AGENCE FRANÇAISE
DE SÉCURITÉ SANITAIRE
DES ALIMENTS

GUIDELINES FOR THE DEVELOPMENT OF MICROBIOLOGICAL PROCESS HYGIENE CRITERIA

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Table of Contents

Introduction

In preparation for the "Hygiene Package" coming into force in January 2006, Afssa, on 20 December 2005 and 24 February 2006, issued opinions on regulatory amendments regarding microbiological criteria applying to foodstuffs. Afssa then received a request on pathogenic microorganisms, and the opinions produced highlighted the interest of conducting discussions on process hygiene criteria.

In response to the requests by the DGAI and the DGCCRF, two opinions were issued on the topic of criteria serving as indicators of process hygiene:

- **The opinion of Afssa of 18 January 2007 on the request to create reference documents on microbial flora which could be used as process hygiene indicators.** This opinion review the concept of indicators and the qualities required for indicator microorganisms, then gives a list of the main indicators used in France in the majority of food-related fields (excluding water for human consumption), as well as the interpretation that can be made of their presence, or of their presence in excessive quantities.
- **The opinion of Afssa of 13 March 2008 on references applicable to foodstuffs as process hygiene criteria.** Afssa is proceeding in this opinion with the analysis of proposals for process hygiene criteria applying to products manufactured and marketed in France, submitted by the professional federations of various sectors of the food industry. The detailed analysis of proposals of process hygiene criteria gave rise to the following comments:

  1. Regarding the interpretation of results and the consideration of analytical tolerance:

  - In light of the uncertainty on the consideration of analytical tolerance in the microbiological limits proposed by the professionals, the reasoning involved orders of magnitude and not absolute values. Indeed, it is impossible to rule out the chance that, among the microbiological limits(*m*) proposed, some of them were developed as values not to be exceeded, while others implicitly accept comparing the results to 3 or 10 times the value of *m*;
  - Furthermore, the question of taking into account the uncertainty of measurement in the interpretation of results was not resolved, and needs clarifications from the European commission.

  2. Concerning the proposed criteria:

  - Harmonisation of proposals of criteria for different categories of foods with regard to the risk of microbial contamination by processes of production and distribution, requested in Afssa note of 27 September 2006, does not seem to have taken place;
  - Several proposals from the federations do not pertain to operations specific to the operator, but to those of suppliers;
  - Certain microbiological limits of criteria appeared to be considerably high and not representative of a company that is efficient in the areas of hygiene and cleanliness.

The questions raised lead one to believe that the professionals absolutely need specific information to allow them to identify in a relevant manner the process hygiene criteria specific to their activity, and so that the entire sector of activity can be effectively harmonised.

Afssa therefore issued an self- request with its "Microbiology" Scientific Panel for the writing of guidelines for the development of process hygiene criteria. These guidelines should help **food business operators or their federations to create their own reference guide.** It is the professionals themselves who best know their own activity, their processes, their work station within its surroundings, and they are therefore in the best position to identify the vulnerabilities of their process to microbial contamination. The reference guide including all of the process hygiene criteria can be written in a general manner for a branch, but **each site is unique** and possesses, outside of specificities of the branch to which it belongs, particular sensitive points regarding hygiene.

Thus, it is clear that the guides to good practices for hygiene and application of HACCP principles (hereinafter referred to as "guides") are the most appropriate documents for the presentation of these criteria. The guides, which are useful to operators as well as inspection services, **result from a thorough study of the sector** or of the process, and are to be implemented by the operator of each trans-

formation or distribution site with any necessary adjustments, related to, for example, the type of activity (traditional or industrial).

Therefore, it is planned to have the **guides written by and for professionals** as a relevant tool for the monitoring and improvement of food safety. These guides will also be used **in supplier/client relations** to demonstrate the implementation of an **effective hygiene policy.** The Afssa experts wish here to make their contribution to reflections by operators to determine the most relevant criteria to reach their objectives. In their archives, the professionals have **a large set of analysis results.** The use of these data, which have been recorded over many years, can be of valuable assistance, because they are **the expression of the hygienic quality of the site itself**, with its environment, its receiving of materials and its manufacturing incidents, as well as showing changes and trends. Based on this history, microbiological accidents can be identified and quantified. This analysis must be used to inform thinking on the hygiene measures to be implemented in order to then arrive at the choice of **process hygiene criteria** designed to serve as a tool for the monitoring of **the effectiveness of the prevention policy that has been developed.**

Principles and methodology for the establishment of process hygiene criteria

I - Definition and objectives of microbiological process hygiene criteria (PHC)

1. Definition of the microbiological criterion

The definition taken from the Regulation (EC) no. 2073/2005 as amended is the following: “criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of micro-organisms and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area or batch.”

A microbiological criterion should only be developed and applied in cases of specific needs when its practical usefulness has been demonstrated.

