INTRODUCTION

In the past, decisions concerning the microbiological quality of food products were ideally taken based on microbial control of 100% of the to be evaluated product. This kind of testing is however too labour intensive, too expensive, too slow and too destructive and therefore representative samples have been taken to be analysed. This latter concept is still applied for food products with an unknown origin. At the moment, the quality and safety of a food product is no longer tested retro-actively but a sound quality assurance system guarantees a stipulated microbial quality. Indeed, modern quality assurance systems, including HACCP, do not longer standardise the product but rather the production process. The analysis of raw materials, intermediate products or end products is therefore not redundant but have another purpose: verification instead of surveillance

The interpretation of the results of microbial analysis is at least that important than the analytical procedure itself. An microbial analysis result needs a specific way of interpretation to make correct decisions. It is not the result on its own that matters for the client but more the consequences of the result. To interpret microbial analyses in a sound way, a thorough knowledge of microbial analysis techniques is essential. However, knowledge of the factors influencing the microbial development, the microbial ecology and statistics are of similar importance.

CHAPTER 1

SAMPLING

1.1 Good sampling for a good beginning

The quality of a microbial analysis is strongly dependent of the way of sampling. Ideally, unitary samples are applied but often **pool samples** are taken. Pool samples are composed out of several sub-samples which are analysed after mixing. In this way, time, labour and money as such is often saved. An important characteristic of pool samples is the fact that all information regarding the variability of the tested parameter is lost as only one result is obtained. The result can for example be strongly influenced by one sub-sample having a high microbial load. This is illustrated in the next example.

Absolute values	Logarithmic values
Sub-sample 1: $4,0x10^4$/g	4,60
Sub-sample 2: $3,0x10^4$/g	4,48
Sub-sample 3: $2,0x10^4$/g	4,30
Sub-sample 4: $5,0x10^4$/g	4,70
Sub-sample 5: $8,0x10^5$/g	5,90
Pool sample: $9,4 x10^5/5g = 1,9 x 10^5$/g	
Logarithm of pool sample: 5,28	Mean of the logarithmic values: 4,80 (corresponds with $6,3x10^4$/g)

It is remarkable that the final result depends on the moment the logarithmic transformation is made. By taking first the logarithm of the results of the sub-samples and taking then the mean value, the effect of an outlier on the results is minimised. As the behaviour of micro-organisms is described as being logarithmic, one should always **work with the logarithmic values of the results**.

The usefulness of a pool sample is strongly dependent on the application. Pool samples can be applied especially for microbial analyses demonstrating the **absence or presence** of a micro-organism. In this case, the variability is of less importance. When the variability of the number of a specific (group) micro-organism(s) is relevant, pool samples won't contain this information so individual samples have to be taken.

As it is stated above and explained briefly in Chapter 1 of Part 1, statistical sampling of a lot is based on random sample survey. Not the entire lot but only a (small) part of it is tested. The reasons for this are:

- Testing the entire lot, which is called **screening**, can be too expensive and too time consuming
- When the sampling is destructive, which is the case for microbial analysis, then screening is not possible

On the basis of results from the random sampling survey, we can make a final decision about the entire lot. This is an extrapolation which brings along a certain risk. We can make a mistake in two ways:

- We can reject a good lot. The risk on this mistake is called "**producer's risk**".
- We can accept a bad lot. The risk on this mistake is called "**consumer's risk**".

The quality of sampling is dependent on both risks. The smaller the risks, the better the quality. Each sub-unit in a lot has an equal chance to be taken in a random sampling survey. This consequence of this characteristic is that a random sampling survey is representative for the lot of which it originates. It will mean that on the basis of such a kind of sampling, we will form a correct image of the lot quality. Imagine that in a (big) lot 1 % defective items are present. This is the percentage (unknown) of the population that we would like to find with our (small) random sampling survey. We do the sampling survey and note down the amount of defectives. We may expect that the percentage of defectives from the sampling will not be equal to the percentage in the lot. Imagine that the sampling resulted in no defectives. In that case we estimated 0 % and we make a mistake of 1 %. Imagine we do another sampling. Then it is possible to estimate 2 % defectives. A third sampling can lead to 1.5 % defectives. This means that every potential sampling survey has an **associated estimation mistake**. If, by incidence, we have a favourable sampling from a bad lot, then we underestimate the percentage of defectives. The estimation mistake is influenced by the **precision** of the sampling. The bigger the sampling the smaller the deviation is between percentage defectives obtained by sampling and the percentage which is actually in the lot. The example above illustrates the importance of choosing an appropriate sampling procedure and a sampling plan to determine the quality of lots.

Sampling can be done for a number of reasons:

- To check if the lots are in accordance with legislation (**EU directive 2073/2005**)
- To obtain general information about the microbiological status of certain products on the market
- Verification of food safety management systems
- To which extent are the different batches in accordance with each other
- Examination of suspicious foodborne outbreaks, complaints
- For identification and gathering of information about new emerging microbial hazards, to generate data to set up risk profiles and risk analysis.

