

Maisons-Alfort, 18 January 2007

## OPINION

### **of the French Food Safety Agency (Afssa) on the request to create reference documents on microbial flora which could be used as process hygiene indicators**

LE DIRECTEUR GÉNÉRAL

The Directorate General for Food (DGAL) and Directorate General for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF) requested the French Food Safety Agency (Afssa) on 12/07/2006 to issue an opinion on the request to create reference documents on microbial flora which could be used as process hygiene indicators.

#### Context

With the Hygiene Package coming into force in January 2006, Afssa received a request at the end of 2005 on keeping certain national microbiological criteria in addition to those featuring in Regulation (EC) No 2073/2005<sup>1</sup>. This request concerned the safety criteria on pathogenic microorganisms, and Afssa's subsequent opinions highlighted the interest of conducting discussions in parallel on process hygiene criteria.

The funding government departments conducted a survey to collect proposals of process hygiene criteria from professional federations. They then carried out a compilation.

In July 2006, the DGAL and DGCCRF requested Afssa about these process hygiene criteria.

This request was threefold:

1. create reference documents on microbial flora which could be used as process hygiene indicators;
2. issue an opinion on the criteria put forward in different sectors and particularly on the interest of the flora selected and contamination levels of foodstuffs by process hygiene criteria microorganisms, on the basis of the proposals presented in an Excel spreadsheet;
3. issue instructions for the inspection departments (this information will be subject to another request).

Responses to this request will be given successively:

- the response to the first part (general datasheet on the microbial flora that may be used as process hygiene indicators) is given in this document.
- an *ad hoc* working group will be created to respond to the second part (opinion on the criteria put forward), as soon as new criteria proposals, in a more aggregate form, requested in a letter dated 27 September 2006, are presented to Afssa.
- the third part will be re-examined once the specific request mentioned by the government departments has been received.

After consulting the "Microbiology" Scientific Panel, which met on 28 September, 24 October and 28 November 2006, Afssa issues the following opinion on the first part of the request:

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<sup>1</sup> Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

## Expert assessment

### Context on the concept of indicators

The DGAL and DGCCRF would like Afssa to establish reference documents on certain flora which could be used as hygiene indicators, particularly for certain flora or microorganisms indicated in the order of 21 December 1979.

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs makes a distinction between food safety criteria and process hygiene criteria. The latter are defined as follows:

**“Process hygiene criterion:** *“a criterion indicating the acceptable functioning of the production process. Such a criterion is not applicable to products placed on the market. It sets an indicative value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law”.*

**Indicators** were defined by J. Ingram in 1977:

*“Markers which, when present at a given number, indicate that good manufacturing and distribution practices have been broken”.*

These definitions refer implicitly to non-compliance with:

- good hygiene practice, whether this concern prerequisite programmes (PRP) or operational prerequisite programmes (PRPo),
- or critical control points (CCP), such as product criteria (e.g. not acidic enough, too much water activity) or process criteria (e.g. temperature and/or heat treatment duration too low, temperature and/or warehousing duration too high).

This non-compliance can make the food unfit to eat (without affecting its safety) because of:

- excess development of the natural microflora in the food
- contamination or the possible multiplication of environmental or faecal microflora.

Certain indicators are clearly associated with a PRPo or a CCP, and are easy to interpret (e.g. the concentration of alkaline phosphatase determines milk pasteurisation). But most are evidence of several causes at once. This often makes the analysis results difficult to interpret.

Certain indicators are pathogenic microorganisms for humans and animals. They come, for example, from the digestive tract, the skin, nasal cavity, lungs or udder (coagulase-positive staphylococci), soil or water (*Bacillus cereus*, *Clostridium perfringens*). When they are highly concentrated, these microorganisms cause digestive disorders, with a possible outbreak. They are used as indicators by looking to see if they are present at a low concentration. This is with a view to detecting poor hygiene practices that may cause health problems by:

- either growth and the production of adverse substances that cooking or any other culinary preparation does not inactivate (e.g. staphylococcus enterotoxin, *B. cereus* emetic toxin),
- or non-compliance with hygiene between the final culinary preparation of a food and its consumption.