A microbiological criterion must mention the following:

1. The reason for which the criterion has been created, in this case process hygiene;
2. The food or the process for which the microbiological criterion has been created;
3. The point at which the sample is taken from the food supply chain or its environment;
4. The identification of microorganisms and/or their toxins or metabolites, of which the presence is undesirable. The importance of microorganisms/toxins or metabolites targeted by a criterion should also be widely recognised for the food or technology in question;
5. The maximum concentration of microorganisms and/or toxins or metabolites deemed to be appropriate for the food at a given stage in the food production chain. This limit value can be obtained by collecting and analysing data during production and distribution of foods in acceptable conditions of good practices of hygiene and of the HACCP system;
6. The analytical methods for the detection and/or quantification of microorganisms or their toxins or metabolites;
7. The sampling procedure: the sampling procedure describes either a sampling design or a process of trend analysis (for example, control chart). The procedure includes decision-making criteria used to declare a result compliant or non-compliant;
8. Actions to be taken for a non-compliant result;

There are two types of microbiological criteria:

- **safety criteria** defining the acceptability of a food on a sanitary level. Non-compliance with a safety criterion leads to withdrawal, recall, reprocessing or reuse.
- **process hygiene criteria** (PHC) are indicators of the acceptability of the hygienic functioning of the production or distribution process. In Regulation (CE) no. 2073/2005 as amended, criteria applying to manufacturing processes were stipulated. However, additional criteria applicable during the manufacturing stage as well as criteria applicable during the distribution stage may be useful or necessary. Non-compliance with a microbiological process hygiene criterion leads to corrective

actions designed to maintain the hygiene of the process (revision of good hygiene practices and of the HACCP system and/or better selection of raw materials). Non-compliance does not lead to the conclusion that the food is unfit for human consumption. Indeed, according to Regulation (EC) 178/2002, article 14, section 2b, “In determining whether any food is unfit for human consumption, regard shall be had to whether the food is unacceptable for human consumption according to its intended use, for reasons of contamination, whether by extraneous matter or otherwise, or through putrefaction, deterioration or decay.” Bacterial concentrations that cause such defects are significantly greater than the *m* concentrations of process hygiene criteria.

Slightly exceeding the microbiological limit of a PHC does not make the food unfit for consumption.

2. Objectives of process hygiene criteria (PHC)

PHC can be used in four distinct situations:

- isolated evaluation of the control of a manufacturing process,
- validation of the effectiveness of good hygiene practices during their setup, before the start of their use,
- monitoring (in the sense of standard NF EN ISO 22000) of the effectiveness of good hygiene practices in order to detect potential deviations in the expected functioning of these good hygiene practices, for example using samples from surfaces with detection of contamination,
- verification of the effectiveness of good hygiene practices to confirm that they are effective in ensuring process hygiene.

II - To what does the criterion apply?

The process hygiene criteria must apply to manufacturing processes in which good hygiene practices have been implemented in a consistent or uniform manner. Three considerations can be taken into account: batch, stage of manufacturing chain and time of sampling. The manufacturing processes must be categorised taking into account the microbiological quality of raw materials and ingredients used in the formulas, steps in the process having a major impact on the evolution of microbial contamination (sanitising steps, storage phases, maturation phases, handling).

III - Choice of indicators

Microorganisms used as process hygiene indicators may be pathogenic or non-pathogenic microorganisms. One should only use microorganisms, toxins or metabolites that possess a real significance, and for which the presence or concentration is correlated to the control of process hygiene. They may be indicators of initial microbial contamination of raw products, of control of contamination after application of sanitising treatments, hygiene of handling, or storage conditions. It is not always easy to choose the most pertinent indicator. Also, it may be wise to track several of them (for example, to monitor faecal contamination: Enterobacteriaceae, thermotolerant coliforms or *E. coli*) over a determined period, in order subsequently to use only the one that seems the most sensitive to deviations in hygiene practices (see figures 1 and 2). Furthermore, it may be useful to broaden the choice of indicators to respond to the specificity of certain branches. For example, yeasts and molds may be used for monitoring of airborne biocontamination. Of course, it is necessary to have a satisfactory analytical method for the chosen indicator.

Process hygiene criteria for one link in the food supply chain may be considered a safety criterion for another link in the chain, and vice versa. For example, within the same supply chain, *Salmonella* is an hygiene indicator for slaughter processes and becomes a safety criterion for minced and cured meats.

IV - Sampling options

The sampling strategy depends on the situation in which one finds oneself when using process hygiene criteria.

When it involves isolated evaluation of the correct application of good hygiene practices for a given manufacturing process, one uses a predefined sampling plan. The sampling plans currently used (based on the examination of 5 units) provide little in the way of guarantee on the control of hygiene of a given process. However, they make it possible to ascertain that there are no major hygiene errors.

When it involves validating the effectiveness of good hygiene practices, the sampling performed must make it possible to demonstrate that those that are under consideration are effective with a certain degree of safety. More samples will be taken when higher levels of safety in the assurance of effectiveness are sought.

When one monitors the effectiveness of good hygiene practices, one must define a strategy of testing based on regular monitoring of microbiological quality. This monitoring must make it possible to identify lapses in the control of process hygiene. Appropriate statistical tools to perform this monitoring are control charts. It is necessary to define the frequency of checks, the number of analyses to be performed during each check and the limits used to identify a lapse in control (see standard ISO 7870-1:2007). This calibration depends on the requirements of the operator in terms of the amplitude of deviations to be detected and the speed of detecting them.

Verification must make it possible to provide evidence that good hygiene practices have been effective. It is performed over a relatively long period that makes it possible to ensure good representativeness of the normal functioning of the company. As in the case of validation, the number of analyses to be performed over the verification period is directly related to the degree of safety desired during this verification. The accumulation of satisfactory results obtained over a long period generally makes it possible to have greater confidence in the correct application of hygiene measures than the isolated evaluation of their correct application through sampling plans with limited effectiveness.

