 7 November 2012 from http://www.lacors.gov.uk/lacors/ContentDetails.aspx?id=3532

[4] The LACORS website will close in 2013: the report was also published in *Communicable Disease and Public Health* / PHLS [2003, 6(1):6-17]

NSW Food Authority
6 Avenue of the Americas
Newington NSW 2127
PO Box 6682 Silverwater NSW 1811
Phone 1300 552 406
Fax 02 9647 0026
www.foodauthority.nsw.gov.au