In some cases, using microorganisms as indicators is less convenient and/or instructive than using their toxic or non-toxic metabolites or molecules, which behave in a similar way to microorganisms of interest. The process hygiene criteria can therefore be based on the quantification or detection of the presence, absence or concentration of either microorganisms or chemical compounds.

Indicators should not be confused with **“indexes”**, defined by J. Ingram as *“markers whose presence in numbers exceeding given numerical limits, indicate the possible occurrence of ecologically similar pathogens”.*

Note that indicators (when they have been well chosen) are a positive indication that good practices have not been complied with. Indexes, however, can only inform us of the possibility of pathogens occurring. Experience has revealed that there wasn't any relationship of constant and universal proportionality between index and pathogen quantities. As a result, pathogens may be absent even when the indexes are high. Conversely, pathogens can be detected without the indicator being detected: this paradoxical observation is explained by the imperfection of the analytical methods.

This is why non-compliance with a criterion for an index is not sufficient to declare that a food is injurious to health, in the meaning of article 14 of Regulation (EC) No 178/2002<sup>2</sup>. Regulation (EC) No 2073/2005 uses one index: *Enterobacteriaceae* as an index of the potential presence of *Enterobacter sakazakii*.

More generally, although other indexes may be useful for food operators, their significance should be assessed on a case-by-case, workshop-by-workshop basis (e.g.: *Listeria* spp. for *L. monocytogenes*).

To date, there are no satisfactory indexes for viruses or parasites.

### The required qualities of microorganism indicators

An ideal microorganism indicator should (Jay 2000):

- be present and detectable in foods for which process hygiene must be monitored,
- have a concentration level inversely correlated to the process hygiene,
- be easy to detect and/or count and easy to distinguish from other microorganisms,
- be able to be detected and/or counted quickly, preferably during the working day,
- not have its growth slowed or inhibited by the rest of the microflora.

In addition, for faecal contamination, microorganism indicators should (Bornert 1998):

- be present constantly and specifically in the digestive tract of humans and animals,
- be abundant in faecal matter, to enable the detection of low contamination,
- not be able to multiply in water, food or the environment,
- resist technological treatments in a similar way to faecal pathogenic microorganisms.

No indicator has all of these ideal qualities. Moreover, the effectiveness of a microbiological criterion applied to a batch depends above all on the detection limit and proportion of false-negatives (sensitivity) and false-positives (specificity) of the analysis technique, the value of the chosen microbiological limit and the sampling plan. The microbiological criteria traditionally used are not particularly severe: the probability of not detecting unacceptable batches (consumer risk) and the probability of rejecting acceptable batches (producer risk) are high. But the systematic use of these microbiological criteria by all producers, year in year out, ensures an improvement of microbiological quality in the long run. Accordingly, the microbiological criteria described in this document have long since proven their usefulness.

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<sup>2</sup> [Regulation](#) (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

### Main indicators

The following table gives the list of main indicators used in France in most food sectors (excluding drinking water), and the possible interpretation of their presence or presence in excess amounts. The list does not claim to be exhaustive. Other indicators may be considered in the future.

The table indicates the appropriate analysis methods, which are ISO methods associated with standard ISO 7218<sup>3</sup> when these exist. Strictly national NF methods can be used as long as AFNOR keeps them in force. The rapid methods validated according to standard EN ISO 16140<sup>4</sup>, as well as international protocols used prior to application of the latter, can also be used. If an operator wants to use other methods, he must prove that these are at least equivalent to the aforementioned methods. The protocol of standard EN ISO 16140 is recommended for this demonstration.

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<sup>3</sup> standard ISO 7218 "Microbiology of food and animal feedingstuffs – General requirements for microbiological examinations"

<sup>4</sup> standard EN ISO 16140 "Microbiology for food and animal feedingstuffs – Protocol for the validation of alternative methods"

Table 1: list of main indicators used in France in most food sectors (excluding drinking water)