V - Methodology to develop process hygiene criteria

1. Deciding the stage of application

When developing a process hygiene criterion, the stage of application of this criterion should be identified. This stage corresponds to a step in the process that has a major impact on the evolution of microbiological contamination. Evolution in this sense means: growth, inactivation or contamination. Poor control of this step in the process will result in degradation of the microbiological quality of the finished product. Thus, for example, the stage of application may be placed after a heating step, or any other “decontamination” process. Similarly, PHC may be implemented at the time of final packaging of the product. At this stage, PHC may involve not only the product but also to the personnel (e.g. hygiene of hands or gloves), the air (airborne biocontamination), surfaces in contact with the foodstuff, etc. Furthermore, one must remember that the stage of application is not necessarily in the middle or at the end of a process, but may involve the very first steps in it. For example, PHC may be defined at the end of thawing of raw materials intended for the manufacturing of products for which the process does not allow significant reduction in contamination.

As a reminder, the indicator micro-organism(s) chosen may vary from one stage of application to another.

2. Creation of a sampling plan: *n* and *c*

- Reminder on sampling plans (see Afssa Opinion of 13 March 2008)

The sampling plans conventionally used in microbiology of foods, two- or three-class plans (attribute control plans) were initially defined by the ICMSF (International Commission on Microbiological Specifications for Foods) and are used in particular by the Codex Committee on Food Hygiene (CCFH).

The following symbols and terms are used in sampling plans:

- ***n***: represents the number of units comprising the sample, which must be taken randomly from a batch. The value of *n* represents the sample size. Depending on the case, *n* may be equal to 1, 2, 3, 4, 5, etc. *n* may vary depending on the risk, batch size and occasionally s on the number of units available. A sample size “n=5” is often used, but this value does not represent the rule to be followed in all cases, particularly when testing for certain pathogenic microorganisms. In these cases, the sampling plans recommended by the ICMSF and standard ISO 2859 can be used.

- ***m***: the numerical value of *m* represents the limit of microorganism concentrations corresponding to satisfactory hygiene of the processes being considered, concentrations which are usually expressed by number of CFU (colony forming units) per g or mL or cm².

- ***M*** (three-class plans only): represents the limit of concentrations denoting unsatisfactory hygiene, usually expressed by number of CFU per g or mL or cm².

- ***c*****:** represents the maximum allowable number of sample units:
    - of acceptable quality for a three-class plan (i.e. the maximum number of values between *m* and *M.*
    - or of unsatisfactory quality for a two-class plan (i.e. the maximum number of values exceeding *m*.

If, depending on the situation, the number of acceptable or unsatisfactory quality units exceeds *c*, the batch from which the sample was taken is unacceptable.

Two-class sampling plan

The two-class sampling plan can be used to qualify each sampled unit simply as satisfactory or unsatisfactory. In certain plans, only the presence of a single specific microorganism, such as *Salmonella*, qualifies a sample unit as unsatisfactory. In other plans, a sample unit containing a limited number of microorganisms may be considered to be satisfactory. In these latter situations, a single limit is set, and is indicated by *m.* The two-class plan rejects a batch if the number of sample units of unsatisfactory quality exceeds *c*.

Three-class sampling plan

Sample units containing a number of microorganisms lower than *m* are considered to be satisfactory or of good quality in terms of process hygiene. Units containing a number of microorganisms between *m* and *M* are deemed to be of acceptable quality, and units containing more than *M* microorganisms are unsatisfactory.

The three-class plan rejects a batch if:

- any single sample unit has a concentration greater than *M*;
- the number of sample units of acceptable quality is greater than *c*.

▪ Case of sample limited to a single unit (*n*=1)

Examination of a single unit for a production batch gives very imperfect information on the microbiological quality of a batch. However, the set of information collected on successive batches of a given product coming from a given supplier or from a given site makes it possible to have an estimate of the microbiological quality of this supplier or this site, with increasing precision over time. Furthermore, it is important to realise that uninterrupted compliance with a microbiological criterion by a given supplier or a given site can only result from continued improvement in hygiene. In these conditions, the common practice, particularly in retail, of sampling plans, with sampling reduced to a single unit (*n*=1) seems to be acceptable.

3. Definition of microbiological limits *m* and *M*

Microbiological limits used to discriminate normal microbial contaminations from abnormal contaminations must be defined using records of analyses of companies applying good hygiene practices (GHP). The limit *m* separating satisfactory results from results considered acceptable is generally located above the 95th percentile in the distribution of results observed when the company correctly applies GHP. In this case, the probability of exceeding this limit when GHP are correctly applied is less than 5%. The limit *M* separating acceptable results from unsatisfactory results is usually set so that *M*=*m*+1 ($\log_{10}$ (CFU/g). This is the rule that is conventionally applied for results obtained by counting on solid culture, but it is entirely possible to propose another limit.

When GHP are incorrectly applied, the results have a tendency to exceed these limits with a greater probability, thus allowing the distinction of two situations: GHP correctly applied / GHP incorrectly applied. This discrimination is all the easier the higher the probability of obtaining a result greater than the limit *m* when the GHP are incorrectly applied. This is the case when the microbial indicator is correctly chosen and its concentration is inversely correlated with the application of GHP. In this case,

limited sampling (*n*=1 or 5) will easily make it possible to conclude on the control of hygiene. If the indicator is less pertinent and its concentration increases little when GHP are incorrectly applied, it will be very difficult to conclude on the company's control of hygiene, and this will result in much more burdensome sampling quotas.

- Computer simulation: an illustration

In this section, the numbers representing bacteria counts are expressed in $\log_{10}$ (CFU/g).

