Microbial update
sampling methods

Produced as a service to the food industry by Frances Presland, development scientist, Oxoid Ltd.

When samples are taken for microbiological testing, it is often so that the condition of a large consignment of food can be evaluated. It is therefore important that established procedures are in place to ensure that a representative sample is taken, as pathogens or toxins will normally be present at a low concentration.

Established procedures are also important if the sample may be used to certify that the bacterial load is within the limits of a legal standard.

Copyright www.corbis.com

Sampling plans

A sampling plan will usually include the following criteria:

- A definition of the micro-organism(s) for which the sample is to be examined.
- The number of samples to be included in the sampling plan 'n'.
- The microbiological limits; these are normally defined as 'm' and 'M'. 'm' represents the upper limit acceptable for Good Manufacturing Practice (GMP), whereas 'M' represents the limit beyond which the sample is unacceptable or hazardous.
  - Satisfactory = ≤ m (less than or equal to the upper limit acceptable for GMP).
  - Marginally acceptable = > m and ≤ M (greater than the GMP upper limit but less than or equal to the limit beyond which the product is unacceptable for hazardous).
  - Unacceptable = > M (greater than the limit beyond which the product is unacceptable or hazardous).
- The number of samples which fall into each of the microbiological limits (satisfactory, marginally acceptable and unacceptable).

Two and three class plans

In the case of a two class plan only one microbiological limit 'm' is involved. The sample is either ≤ m or > m.

The second attribute is the maximum number of samples allowed to be outside these limits and this is defined as 'c'.

Copyright www.digitalvisiononline.co.uk

This means that if n samples are tested and are found to be ≤ m, the batch is accepted.

If ≤ c of n samples are > m, the batch is accepted.

If > c of n samples are > m, then the batch is rejected.

Three class plans use 'm' and 'M' attributes together with 'c' the maximum number of samples allowed outside the limits, as the third attribute.

If n samples are tested and are found to be ≤ m, the batch is accepted.

If ≤ c of n samples are > m and ≤ M, the batch is accepted.

If > c of n samples are > m and ≤ M, the batch is rejected.

Any sample > M is rejected.

Selecting an appropriate plan

Generally, where an organism is not permitted in a food (presence/absence test) a two class attributes test is used. Where a certain concentration of micro-organisms is allowed, then a three class attribute test is normally used.

However, the acceptable limits can be adjusted for both plans by adjusting n and c, and the values of m and M.

Statistically based sampling plans should be used at every stage of the production process where a determination is to be made.

However, the assurance it provides that a pathogen is not present is poor, as these plans usually make assumptions about the normality of distribution.

As well as the problems created by an uneven distribution of organisms within the sample, the microbiologist must also be aware that inaccuracies may occur if the food sample contains inherent antimicrobial substances.

Anthocyanines in chocolate and ovotransferrin, which is present in egg white, may inhibit salmonella for example. Where there is a competing flora, pathogenic organisms may not grow and this suppression of the pathogen in mixed cultures may limit detection techniques.

Sample handling

Correct sampling requires that great care is taken to avoid contamination of the sample and to prevent any changes to the microbial numbers occurring due to incorrect handling.

Training is a very important element in ensuring that samples are taken correctly, labelled appropriately, and that they are transported and stored properly.

Continued on page 15

Continued from page 13

Sampling equipment

Sampling equipment should be sterile before use and sampling carried out using an aseptic technique. Stainless steel spoons, knives, scoops, forceps, spatulas, scissors and other equipment should be sterilised before use, preferably in an autoclave.

Finished products

Unopened containers of the product should be obtained for sampling where possible, especially where these are products offered for sale. Sampling from the original container eliminates the possibility of the sample being contaminated when it is opened under conditions outside the control of the testing laboratory. Samples should be labelled with a sample code and processing information should be recorded.

Bulk materials

As food batches are often made in bulk, representative samples should be taken aseptically and then transferred to sterile, leak-proof containers. These should be carefully labelled with the details of the sample before sending to the testing laboratory without delay.

In the case of liquid product, samples should be taken after the food mass has been mixed, to ensure that the samples will be homogeneous. Where mixing is not possible, multiple samples will need to taken to ensure representative sampling of the batch.