For each of these reasons, different approaches to sampling are required. Sampling plans are executed on different levels such as:

- government
- sector
- company

1.2 Sampling plans

1.2.1 Intuitive sampling plans

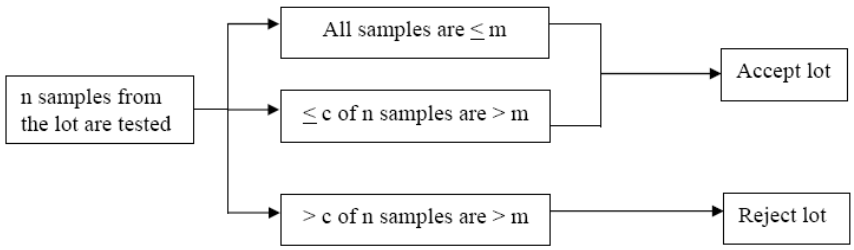
In view of the clarity and simple structure of the usual microbiological sampling design it is not surprising that plans of this kind are often drawn up intuitively. This expression characterizes a procedure in which the sample size is determined mainly from the point of view of what is financially acceptable between parties involved (who pays the bill?). The use of such intuitive sampling plans in practice often forms no problem. Discussions will however occur when the producer or the client do not agree with the reliability of a microbial analysis that is based on an intuitive sampling plan. When intuitive sampling plans are used, it is therefore essential that all involved parties have agreed prior to applying the selected sampling plan.

1.2.2 Attributive sampling plans

The simplest concept to control an alternative characteristic is to determine the number of random samples required, and then to determine how many sample units may exceed the limit **m** without rejecting the lot (acceptance number **c**).

Two class plans

The decision of a two-class plan is based on two numbers, i.e. **n** (number of samples to be tested) and **c** (the maximum number of samples allowed to have a unsatisfactory result). This can be the presence of a micro-organism or colony counts above a specific value **m**. Two possible classifications are possible: satisfactory and unsatisfactory.



Example

Criteria for a two-class plan are:

n = 5

c = 2

m = 1000 CFU/g

Five samples were taken of a batch and every sample was individually analysed. The results of the microbiological analyses (CFU/g) are summarised in the next table:

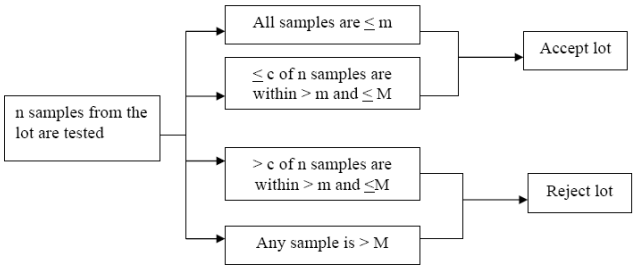
	Case 1	Case 2	Case 3	Case 4	Case 5
Sample 1	450	2200	2200	2200	450
Sample 2	700	700	700	5000	700
Sample 3	800	8000	800	800	800
Sample 4	1200	1200	1200	1200	5200
Sample 5	300	300	1700	300	300

Which cases are accepted and which are rejected?

Besides singular attributive sampling (explained above) where decisions are made after taking one random sampling survey, it is also possible to execute double attributive sampling which includes two sampling surveys. First a sampling survey is done with n1 elements and the number of defectives is determined. If this number is not more than Ac1, then the lot is accepted. If the number of defectives is not smaller than Re1, the lot is rejected. When the number of defectives lays between Ac1 and Re1 then an additional sampling survey of n2 elements is done. When the number of defectives of both sampling surveys is not exceeding Ac2 then the lot is accepted, if not rejected.
Example: the singular plan (131,5) and the double plan (82,2,5) and (82,6) are equal based on the severity of inspection/sampling. This means that rejecting/accepting of a lot is for both plans the same on an average base. When sampling is done according to the singular sampling then 131 items should be investigated each time. When a double plan is performed, then in some cases only 82 samples are investigated which brings along a big profit compared to a singular plan. But it can also happen that 164 items are necessary for examination which makes the double plan more expensive. However, in the long run, the double plan will be more efficient than the singular plan, thus on an average basis smaller amounts of items need to be controlled. This is in general the case: double plans are less expensive than singular plans.

Three-class plans

Three-class plans are designed for situations where the quality of a product, depending on the number of a specific target organism in the samples, can be divided in three classes of microbiological quality: the **good quality range** from 0 to **m**, the **marginally acceptable class** between **m** and **M** and the so-called **class of defective quality** above M. An acceptance number **c** is assigned to each of the two limits **m** and **M**: the value assigned to **M** is always **0**. A lot will be rejected if at least one unit in a sample exceeds the limit M and/or if more units range above m than the acceptance number c would permit.