An example of the technique is illustrated by figure 1. In the case of a company correctly applying GHP (Fig. 1a), the accumulation of results of analysis over a long period representative of the applied practices makes it possible to characterise the "normal" distribution of contamination. The mean of this contamination is 2.3 and its standard deviation is 1.1. The distribution of the results obtained by a company making the same type of product not having implemented hygiene procedures or obtained by the same company when the hygiene procedures incorrectly applied makes it possible to characterise the "abnormal" distribution of contamination (Fig. 1b). This has a mean of 6.1 and a standard deviation of 1.0 (Fig. 1b).

In this hypothetical case, it is possible to propose a limit *m* of 4.2, which will only be exceeded in 5% of cases when GHP are correctly applied. The limit *M* separating acceptable results from unsatisfactory results can be set at 5.2. When GHP are applied, the limit *M* is only exceeded in 1% of cases (Fig. 1a). On the other hand, when GHP are not correctly applied (Fig. 1b), only 1% of results are lower than *m* and 17% are greater than *M.*

In the case of such a gap between the results obtained by applying or not applying GHP, the results of analysis make it easy to conclude on whether or not GHP were correctly applied. If one applies the classic sampling plan n=5, c_m=2, c_M=0, the probability of passing this plan is 95% when GHP are correctly applied, and the probability of not passing it is 100% when hygiene measures are incorrectly applied. In this case, one can then specify that the contrast is so great that a simple plan n=1 c_m=c_M=0 effectively allows discrimination between the two situations.

If, on the other hand, the incorrect application of GHP is reflected by a distribution of results with a mean of 3.6 and a standard deviation of 1.0 (Fig. 2b), it will be much more difficult to conclude on the correct application of GHP. Indeed, using the same limits *m* and *M* and applying the plan n=5, c_m=2, c_M=0, the probability of not passing this plan in case of incorrect application of hygiene practices is only 35%. To increase the power of discrimination, one must then considerably increase the sampling. It may also be interesting to consider the pertinence of the indicator used, which seems to be poorly sensitive to changes in hygiene practices.

These two situations illustrate the technique that should be used when one wants to set microbiological limits separating the acceptable from the unacceptable. They illustrate the fact that it is difficult to define rules applicable to all situations, and that analysis of the records of results is an indispensable prerequisite to setting these limits.

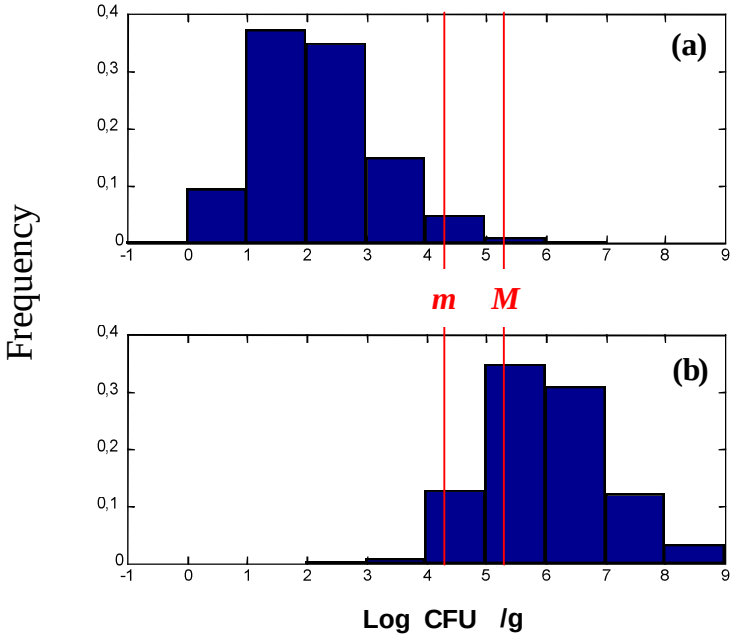

Figure 1. Example illustrating distributions of results of microbial contamination (log CFU/g) in two facilities manufacturing the same products, when hygiene practices are correctly applied (a) and when these practices are incorrectly applied (b). This example shows a case in which the indicator microorganism was correctly chosen, and in which it is possible to conclude on whether or not GHP were correctly applied.

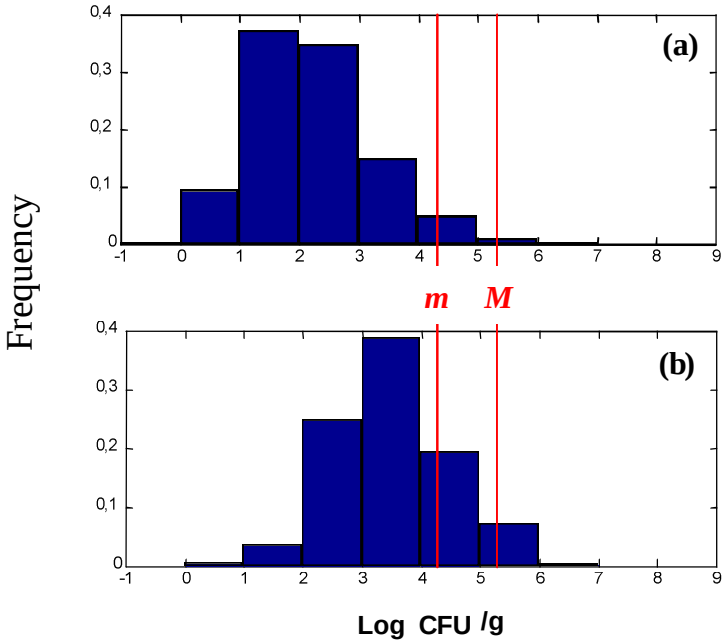

Figure 2. Example illustrating distributions of results of microbial contamination (log CFU/g) in two facilities manufacturing the same products, when hygiene practices are correctly applied (a) and when these practices are incorrectly applied (b). This example shows a case in which the indicator microorganism is not pertinent, because it is difficult to conclude on whether or not GHP were correctly applied.

