Microbial update
method evaluation

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Whenever new routine methods of testing are introduced into food microbiology laboratories, Good Laboratory Practice dictates that the laboratories should not only be able to demonstrate its suitability for their intended purpose, but that they can also use the method correctly.

This applies equally to the modification of existing methods and the extension of method usage to encompass different types of sample. The process is known as method evaluation or 'validation', and may also be used to assess the continuing suitability of established tests.

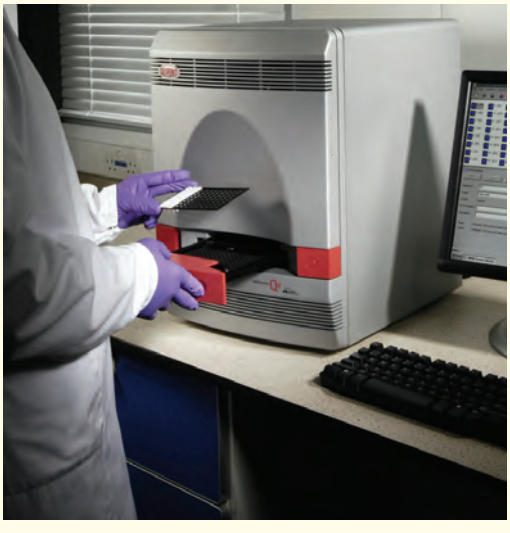
It is not expected that laboratories should have to validate a method from first principles, since this is the role of method standardisation bodies such as BS/ISO, the manufacturers of the testing products themselves and independent bodies such as AOAC, Association Français de Normalisation (AFNOR) and European Microbiological Method Assessment Scheme (EMMAS). From the laboratory's point of view, method evaluation is primarily concerned with demonstrating 'fitness for purpose' and 'suitability for use' in the context of customer requirements.

Establishing a method's 'fitness for purpose' in-house is beneficial for laboratories in several ways. Firstly, it can imbue a sense of confidence in the reliability and consistency of the method for the laboratory's particular purpose by focusing on its performance for specific types of samples.

Secondly, it enables laboratories to show that the method meets their own specifications and those of their companies' customers. Method evaluation is a key part of company 'due diligence' regimes and is, of course, a requirement of laboratory accreditation.

It could be argued that external evaluations carried out by independent bodies such as AFNOR or EMMAS should be sufficient to demonstrate the efficacy of a particular method. However, evaluations by such bodies cannot take account of all types of samples and industry situations.

External evaluation data provides a useful indication of a method's performance in terms of selectivity, sensitivity, reproducibility and repeatability, but the only way for laboratories to prove that a method will work satisfactorily in their particular circumstances is to validate it for themselves.



Rising to the challenge

As with most exercises of this kind, advance planning is the key to a successful method evaluation and will help to minimise disruption in the laboratory. Some useful guidelines have been published by the Campden & Chorleywood Food Research Association Group (CCFRA) which define the essential steps required to achieve a valid assessment and provide practical advice on how these can be performed. Another useful source of guidance is the BS EN ISO 16140:2003 standard which establishes the general principle and technical protocol for the validation of alternative testing methods.

No guidelines, however, can be expected to cover all eventualities, and it is up to individual laboratories to decide which approach to method evaluation is most appropriate for them.

First and foremost, it is necessary for a laboratory to establish exactly what it is trying to achieve and why. Those involved should be clear about what they want from the method, what limitations (if any) they are prepared to tolerate and what are the positive requirements that absolutely must be met.

Ideally, the acceptance criteria set should be quantifiable and must have practical significance in the context of the laboratory's market sample matrices. The criteria may also include tangible benefits such as the cost of using the method, turnaround time and the expertise required.

It is important to note that laboratory accreditation bodies will not set acceptance criteria on laboratories' behalf, and it is up to individual laboratories to determine the operating characteristics that it is prepared to accept.

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The role of accreditation bodies is to verify these criteria to ensure that they are appropriate in terms of laboratory operations and customer requirements. They will also seek to ensure that appropriate statistical analyses have been performed, and that the results obtained agree with the conclusions drawn.