It is important to make a difference between **m** and **M**. **M** is a contamination level, caused by bad hygienic practices, endangering the public health or being unacceptable. Therefore, all lots with values > M have to be rejected. The level of M can be based on different grounds (ICMSF, 1986):

- As a **utility** (spoilage or shelf-life) **index**. Relate levels of bacteria to detectable spoilage (odour, flavour) or to a decrease in shelf-life to an unacceptable short period.

- As a **general hygiene indicator**. Relate levels of the indicator bacteria to a clearly unacceptable condition of hygiene – whether contamination or growth, or a combination of these factors.
- As a **health hazard.** Relate levels of bacteria to illness
- It is important to realise that M is defined by the hazard. The value of m is available and defined by GCP (Good Commercial Practice), and may alter with time. The values of m and M are independent of each other and have no constant relationship. Figure 1 illustrates the effect of various frequency distributions of microbial content, within a lot, on the location of m and M for 3-class plans.

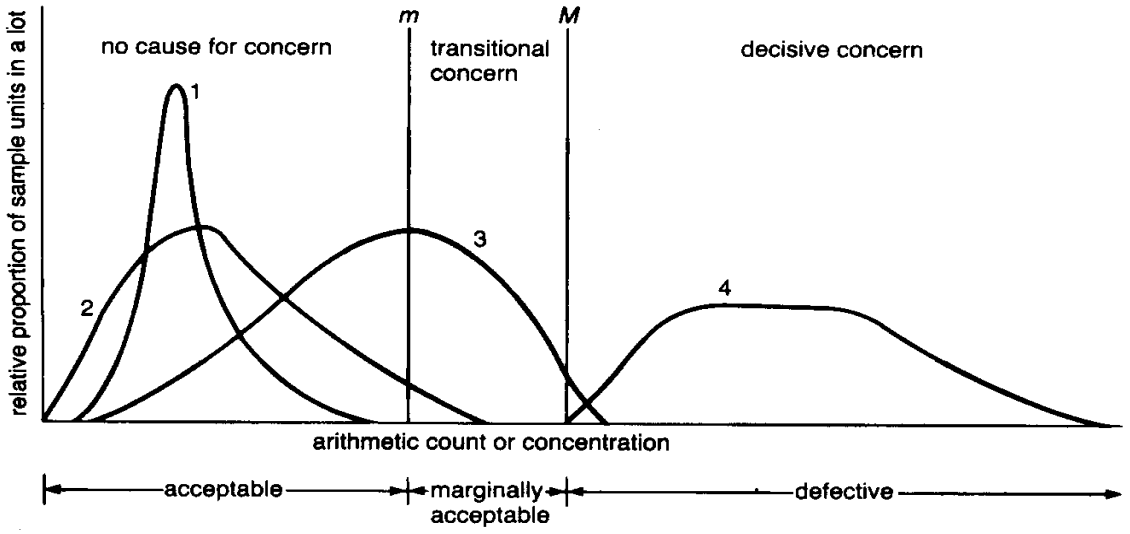


Figure 1: Considerations in choosing m and M (ICMSF, 1986)

Example

Criteria for a three-class plan are:

n = 5

c = 2

m = 1000 CFU/g

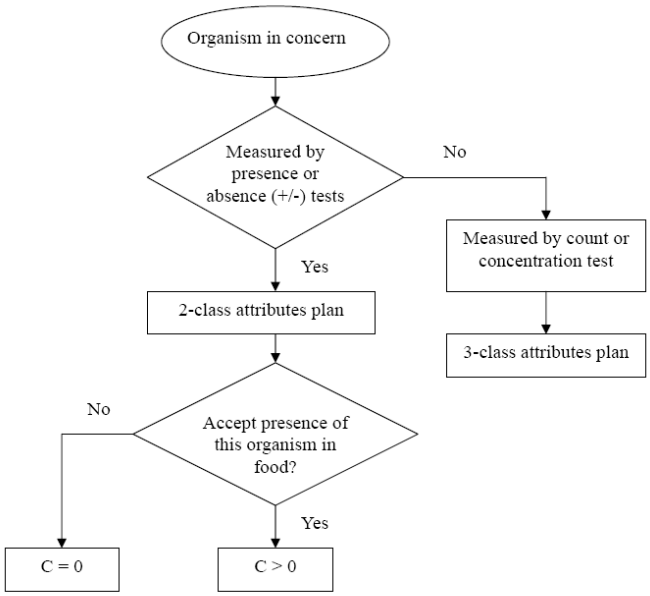
M = 4000 CFU/g

To which categories belong the samples mentioned in the example for the two-class plan?

Choice between two-class and three-class plans

If the microbial analysis is supposed to determine the **presence or absence** of a micro-organism (mostly pathogens), then a **two-class plan** is always chosen. C=0 in the case the pathogen can't be tolerated in the product and c>0 when the pathogen can be tolerated in a limited extent.