4. Use of control charts

Control charts are used to monitor the evolution of the microbiological quality of a process. These charts are traditionally in the form of graphs on which the monitored parameters are recorded chronologically (microbial contamination, in our case) during each check. One generally indicates the target level of the parameter monitored and control limits used to demonstrate an out of control of the proc-

ess when the parameter is found outside of the limits (Fig. 3). In the case of microbial contamination, one can thus list an individual result or a mean of several counts (measurement control charts) or a number of unsatisfactory results due to, for example, the presence of a pathogenic agent (attribute control charts). This monitoring can also involve cumulative results from several consecutive dates of observation (cumulative control charts).

The performance of control charts is characterised by their ability to rapidly detect an out of control. The closer the control limits are to the target limit, the earlier the detection of a deviation will be. On the other hand, this tightening of limits is also accompanied by an increase in the risk of a false alarm: the limits are exceeded even though the process is perfectly controlled. It therefore necessary to find a balance between the necessity of rapidly detecting an out of control process and the necessity of not implementing unwarranted corrective actions too often. The charts are therefore usually set up by first establishing control limits involving an acceptable risk of false alarm, then their efficiency at detecting certain deviations is evaluated. As in the case of sampling plans, more samples will be taken when the contrast between the situation of control and the deviations to be detected is low.

An example of the use of control charts is presented in annex 1.

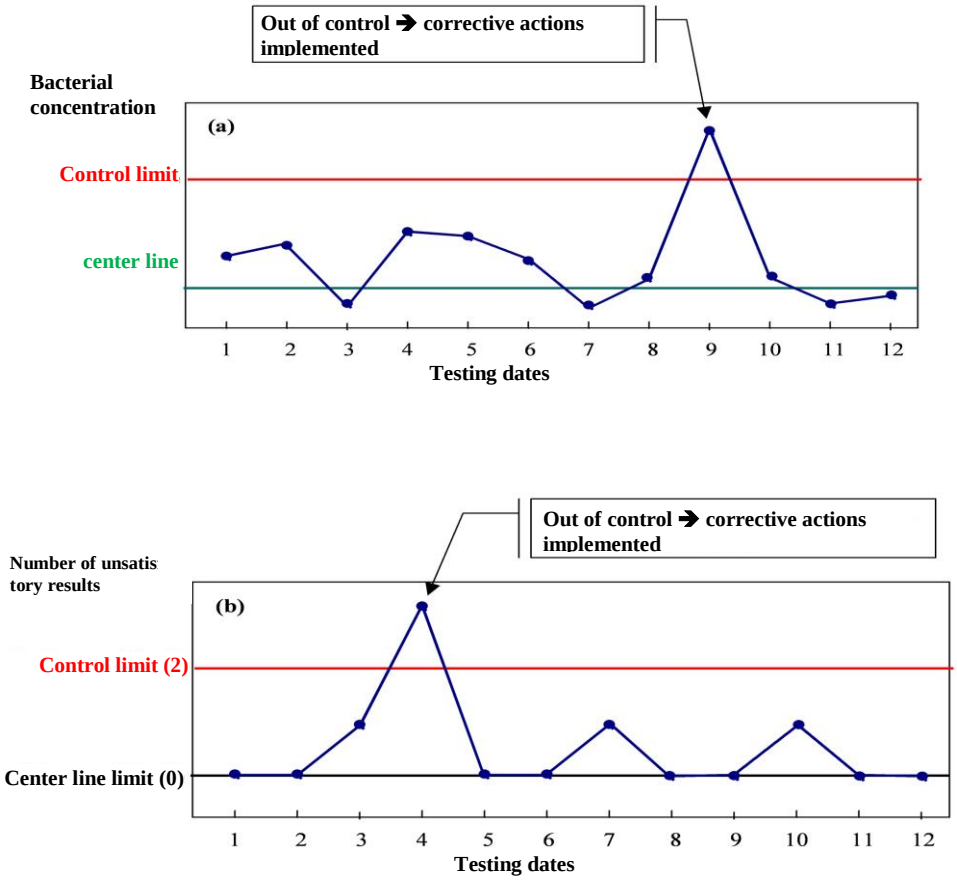

Figure 3. Examples of measurements a) (and attributes (b) control charts

5. Case of criteria to be included in the supplier's specifications

PHC can be applied at the stage of production and distribution for retail companies in which the selling of products takes place after handling performed on site (restaurants, deli, etc.). The criteria must take into account the reasonably foreseeable evolution of the microbial flora. For retail, one must distinguish criteria pertaining to specifications (qualification of the supplier) from those evaluating hygiene practices of the retailer.

It is important not to confuse indicators pertaining to processes under the operator's control, and those that must be included in the suppliers' specifications.

A dialogue is indispensable between the supplier and the buyer to establish the *m* and *M* limits by common agreement, taking into account the constraints of each profession, and clearly defining the intended uses and microbiological shelf life.