Solid bulk materials should be sampled from several areas to ensure representative sampling. Where the bulk material is frozen, it will need to be sampled with sterile auger bits, or test portions can be removed with sterile knives and forceps. Again it is important to sample over the entire bulk of the product in order to obtain a representative sample.

Transport and storage

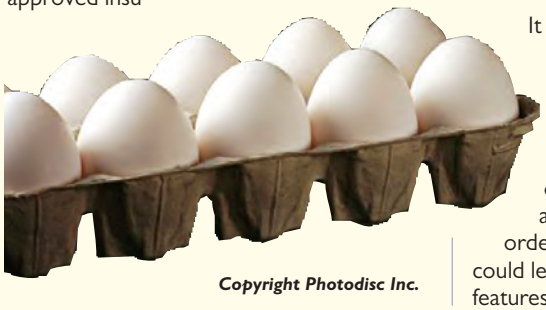
It is important that the original storage conditions are maintained as far as possible, to avoid microbiological changes occurring in the sample. Frozen and chilled samples should be transported in approved insulated containers and frozen foods should remain solidly frozen at all times, by using dry ice during transportation.

When transporting chilled samples, free ice should not be used, as it may contaminate the samples. Water frozen in leak proof containers or bought 'ice bricks' can be used and should keep the samples at 0-4°C for up to 48 hours.

For longer transit times it is advisable use dry ice, but the samples will need to separated from the dry ice to avoid free Non-perishable foods can transported ambient temperatures.

Samples should be examined immedia... on receipt if possible, but if analysis must be postponed, frozen samples should be stored at -20°C until they are tested.

Chilled samples can be stored at 0-4°C for up to 36 hours and non-perishable, canned, or low-moisture foods can be stored at room temperature until analysis. When samples arrive, the condition, time and date of arrival should be recorded.



Copyright Photodisc Inc.

Homogenising and diluting

Various forms of blender or Stomacher can be used to produce a homogenised sample. The quantity of food required by the specification is weighed out and normally diluted 1in 10 with a suitable diluent.

The sample is then homogenised until it is uniform to produce a 10-1 dilution. Further 1 in 10 dilutions can then be made as required, preparing all decimal dilutions with 9ml of diluent to every 1ml of sample.

The dilutions should either be mixed on a mechanical mixer or they should be shaken vigorously 25 times before plating out.

In order to produce an accurate sample, dilutions should be carried out within 15 minutes of the original sample being prepared.

Sample testing methods

The performance of microbiological methods is influenced by a number of factors, including the efficiency of the method, the physiological state of the micro-organism and the accuracy of the enumeration procedure that is used.

Plate counts

It is essential that the sample plated out is homogenous, so that any micro-organisms present are evenly suspended in the plating media. Most plate counting methods recommend a minimum of 15/30 colony forming units (CFU) and a maximum of 150/300 CFU in order to avoid overcrowding, which could lead to inhibition or loss of diagnostic features.

Indicator organisms

These organisms are easier to detect than enteric pathogens themselves and use simpler detection methods. Their presence indicates that the sample has been exposed to conditions which could allow the growth of hazardous pathogens, even if the pathogen is not present in the sample tested.

Total viable count

Total viable counts are used to detect a large range of micro-organisms. The media type, the atmosphere during incubation (aerobic or anaerobic) and the temperature and time of incubation will determine which organisms will grow.

Presence/absence testing

This is used to detect low numbers of pathogens and spoilage organisms, and where these are detected, the Most Probable Number method may be used to assess the number of micro-organisms present. ■

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

References:

● Kilsby D. C. and Pugh M. E. The Relevance of the Distribution of Micro-organisms Within Batches of Food to the Control of Microbiological Hazards from Foods. Journal of Applied Bacteriology 1981, **51**, 345-354.

● Agmar A. (Editor) Proceedings of the Third ASEPT International Conference. June 1994. Baird – Parker A. Difficulties Encountered with the Analysis of Results. PP37-43.

● Vanderzant C. and Splittstoesser D. F. (Editors). Compendium of Methods for the Microbiological Examination of Foods. Third Edition ISBN 0-87553-173-3 APHA.