When microbial analyses tolerate the determination of counts (**enumeration**) there is by preference chosen for a **three-class plan**. The three-class plan gives the opportunity to 1) define a specific count **m** which is the goal if work has been performed under GMP and 2) define a maximum count **M** which is related with a main public health problem. A limited number of samples are allowed in the marginal area (between m and M) which takes into consideration the fact that occasionally higher values than the target value (m) can be obtained under GMP without immediate risk for public health. This is especially applied for indicator organisms or food toxicants. A three-class plan allows also to set up a trend analysis whereby an increasing result in the marginal area, indicates a less adequate GMP or bad functioning of the preventive quality system.



1.2.3 Variable plans

If the result of a bacteriological examination is available in the form of a bacterial count, the use of attributive plans always means a loss of information. The degree to which results fall above or below the limit is not taken into account; also true variability is not included in the construction of the plan. The use of real standard deviations can thus lead to an improved decision making process and even to economic advantages. But those plans require the data to follow a normal or log-normal frequency distribution. Many surveys show that the log-normal distribution model is applicable to the behaviour of bacterial counts in parallel samples from a lot. The fact that micro-organisms grow exponentially is itself already a plausible explanation for this assumption.

k_1 values calculated using the non-central t distribution safety/quality specification (reject if $\bar{x} + k_1 s > V$)

Probability (P) of rejection	Proportion (p_d) exceeding V	Number of sample units							
		3	4	5	6	7	8	9	10
0.95	0.05	7.7	5.1	4.2	3.7	3.4	3.2	3.0	2.9
	0.1	6.2	4.2	3.4	3.0	2.8	2.6	2.4	2.4
	0.3	3.3	2.3	1.9	1.6	1.5	1.4	1.3	1.3
0.90	0.1	4.3	3.2	2.7	2.5	2.3	2.2	2.1	2.1
	0.25	2.6	2.0	1.7	1.5	1.4	1.4	1.3	1.3

In the field of quality control, the so-called **mean value plan** is often chosen because it is easier to understand and apply. The general focus of this kind of test for significance is the decision as to whether the **mean of the (log-) transformed colony counts exceeds** significantly the limit **m**. From the measured values an average value ($\mathbf{x_{mean}}$) and a standard deviation (**s**) can be calculated. These values are used to decide if the limit **V** (logarithm of the postulated limit) is significantly exceeded. The lot is rejected when $\mathbf{x_{mean}+k_1.s>V}$. The value of k_1 can be derived from tables.

The above given Table tabulates the k_1 values for a number of samples between 3 and 10. To define k_1 a decision has to be made regarding the maximal fraction of units in a lot that can have a value above the limit value **V** (=**Pd**) and about the accepted chance of rejecting a lot having at least a fraction P_d above V.

For example when 5 samples are analysed per lot and it contains 10% of units exceeding V, it should be rejected with a chance 0,95. k_1=3,4 will be used. The following table gives the results of the aerobic total plate count of five sample units obtained from a lot of poultry.

An example of aerobic plate counts for a sample of poultry ($n = 5$)

APC	$\log_{10}$ (APC)	Mean log ($\bar{x}$)	Standard deviation (s)
40,000	4.602		
69,000	4.839		
81,000	4.909	5.039	0.378
200,000	5.301		
350,000	5.544		

Possible values for a sampling plan could be: P=0,90, Pd = 0,25 with a limit of V=7. The corresponding k1-value is 1,7. When the above mentioned formula is used, a value of 5,039+1,7x0,378 = 5,682 is obtained. This value is clearly lower than the limit of 7, meaning that the lot can be accepted.

1.2.4 Sampling plans in relation to reliability of accepting a lot

When more samples are tested, the chance to make a wrong decision will be smaller (accepting a lot which is too highly contaminated or rejecting a lot of good quality). But because the lot is never completely

tested, a decision is made on the basis of a sampling survey and therefore the reliability of the decision will never be 100%.

To acquire insight about the reliability of a decision on basis of a certain sampling plan (with n and c) , Operating Characteristic curves (abbreviated as **OC** curves) and tables are observed from which the chance of accepting a lot with a certain percentage of defectives can be derived.