VI - Analytical aspects

1. Methods of analysis and analytical tolerance

- General recommendations on analytical methods (see Afssa Opinion of 13 March 2008)

In the context of self-inspections, the following types of analytical methods are recommended in decreasing order of priority when choosing the analytical methods to verify compliance with process hygiene criteria, , except in case of a specific agreement between the parties:

- AFNOR standardised methods which are identical to CEN and/or ISO standards, or otherwise, methods standardised only by AFNOR;
- Commercial methods (commercial kits), provided that these have been validated by AFNOR Certification in accordance with Standard NF EN ISO 16140;
- Internal methods, provided that these have undergone an appropriate validation. Currently, there is no standard-based protocol for the validation of internal methods in food microbiology. Such a protocol will be defined by one of the parts of the future revised version of the standard NF EN ISO 16140. In the meantime, the validation dossier should be compiled with reference to intralaboratory validation protocols defined by the standard NF EN ISO 16140 for qualitative methods (article 5.1) and for quantitative methods (article 6.2).

In the 2nd and 3rd cases, attention should be paid with regard to the effective validation of the analytical method for the couple (microorganism, matrix) in question.

It should be noted that the new version of the standard NF EN ISO 7218 requires the use of one dish per dilution for counting methods; this rule has been applied since the publication of the standard (October 2007) for all of the standardised counting methods.

- Measurement uncertainty and analytical tolerance

Estimation of measurement uncertainty and analytical tolerance

With regard to quantitative determinations, it is recommended that the general tolerance of 3 times the value of *m* for counts in solid medium, introduced in the order of 21 December 1979 no longer be applied but that the approach recently selected by ISO/TC 34/SC 9 for microbiological analysis of foods, an approach described in the Technical Specification ISO/TS 19036 and published in 2006, be adopted.

The approach adopted in ISO/TS 19036 is universal, experimental and founded on the total results variability. It includes the variability related to the heterogeneity of contamination of samples for trials. This variability is quantified by the standard deviation of reproducibility (s_R). ISO/TS 19036 proposes the following three options to estimate the standard deviation of reproducibility (s_R):

- intralaboratory standard deviation reproducibility, estimated by each laboratory (the preferred option);
- interlaboratory standard deviation reproducibility, estimated in the context of an interlaboratory method validation test;
- standard deviation of interlaboratory reproducibility, estimated in the context of an interlaboratory performance test;

A certain number of rather restrictive conditions are required in order that a laboratory may use the latter two options. ISO/TS 19036 is not formally applicable to methods based on the most probable

number principle, but the defined approach can also be followed for these methods. Furthermore, the case of low counts is not addressed by the first version of this standard, but will be soon in an amendment currently being prepared, which will introduce into the estimation of measurement uncertainty a component related to a Poisson distribution of microorganisms.

It is recommended that the values of measurement uncertainty, estimated according to the approach described above, respect the maximum guidelines values described in the table below. These are mean values, and it is possible that in particular cases (such as very heterogeneous products for certain microorganisms), these values may not be met. In such cases, these exceptions should be justified.

Table 1 : Acceptable measurement uncertainty for bacterial counts (in log CFU) established based on the work of Augustin and Carlier (2006) and Ah Soon and Cornu (2004)

	Homogenous matrix		Heterogeneous matrix	
Total number of colonies counted on the dish(es) selected for counting	Method without confirmation	Method with confirmation	Method without confirmation	Method with confirmation
≤ 5	0.7	0.7	0.7	0.8
6-10	0.5	0.6	0.6	0.7
11-15	0.4	0.5	0.5	0.6
>15-150 or 300 depending on the case	0.3	0.5	0.5	0.6

The following matrices can be considered to be homogenous:

- liquids (for example, milk, water, beverages) and powders (for example, powdered milk, egg);
- mixtures of solids (mincemeat, mechanically separated meat, sausage meat, ground meat, whipped cream, soy cream, etc.).

The other types of matrices will be considered to be heterogeneous.

Taking the measurement uncertainty into account in the interpretation of results

Regulation (EC) no. 2073/2005 states in one preamble that, in order to observe “Article 4 of Regulation (EC) No 852/2004, food business operators are to comply with microbiological criteria... It is therefore appropriate to lay down implementing measures concerning the analytical methods, including, where necessary, the measurement uncertainty, the sampling plan, the microbiological limits, the number of analytical units that should comply with these limits.” Regulation (EC) no. 2073/2005 therefore sets microbiological criteria based on this preamble, and states that meeting a criterion implies “the acceptability of a product, a batch of product or a process.” This regulation did not define the method for taking into account measurement uncertainty in the interpretation of results. Lacking this, and in keeping with the spirit of the text of this regulation, unadjusted results must be in compliance with the limits of the criteria.

For **process hygiene criteria**, considering that the limits are normally set based on retrospective data from testing of the products in question, **we consider that the measurement uncertainty was incorporated when these limits were set.** For this reason, we recommend applying the following rule for the interpretation of results:

$$\mathbf{R \leq m}$$

R being the result of analysis.

Thus, the term “limit” can retain its usual meaning of “value not to be exceeded.”

Once it has been verified by laboratories that their measurement uncertainty is in keeping with the table defining the acceptable measurement uncertainty for bacterial counts, the interpretation of an analytical result is simple: it must be less than or equal to *m*.

2. Sampling techniques

The sampling techniques must be in compliance with the dispositions of Regulation (EC) No. 2073/2005, the standard NF EN ISO 7218[1] and standards setting forth the methods of sampling and analysis of foods samples (see Annex 2).