Consider your approach

Laboratories seeking to replace an existing test method with a new method will wish to establish the new method's track record by comparing its performance with that of the old one over time.

In such cases, it is important that the old method should itself be a standard/reference method, or one which has been proven to produce equivalent results.

If the old method fails to meet these criteria, it may be appropriate to compare results for the same samples with those obtained by another laboratory which does routinely utilise a standard/reference method.

External comparison of duplicate analyses may also be appropriate when a laboratory has no previous experience of testing for a particular micro-organism.

When modifying the use of an existing method, it will be necessary to evaluate the modified version of the method against the original, unmodified, one. This is appropriate when changes are made in respect of technical aspects of the method such as confirmation procedure, incubation temperature or incubation time.

First steps

Before starting to evaluate any new method, it is always a good idea to review all the available literature, starting with information available from the manufacturer. This will indicate the status of the method in terms of BS/ISO conformity/equivalence, whether any formal appraisals have been carried out by recognised method validation schemes, or whether it has been collaboratively trialled by any laboratory groups.

Any relevant data available in published literature should also help to provide information about the overall performance of the method and any possible problems/limitations that may be associated with it, thereby assisting with the design of the evaluation protocol.

It should, of course, be borne in mind that any published trials may have been conducted using different kinds of samples from a different industry sector. Such trials may not, therefore, reflect the same challenges faced by other laboratories wishing to change over to the method.

A literature review is helpful in gaining an initial understanding of the principles of the method and the techniques involved in using it.

Following on from this, appropriate training in use of the method, to avoid results becoming statistically skewed by incorrect technique, should be provided to all the staff that will be performing evaluation tests.

Choice of samples

Whatever the acceptance criteria set for an evaluation, sufficient samples must be analysed in order to facilitate analysis of results in a meaningful way.

The samples tested should be appropriate in terms of those which would normally be tested in the laboratory (both product-based and environmental), and should ideally include naturally contaminated samples as well as those which have been spiked. Inclusion of negative samples may also be appropriate in order to demonstrate that the method does not give false positive results.

Samples from external proficiency testing schemes may be included in the trial, but as these may not be representative of the laboratory's routine workload, this should not be regarded as the only appropriate source.

When spiking samples, it is important to ensure that the levels of contamination reflect those likely to be encountered in routine testing. For the same reason, organisms purchased from recognised culture collections should ideally originate from a food derived background, rather than being clinical isolates. The rigour of the evaluation may be strengthened by inclusion of atypical strains, sub-lethally injured cells and competitor organisms which might give rise to false positives.

If the laboratory already has its own culture collection of organisms which have been isolated from products or environmental samples in the past, these are worthy of inclusion, since the chances of them being encountered routinely are high.

In order to make a full appraisal of a new method's ability to recover target organisms, samples with low spike levels should be included to demonstrate detection limits. A mixture of low, medium and high spike levels should be utilised for assessing the effectiveness of enumeration methods. In order to demonstrate the validity of results, it is advisable to set up uninoculated sample controls at the same time as spiked samples are being prepared.

Audit requirements

Results from method evaluations will come under scrutiny when a laboratory is audited for the purposes of accreditation.

Auditors have to look at the data generated and verify not only that the method is reliable in the laboratory's hands, but also that it can achieve the sensitivity required. In addition, they need to satisfy themselves that the laboratory is competent in use of the method and can isolate/recover micro-organisms from typical samples in the presence of competing flora.

In some cases, the accreditation body will ask a laboratory to verify the identification of organisms reported as positives in method evaluations – a requirement which is best satisfied by purifying colonies and confirming their identification by biochemical or serological means. Verification of true negatives may also sometimes be required.

Method evaluations require careful planning and execution before data can be collected and conclusions drawn. When results are meticulously obtained and reported, however, the process of verification through audit should hold no fear. ■

FaxNOW +44 1256 329728
✉ val.kane@oxoid.com

Reference

● Campden & Chorleywood Food Research Association Group. Guidelines for Establishing the Suitability of Food Microbiology Methods. 2001; Guideline No. 29.

● BS EN ISO 16140:2003. Microbiology of food and animal feedings stuffs – Protocol for the validation of alternative methods.