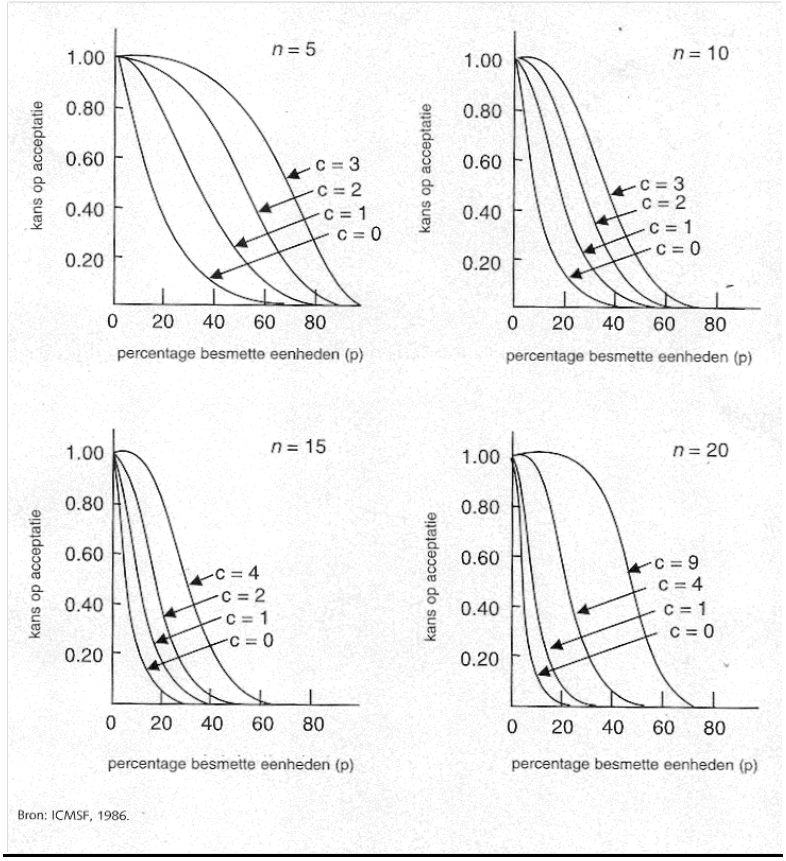


Figure 2: Characteristic operating curves for a different amount of samples of a lot (n) and different acceptance criteria (c) for a two-class plan

Imagine that a lot is examined for the presence of *Salmonella,* then n samples of 25g are taken by a sampling survey from the lot and examined for the presence/absence of *Salmonella*. The samples may or may not be contaminated and the number of contaminated samples is called **k**. The number **k/n** is the estimation of the percentage of the lot that is contaminated. The actual percentage of the lot that is contaminated is indicated with **p**. Low values of p indicate good microbiological quality; high values indicate bad microbiological quality.

For instance n=10, c=0

The chance of acceptance is equal to the chance of 10 measurements leading to no single positive result (positive is presence of *Salmonella*). This will be determined by the actual percentage which is contaminated (the higher the contamination, the higher the chance of positive results to be found). The

number of contaminated samples in the sampling survey will follow a **binomial distribution**. The chance of acceptance with this sampling plan will be calculated for different values of p.

P	**chance of acceptance**
0.005	0.95
0.01	0.90
0.05	0.60
0.10	0.35

In the case that not 10 but 5 samples are taken, the chance to find at least 1 contaminated sample will be smaller. The chance of acceptance will increase independently of the actual percentage of contaminated items. This means that when the number of samples taken in the sampling survey decreases, the severity of the sampling plan also decreases. When the value c (the number of samples within the sampling survey which is allowed to exceed m) increases, then the severity of the sampling plan will decrease.

The chance of acceptance with a relatively high percentage of contaminated samples can sometimes be high. For example, with n=5, c=0: when a lot contains 10% defective samples, the chance of acceptance will be ca. 70%. Certainly in the case that the contamination is due to pathogens, there is a high consumer's risk. But it means that in 3 out of 10 cases, rejection will occur. This confrontation of the producer with these high chances of rejection of the lot will lead to sufficient pressure to adjust the production process or to introduce more severe hygiene measurements or at least to control them precisely.

OC curves and tables illustrating the chance of acceptance in a certain sampling plan of a lot with a specified percentage of defectives (two-class plan) or a lot with a certain number of items of marginal quality and bad quality (three-class plan) can be found in the ICMSF (1986, 2001) book.

The severity of a sampling plan is based on the hazard for the consumer coming from pathogens and their toxins or the potential to spoil food; also the kind of micro-organism present and their numbers are important. Some micro-organisms cause mild disease which seldom spread while others cause mild illnesses which may spread rapidly; and yet others cause severe illness. In some cases the food acts as a vehicle for transmission of the infectious organism. Tampering in the normal course of distribution, storage, and preparation for consumption may decrease, leave unchanged, or increase those numbers. The choice of a plan must therefore consider: (i) the type and severity of hazards implied by the micro-organisms for which the test is to be made; and (ii) the conditions under which the food is expected to be handled and consumed after sampling. ICMSF classifies 15 different cases of sampling plans on a two dimensional grid taking into account these factors. The stringency of the sampling plan increases with the type and degree of hazard: from a situation of no health hazard but of utility only, through a low indirect health risk (as implied by the presence of indicator organisms), to direct health risks related to disease of moderate or severe implication. The stringency of the sampling plan also changes according to the conditions under which the food is expected to be handled. Hazards may be reduced by cooking, or increase by subsequent growth of micro-organisms.