- Case of a homogenous matrix

In the case of homogeneous products such as liquids for which one can suppose homogeneous distribution of microorganisms within a given batch, it is sufficient to take a few units per batch, which will constitute the sample. Liquid products must be well stirred or homogenised using any other appropriate method before the sample unit is taken.

- Case of a heterogeneous matrix

For foods with very high observed heterogeneity of contamination, which is true for many food matrices (such as salad, pizza, bags of frozen fries, etc.), it is not only essential to define a sampling plan based on the type of product, but also to define procedures for elementary sampling and for preparation of samples for the laboratory. In particular, the following should be specified:

- location from which samples should be taken,
- sampling equipment to be used,
- conditions of mixing of the total sample, if applicable,
- conditions and tools for dividing the total sample up into test portions for the laboratory.

- Samples in the environment

When a PHC involves surface samples, the techniques to be used are described in the standard NF ISO 18593.

VII - Actions in case of unsatisfactory results

Non-compliance with process hygiene criteria shall lead to the implementation of corrective actions (in the sense of NF EN ISO 22000). Although the tested or counted microorganisms may be pathogenic germs, the quantities detected or the technological treatments set to take place later mean that non-compliance with these criteria does not represent an immediate threat to consumers' health. Therefore, corrective actions generally do not involve products (withdrawal, recall, reprocessing or reuse), but only the processes, in order to improve the hygiene of the practices with detected problems.

As was explained above, *m* concentrations are significantly lower than the concentrations at which the food becomes unfit for human consumption. If the alert thresholds above which foods should not be placed on the market had been created by professionals and/or administration, they would be much higher. If the microorganisms used as indicators of the possible presence of pathogenic microorganisms or toxins were to exceed these alert thresholds, it could be appropriate to perform targeted testing for these pathogenic microorganisms or these toxins in foods not meeting hygiene criteria.

Recommendations

When establishing process hygiene criteria, the following points should be taken into consideration:

1. sensitive points at which PHC are useful may, of course, involve ingredients or developed products, as well as personnel (e.g. hygiene of hands or gloves), air (airborne biocontamination), water (and pipes and taps), surfaces in contact with ingredients or developed products, and surfaces from which microorganisms may be dislodged by personnel or moving equipment (carts, etc.) and contaminate the ingredients or developed products.
2. the relationship between the nature of the indicator (microorganism, toxin or metabolite) and the hygiene defect must exist. In other words, the pertinence of the indicator must be proven. For example, there is no reason to test for sulphite-reducing bacteria growing in anaerobiosis in bread or

[1] ISO 7218 : Microbiology of food and animal feeding stuffs — General rules for microbiological examinations

Salmonella in a dry biscuit, etc. Furthermore, there must be a significant difference in concentration of the indicator depending on whether the practices are hygienic or not.

3. Compliance with a PHC should be verified at the sensitive point for which it was created, and not at some later step in the food supply chain. Between the sensitive point and a subsequent point, the concentration of a microbiological indicator may have increased due to microbial growth, or decreased due to sanitising treatments.
4. A PHC must be created to validate, monitor or verify a step in the food supply chain. Thus, for retailers of products remaining packaged until their sale, PHC are limited to verifying compliance with the supplier's specifications. On the other hand, when distributors remove products from their immediate packaging and/or cut them up and serve them by the slice, they must additionally use PHC to monitor and verify their good hygiene practices.
5. In the creation of specifications intended for a supplier, the choice of indicators and limits must be done in coordination between the supplier and the buyer.
6. PHC are particularly useful for monitoring performance of the sanitary control plan over time. The control chart is a statistical tool that should generally be used. The control chart helps answer the following questions: Is it a deviation or an accident?
7. A non-compliant result should not give rise to a decision of the same nature as a non-compliant result pertaining to a microbiological safety criterion: a hygiene defect does not automatically make the food unfit for consumption.
8. The measurement uncertainty is taken into account during the development of the sampling plan, and therefore in the choice of the values *m, M, n, c.* Consequently, a microbial count greater than *m* must be considered to be non-compliant. When professionals or their interprofessional organisations that had not taken the above into account apply the above recommendation, it will be simple for them to multiply by an appropriate factor (see table 1) the numbers that they had previously proposed for *m.*
9. It is advantageous, both for professionals and for inspection services, that PHC resulting from a collective discussion process be used as a common reference within a profession. It is desirable for them to be brought to the attention of interested parties, preferably by including them in guides to good hygiene practices and of application of HACCP principles. Of course, this does not prevent any food business operator from adjusting PHC to the particularities of their company and/or their clients' wishes.

Conclusion

For food business operators, PHC are tools to validate, monitor and verify steps in the food supply chain; it is highly unlikely that the application of only PHC from the European regulations will be sufficient to reach these objectives. However, the cost of analyses is a limiting factor, and this is all the more true for smaller companies. Operators must therefore optimise the choice and use of PHC. The above guidelines should help them select the most relevant PHC.

Bibliographic references

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ANNEX 1: Example of control chart use

Microbiological limits may be used for the monitoring of trends in microbiological quality using control charts. The professional can, for example, perform a single analysis at a regular time interval in order to monitor the absence of a deviation in microbiological quality. Considering that there is an out of control as soon as a result exceeds the control limit set at *m*, there is very little chance (1%) of meeting this criterion in case of high "abnormal" contamination (Fig. 1b). However, in the case of low "abnormal" contamination (Fig. 2b), on average it will not pass one of every 4 tests.[2] This approach is therefore relatively effective for quickly detecting an out of microbiological control. It may, on the other hand, lead to a risk of false alarm that is too high. Thus, if the contamination is "normal" (Fig. 1a), the criterion will not be met on average once every 20 tests,[2] which will lead to the implementation of unnecessary corrective actions.