Indicator	Analysis technique	Interpretation	Main foods concerned (non-exhaustive list) - comments
Aerobic microorganisms growing at 30°C or “mesophilic aerobic flora”	NF V 08-051 NF EN ISO 4833	<ul style="list-style-type: none"> <li>Indicates a general level of hygiene and/or alteration flora</li> <li>Reflects the product’s history (poor management of the temperature/duration combination, disruption of the cold chain)</li> <li>This flora can comprise/include bacteria that multiply at fridge temperature</li> </ul>	<ul style="list-style-type: none"> <li>Most products, but must be interpreted particularly when a technological lactic flora is present</li> <li>For only slightly contaminated products, this indicator can be useful, even if it does not indicate the type of failings observed</li> </ul>
Lactic bacteria growing at 30°C	ISO 15214	<ul style="list-style-type: none"> <li>Lactic flora is found in the composition of mesophilic flora, but not always detected by the mesophilic flora counting method</li> <li>Alteration flora for products whose technological flora does not comprise any</li> <li>Flora related to long storage</li> </ul>	<ul style="list-style-type: none"> <li>Fermented products are of no concern</li> <li>Large pieces of refrigerated meat that is vacuum-packed or packaged in a protective atmosphere and has a shelf-life of several weeks or months, when the analysis focuses on the surface of the product</li> </ul>
<i>Brochothrix thermosphacta</i>	NF V 04-505 ISO 13722	<ul style="list-style-type: none"> <li>Alteration flora, indicates environmental contamination, not controlled by technological treatments</li> <li>Multiplies from 2°C</li> </ul>	<ul style="list-style-type: none"> <li>Meat</li> </ul>
<i>Pseudomonas</i>	NF V 04-504	<ul style="list-style-type: none"> <li>Alteration flora, indicates environmental contamination, not controlled by technological treatments</li> <li>For products that are vacuum-packed or packaged in a protective atmosphere: indicates the presence of oxygen (poor vacuum, leak)</li> <li>There are many species that multiply/ at fridge temperature</li> <li>Cardinal temperatures: 4-42°C</li> </ul>	<ul style="list-style-type: none"> <li>Meat</li> <li>For meat stored in the presence of oxygen, this indicator indicates the storage conditions. Storage for too long or inappropriate conditions cause marked growth of this flora. In addition, for meat packed without oxygen, <i>Pseudomonas</i> clearly identify a packaging defect (presence of oxygen)</li> </ul>

Yeasts and moulds	XP V 08-059 NF ISO 7954	<ul style="list-style-type: none"> <li>Indicates environmental contamination, not controlled by technological treatments</li> <li>Alteration flora</li> <li>There are many species that multiply at fridge temperature</li> </ul>	<ul style="list-style-type: none"> <li>All products except fresh fruit and vegetables, cheese (excluding fresh cheese), starters of bakery products and raw croissant-type pastries</li> <li>To be monitored for processed vegetables</li> </ul>
<ul style="list-style-type: none"> <li>Bacillus in the broad sense of the term</li> </ul>	XP V 08-058 NF EN ISO 7932	<ul style="list-style-type: none"> <li>Indicates environmental or telluric contamination, not controlled by technological treatments</li> <li>Multiplies between 5 and 55°C</li> </ul>	<ul style="list-style-type: none"> <li>Dishes simmered at below 63°C (rice for example)</li> <li>Long shelf-life cooked dishes when the cold chain has not been respected</li> </ul>
<p>a – Enterobacteria growing at 30°C (commonly and wrongly called enterobacteria in general)</p> <p>b – Coliforms growing at 30°C (commonly and wrongly called total coliforms or coliforms 30)</p> <p>c – Thermotolerant coliforms growing at 44°C (commonly and wrongly called faecal coliforms, thermotolerant coliforms or coliforms 44)</p> <p>d - <i>Escherichia coli</i> with beta-glucuronidase and growing at 44°C (commonly and wrongly called <i>E. coli</i>, although there are <i>E. coli</i> without glucuronidase)</p>	<p>a. NF ISO 21528-2 or possibly NF V 08-054</p> <p>b. NF V 08-050</p> <p>c. NF V 08-060</p> <p>d. NF V 08-053, NF ISO 16649-2</p>	<ul style="list-style-type: none"> <li>Indicators associated mainly with human or animal faecal contamination, as well as environmental contamination, not controlled by technological treatments</li> <li>In order of increasing faecal specificity: enterobacteria growing at 30°C, coliforms growing at 30°C, thermotolerant coliforms growing at 44°C, <i>Escherichia coli</i> with beta-glucuronidase and growing at 44°C. Analyses detecting enterobacteria growing at 30°C or coliforms growing at 30 or 44°C are not characteristic enough of faecal contamination. Accordingly, raw vegetables (parsley or tomatoes for example) usually carry coliforms growing at 30 or 44°C</li> <li><i>E. coli</i> multiplies between 7 and 46°C</li> </ul>	<ul style="list-style-type: none"> <li>All handled products</li> <li>Although enterobacteria growing at 30°C are a very imprecise indicator, their use as indicator is useful, especially since, on some products, the more specific indicators (e.g.: <i>E. coli</i> with beta-glucuronidase and growing at 44°C) are extremely few and therefore uncountable. The choice between enterobacteria growing at 30°C, coliforms growing at 30°C, thermotolerant coliforms growing at 30°C and <i>E. coli</i> with beta-glucuronidase and growing at 44°C should be based on the step in the food chain being considered and product type</li> </ul>
Staphylococci with coagulase	NF V 08-057-1 or NF V 08-057-2 NF EN ISO 6888-1 and A1 or NF EN ISO 6888-2 and A1	<ul style="list-style-type: none"> <li>Indicate contamination by personnel (dirty hands or skin, nose or throat infection) or by contaminated milk (mastitis).</li> <li>There are many healthy carriers</li> <li>Multiplies between 7 and 45°C, does not produce toxin below 10°C</li> </ul>	<ul style="list-style-type: none"> <li>All handled products</li> </ul>