CHAPTER 2

MICROBIOLOGICAL CRITERIA

2.1 Introduction

Microbial criteria are inevitably prone to discussion. Indeed, microbiological analyses can never guarantee for 100% product safety or product quality. This is mainly due to the limitations of sampling and the non-uniform distribution of micro-organisms on or in food products. Nevertheless, criteria are necessary and can be very useful for the food industry when well applied.
Microbiological criteria can be divided into three categories: **standards**, **guidelines** end **specifications** (ISFT, 1997).

Standard: This is a microbiological criterion contained in a law or a regulation where compliance is mandatory. These are introduced by governments or regulatory authorities. Typical examples include most criteria in European Community (EC)/European Union (EU) Directives and national legislations. The food industry must ensure full compliance with these standards which are monitored by enforcement authorities.

Guideline: A microbiological guideline is a criterion applied at any stage of food processing and retailing which indicates the microbiological condition of the sample and aids in identifying situations requiring attention for food safety or quality reasons. Results obtained from testing against microbiological guideline also assist in trend analysis. Results that deviate significantly from the trend may indicate a tendency towards a situation which is out of control and highlights the need for attention before control is lost. Guidelines are usually self-imposed by the food industry but may occasionally be included in legislation. Guidelines on the levels and types of micro-organism relevant in specified foods produced under good manufacturing practice may also be provided by industrial associations for their members. In some EU directives there are guidelines for indicator organisms e.g. Directive 92/46/EEC for milk and dairy products).

Specification: This is a microbiological criterion applied to raw materials, ingredients or the end product which is used in a purchase agreement. Criteria may include pathogens, toxins, indicator organisms or spoilage organisms where non-compliance may affect product safety and/or quality during shelf life. End product specifications are usually more stringent than microbiological standards in order to provide a margin of safety. Products not complying with specifications should be investigated to determine the cause. Rejection of products may occur even if they are not hazardous or unwholesome at the time of testing. It is important to ensure that components of microbiological specifications are relevant and realistic and fully understood by both parties in the agreement.

2.2 Components of microbiological criteria

A microbiological criterion consists of a statement of at least the following:

- Micro-organism or microbial toxin of concern
- Food concerned and sample type
- Sampling plan
- Microbiological limit(s)
- Moment of sampling

The contaminants detailed in the criterion may be foodborne pathogens, food spoilage organisms, indicator organisms or microbial toxins. Pathogen testing is applied for reasons of public health where evidence exists of a potential hazard to health from a specific food/organism. Testing for indicator micro-organisms is used for the following reasons:

Cost effectiveness*:* More analyses can be conducted for microbial indicators of unhygienic practice than for specific pathogens because testing for pathogens is usually more expensive.

Simplicity*:* Test for indicator micro-organisms are frequently simpler to conduct than tests for specific pathogens.

Rapidity: Tests for indicator organisms usually provide results more rapidly than those for pathogens, thus allowing faster remedial action.

Trend analysis: Indicator organisms are usually present in higher numbers than pathogens and indicate unsatisfactory conditions of production when levels increase significantly. Since these levels can be monitored, trends can be established so that trend analysis can identify situations before they become out of control.

A serious shortcoming of many applied criteria is the fact that only one criterion for different groups of micro-organisms is given with or without a tolerance. For microbiologically stabile products such criteria are realistic and applicable. However, for microbiologically unstable products, for which the microbial population can increase in number between the day of production and the expiry date, these criteria are often not applicable. For this reason, Laboratory for Food Microbiology and Food Preservation of Ghent University together with the food industry, catering industry and industrial kitchens developed criteria for different categories of food products with comparable intrinsic parameters and corresponding spoilage flora.

In these criteria only parameters that are ecologically relevant are included, i.e. only the groups of micro-organisms that are responsible for spoilage or can endanger public health, taking into account the type of food product,. For every parameter 3 values are given, i.e. the contamination level on the day of production that should be the **objective** of the producer (O), a **tolerance** on the objective (T) and the contamination level on the expiry date. For all the stipulated groups the criteria were successfully validated.

These criteria, that take into account the **growth kinetics** of micro-organisms in food products, are also useful for the evaluation of challenge tests and for predictive microbiology. Both concepts are applied in regard to shelf life determinations and microbial safety in, for example, the framework of a HACCP concept (Hazard Analysis Critical Control Point). A number of these criteria are given in Addendum.

Many discussions were raised regarding the total aerobic plate count as a parameter for the microbial quality of food products. For this reason, the mentioned criteria take into account the development of different groups of spoilage organisms that are able to proliferate in a specific food product and can cause deterioration of the food product. For example, it is known that Gram-negative bacteria cause much faster spoilage (at lower numbers) when compared to micrococci or lactic acid bacteria. For the interpretation of the results one can argue for a less strict interpretation of the total plate count. In many products it is recommended to perform an additional sensorial test when the total count exceeds the limit if it is proven that the total count is in majority composed out of lactic acid bacteria.