To reduce this risk of false alarm, it is possible to tolerate the appearance of results greater than the limit *m*. One can, for example, on each testing date, look at the last 5 analyses and tolerate the presence of one result between *m* and *M* from among the 5. In this case, the control chart will incorrectly detect an out of control only once every 68 tests,[2] on average, if the contamination is "normal". Low "abnormal" contamination (Fig. 2b) is then detected on average within 8 tests[2] and high "abnormal" contamination (Fig. 1b) is detected on average within 1.2 tests.[2]

Control charts can also be constructed directly from the count results obtained. One can, for example, monitor mean microbial contamination (in $\log_{10}$ CFU/g) obtained over the last 5 controls and verify that this mean contamination remains below a control limit. If, for example, this limit is set at 3.1, it will only be exceeded once every 54 tests[2] on average if the contamination is "normal". This risk of false alarm may be considered to be acceptable in case of weekly testing, since it will occur on average every 54 weeks. If contamination becomes high "abnormal" (Fig. 1b), the mean contamination will exceed the test limit of 3.1 once every 1.6 tests[2] on average, and if it becomes low "abnormal" (Fig. 2b), the limit is exceeded after 4 tests[2] on average.

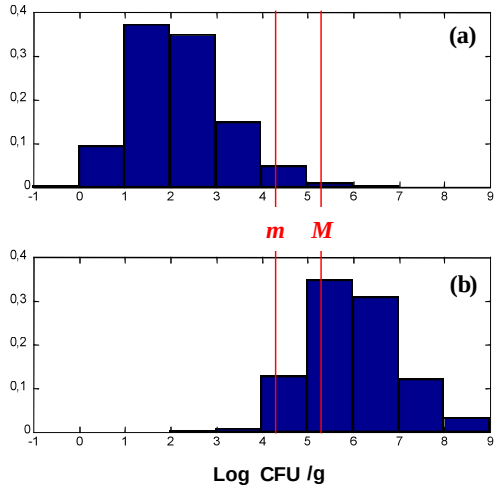

Figure 1. Example illustrating distributions of results of microbial contamination (log CFU/g) in two facilities manufacturing the same products, when hygiene practices are correctly applied (a) and when these practices are incorrectly applied (b). This example shows a case in which the indicator microorganism was correctly chosen, and in which it is possible to conclude on whether or not GHP were correctly applied.

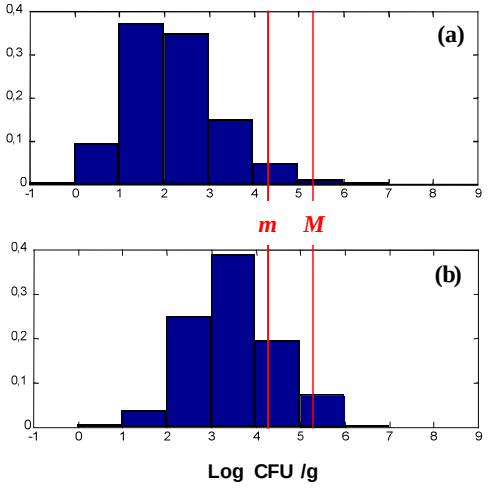

Figure 2. Example illustrating distributions of results of microbial contamination (log CFU/g) in two facilities manufacturing the same products, when hygiene practices are correctly applied (a) and when these practices are incorrectly applied (b). This example shows a case in which the indicator microorganism is not pertinent, because it is difficult to conclude on whether or not GHP were correctly applied.

[2] These results were obtained by computer simulations

ANNEX 2: Sampling standards specific to foods

General information

- ISO 2859: Sampling procedures for inspection by attributes
- Collection 3190861CD: Feb. 2007: Sampling and inspection of food products
- ISO 7002:1986: Agricultural and food products. Presentation of a normalised method for sampling from a batch
- ISO 7870-1:2007: Control charts - Part 1: general guidelines

Milk and milk products

- ISO 707:1997: milk and milk products - Guidance on sampling. (Except for sampling of milk falling under the scope of quality payment systems)
- ISO 5538:2004: Milk and milk products - Sampling - Inspection by attributes
- ISO 8086:2004: Dairy plant - Hygiene conditions - General guidance on inspection and sampling procedures
- PR NF ISO 5538: May 2008: Milk and milk products - Sampling - Inspection by attributes - Sampling plan

Meat, meat-based products or products of animal origin

- NF V04-416: Dec. 1999: Meat and meat-based products - Samples preparation for composition analysis

Prepackaged and prepared foods

- NF V45-074: Apr. 1999 : Processed fish - Deep frozen filet portions - Specifications
- NF V45-065: August 1997 : Processed fish - Smoked salmon

Fruits and vegetables

- ISO 874:1980: Fresh fruits and vegetables - Sampling

Spices:

- ISO 948:1980: Spices and sampling

Cereals

- ISO 13690:1990: Cereals, pulses and milled products: Sampling of static batches
- ISO 6644:2002: Flowing cereals and milled cereal products - Automatic sampling by mechanical means
- PR NF EN ISO 24333: Jan. 2007: Cereals and cereal products - Sampling

Surfaces

- NF ISO 18593:2004: Microbiology of food and animal feeding stuffs - Horizontal methods for sampling techniques from surfaces using contact plates and swabs