<p>a. Sulphite-reducing bacteria growing in anaerobiosis</p> <p>b. <i>Clostridium perfringens</i></p>	<p>a. XP V 08-061 and NF ISO 15213</p> <p>b. NF V 08-056 and NF EN ISO 7937</p>	<ul style="list-style-type: none"> <li>• Both these indicators indicate telluric contamination, not controlled by technological treatments</li> <li>• <i>Clostridium perfringens</i> is found in the composition of sulphite-reducing flora. The same indicator should always be sought. <i>Clostridium perfringens</i> is preferable as it is normally found in the digestive tract of animals and can cause health problems</li> <li>• Counts of sulphite-reducing bacteria growing in anaerobiosis at 46°C (XP V 08-061) may be an alternative to <i>C. perfringens</i> counts.</li> <li>• <i>C. perfringens</i> multiplies between 10 and 51°C</li> </ul>	<ul style="list-style-type: none"> <li>• Dishes kept for too long between 10 and 63°C after cooking (beef tongue in sauce, poultry, pulses)</li> <li>• Vacuum-packed products with a long microbiological life (if the cold chain is insufficiently controlled)</li> </ul>
<p>Total volatile basic nitrogen and trimethylamine</p>	<p>Regulation (EC) No 853/2004 of 29 April 2004                  Regulation (EC) No 854/2004 of 29 April 2004                  Regulation (EC) No 2074/2005 of 5 December 2005</p>	<ul style="list-style-type: none"> <li>• Increases during bacterial growth, indicates product freshness</li> <li>• The values adopted in the Regulation are emission limit values: reference should be made to other values for use as an indicator of process hygiene</li> </ul>	<ul style="list-style-type: none"> <li>• Flesh of fish and cephalopods</li> </ul>
<p>Activity of alkaline phosphatase</p>	<p>Order of 5 February 1980 (OJ of 10 April 1980) or NF EN ISO 11816-1 (classification index: V04-054-1)</p>	<ul style="list-style-type: none"> <li>• Heat-sensitive enzyme whose inactivation indicates pasteurisation (heating for at least 15 seconds at 72°C)</li> </ul>	<ul style="list-style-type: none"> <li>• Cheese, processed cheese, milk and dairy products</li> </ul>

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### Conclusion

These are the elements that the French Food Safety Agency is able to present to the Directorate General for Food and Directorate General for Competition Policy, Consumer Affairs and Fraud Control for passing on to the professionals concerned.

Key words: microbiological criteria, foodstuffs, hygiene package, microbial flora, process hygiene indicators

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