Microbiological criteria have to be developed but also interpreted with a knowledge of: (a) quality and type of raw materials; (b) the applied technological processes; (c) the control of critical control points in the process with special attention for temperature control and cross contamination; (d) the way of storage and storage temperature during distribution, sale and at the consumer (e) the intrinsic parameters of the food product; (f) the normal microflora of the product and the behaviour of the microflora; (g) the type of packaging and effect on product properties; (h) shelf life to be expected and (i) the treatment of the product in the distribution chain and by the consumer.

2.3 Limitations of microbiological criteria

The tests used to assess whether a food complies with a microbiological criterion are subject to a number of factors which should always be clearly understood when using them:

***Methods**:* No microbiological method is capable of detecting all representatives of the target micro-organism being sought. The presence of sub-lethally injured cells or cells that may be viable but not culturable, contaminants present in the food but at a level below sensitivity of the method and variants not capable of growth under the conditions of the test will all result in less than total recovery of the target organism. Confidence limits for results from enumerative techniques are also relative wide.

***Sampling**:* Micro-organisms are rarely distributed homogeneously in a sample or a product batch and pathogens, if present, are usually at low levels. Any single test for detection or enumeration only reflects the situation in that particular sub-sample of the batch.

Laboratory competence: Laboratory staff competence and the operation of quality systems in laboratory are very important for achieving valid microbiological results. Independent auditing, use of external quality assurance schemes and accreditation of laboratories help provide assurance of laboratory competence.

***Cost effectiveness**:* Because of the high costs involved in microbiological testing, the number of samples and the number of microbial parameters that have been analysed is often too limited to

take decisions with 100% certainty. It is therefore essential to choose the right parameters to obtain a maximum of information with a limited budget.

2.4 Examples of microbial criteria

On the European level a new EU regulation (EU Regulation 2073/2005 on microbiological criteria for foodstuffs; part of this document is added in addendum 2) has recently been published. This regulation has come into force on 1 January 2006. These EU criteria have replaced the existing legal criteria that were included in various Royal and Ministerial Decisions at the Belgian level. In this regulation, a distinction is made between food safety criteria (product criteria) and process hygiene criteria.

Food safety criteria are criteria that guarantee the food safety of products that are placed on the market. The criterion is used to determine acceptability of a product or a batch of food products, and is applicable to products that are placed on the market (distribution and import). These criteria do not apply to food products that are further processed by a next step of the food processing industry and where the danger under consideration is still eliminated. The criteria apply to products that are delivered to the retail and that are intended for the consumer. When exceeding the criteria, action needs to be taken immediately in order to guarantee the protection of the public health of consumers (i.e. not placing products on the market or recall or, if possible, subjecting the products to an additional treatment).

Process hygiene criteria on the other hand are criteria that give an indication whether the production process was acceptable and hygienic and they constitute a verification of the preventive measures taken by the producer to operate hygienically and to guarantee food safety. The parameters included in the process criteria need to be checked regularly in order to enable a trend analysis and to make it possible to show a good control of the manufacturing practices. Exceeding the process criteria does not immediately lead to a danger for public health. If the criteria are not reached, corrective measures need to be taken to make sure that the process hygiene remains in accordance with Regulation (EC) 178/2002. The criteria are applicable **during processing** (and not during the retail phase or import). When the process criteria are exceeded, the operator needs to improve the hygienic conditions during production and revise the auto-control process.

As previously mentioned, the 'International Commission on Microbiological Specifications for Foods' formulated in 1986 criteria for different food products. They are based on two or three-class sampling plans. Examples of these criteria are given in annex 2.

The criteria, developed in the Laboratory for Food Microbiology and Food Microbiology, Ghent University (Prof. dr. ir. M. Uyttendaele) are added in annex 3.

2.5 Trend analysis – Control charts

Random sampling plans discussed previously serve to evaluate uniform, discrete, defined lots. The information from a previous test do not influence the next decision. Such acceptance sampling plans for quality control of lots can also in principle be used for the **continuous control of production stages**.

However, they represent a procedure which is more passive than active and cannot be reconciled with the essential goals of quality control. A good quality control strategy should:

- Yield information about characteristics of the process, especially average quality, as well as unavoidable variations.
- Maintain proper production as long as possible.
- Clearly show deviations from the quality standard so that production of faulty units is recognised early?

The task of lot testing (namely to accept or reject certain production units) becomes, due to the implementation of HACCP, of less significance. A **control chart** is employed for continuous evaluation of quality control to determine the agreement between fixed standard and reality of practice (=verification).

Every control chart where random sampling results are recorded in chronological order requires the sample size to be established, as well as sampling frequency, target organism, observed statistical parameter, critical limits and fixed corrective actions in case of rejection. Most quality control charts have 95% limits as attention limits and 99% limits as control limits, where a single event exceeding the attention limit -in practice the first warning step- provides a higher level of caution, while results outside the control limits demand immediate remedial action and countermeasures in the production process.

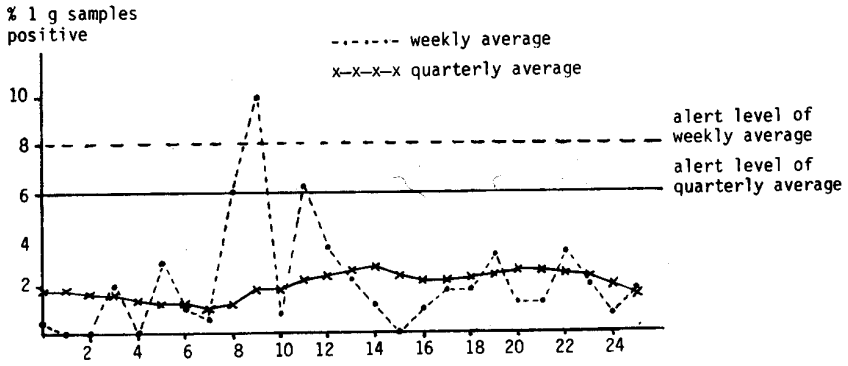


Figure 3: Example of control chart

2.6 Conclusions

Microbiological analyses are strongly different from most other analyses in the food industry (e.g. chemical analyses). This is mainly due to the biological character of the determining parameter which inherently contains great variability. It is therefore essential to interpret microbial analysis results in this context. For the interpretation of results from microbial analyses, one can apply existing criteria from which the most important are mentioned in this document. It is however important to realise that good interpretation of microbial results demands more than the knowledge of analytical techniques. The ultimate purpose of analyses is to take the right decision. This decision can only be taken appropriately with a knowledge of food science in its broad sense and essential mathematics.

Annex 1

Example of microbiological criteria from EU Regulation 2073/2005 on microbiological criteria for foodstuffs

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Food Safety criteria:

Food category	Micro-organisms/their toxins, metabolites	Sampling plan (1)		Limits (2)		Analytical reference method (3)	Stage where the criterion applies
		n	c	m	M		
1.1. Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes (4)	*Listeria monocytogenes*	10	0	Absence in 25 g		EN/ISO 11290-1	Products placed on the market during their shelf-life
1.2. Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g (5)		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life
		5	0	Absence in 25 g (7)		EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
1.3. Ready-to-eat foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes (4) (8)	*Listeria monocytogenes*	5	0	100 cfu/g		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life
1.4. Minced meat and meat preparations intended to be eaten raw	*Salmonella*	5	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf-life
1.5. Minced meat and meat preparations made from poultry meat intended to be eaten cooked	*Salmonella*	5	0	From 1.1.2006 Absence in 10 g From 1.1.2010 Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf-life

Process hygiene criteria:

Food category	Micro-organisms	Sampling plan (1)		Limits (2)		Analytical reference method (3)	Stage where the criterion applies	Action in case of unsatisfactory results
		n	c	m	M			
2.1.1. Carcases of cattle, sheep, goats and horses (4)	Aerobic colony count			3,5 log cfu/cm² daily mean log	5,0 log cfu/cm² daily mean log	ISO 4833	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
	Enterobacteriaceae			1,5 log cfu/cm² daily mean log	2,5 log cfu/cm² daily mean log	ISO 21528-2	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
2.1.2. Carcases of pigs (4)	Aerobic colony count			4,0 log cfu/cm² daily mean log	5,0 log cfu/cm² daily mean log	ISO 4833	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
	Enterobacteriaceae			2,0 log cfu/cm² daily mean log	3,0 log cfu/cm² daily mean log	ISO 21528-2	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
2.1.3. Carcases of cattle, sheep, goats and horses	*Salmonella*	50 (5)	2 (9)	Absence in the area tested per carcase		EN/ISO 6579	Carcases after dressing but before chilling	Improvements in slaughter hygiene, review of process controls and of origin of animals

Annex 4

Example of microbiological criteria and guidelines from The Laboratory for Food Microbiology and Food Preservation (LFMFP), Ghent University

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CAT 1: Milk and dairy products

CAT 1A: Raw milk (cow milk) as raw material

Parameter	T(arget)	T(olerance)
Total aerobic mesophilic count [1]	**10^5**	**10^5 (a)**
E. coli [2]	10^2	10^2
Coagulase positive staphylococci	10^2	10^3
Salmonella spp.	Absence in 25 g	Absence in 25 g
Listeria monocytogenes	Absence in 25 g	Absence. in x g (b)
E. coli O157:H7	Absence in 25 g	Absence in 25 g
Campylobacter spp.	Absence in 25 g	< 10^2
Bacillus cereus	10^2	10^3

(a): other criteria are applied to milk for raw milk products and dairy farm (see EU VO 853/2004 - Section IX).
(b): Depending on the growth potential of *L. monocytogenes* – when not aware of growth, absence in 25g is respected.

[1] Legal criterium for total aerobic mesophilic count : EU VO 853/2004 – section IX.

[2] Advice Health Council.