

# Hygiene Indicators in Food Microbiology

## Definitions

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A **hygiene microbiological indicator** (or hygiene process criteria) is a single microorganism/group of microorganisms (which may/may not be pathogenic) or a metabolic product, reveals a potential problem of quality, hygiene and even safety given a minimum level of contamination (or dose).

It may comprise the following:

- potential presence of a consumer health hazard : risk of presence of a pathogen germ or a toxic metabolite (toxins);
- non-compliance to Good Hygienic / Manufacturing Practices (GHPs/GMPs), of storage (cold chain, warm chain) and distribution;
- initial contamination of raw materials;
- commercial risk : rapid spoilage and/or alteration/deterioration of organoleptic quality of food products.

**Safety microbiological criteria** define if a food product is fit for human consumption or not. Unlike them, an out of specifications Hygiene process criterion does not necessarily mean that the food product is unfit to human consumption and does not lead automatically to its withdrawal or its recall, but rather to the implementation of appropriate corrective actions, as for example the review of GMPs, HACCP system or the process itself.

Another term, **Index**, is employed suggesting a health risk, gives an indication of the likelihood of the presence of pathogens. For example, *E.coli* is an index for *Salmonella* or other pathogenic germs of fecal origin in drinking water. As hygiene process criteria, an out of specifications result does not allow to conclude that the food product is unfit to human consumption.

Some microorganisms can play a multiple role: both as an index (presence of a health risk for human) and as an indicator of poorly controlled process, an issue during the process (too long storage at inappropriate temperatures, insufficient heat treatment, etc...).

Microbiological indicators are naturally present in food products. For purposes of process validation, indicators may be artificially introduced as inocula. Called **surrogates**, indicators usually replace  
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pathogens in order to study the efficiency of treatments, processes, etc. For example, *Clostridium botulinum* are frequently replaced by *Clostridium sporogenes* (historically known to be non-pathogenic) in low-acid canning industry to validate the thermal treatment process.

## *Why indicators ?*

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In Europe, according to the current regulation (EC 2073/2005), manufacturing, storage and distribution processes have to be assessed. Indicators can be used as a tool for process assessment as part of preventive approaches within HACCP plans or GMPs.

Conversely, some pathogenic microorganisms show a low prevalence and/or a low concentration in food products due to the efficiency of procedures implemented by industrials for hygiene control. The effect of low prevalence results in:

- difficulty to detect the pathogen (effect of sampling);
- lack of sensitivity of current methods for some microorganisms;
- absence of detection methods, especially emerging microorganisms;
- no enumerative data to exploit.

“Better” detection methods can also be too expensive or technically complicated to be used routinely.

**So, hygiene indicators are really complementary to safety criteria.**

## *How to choose them ?*

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Some process hygiene indicators are established by the regulations (e.g., Regulation EC 2073/2005 modified by 1441/2007).

Other process hygiene indicators not established by the regulations are defined by specific industry sectors. In France, these criteria are available on DGAI (Direction Générale de l’Alimentation) website for meat products and delicatessen, poultry, catering and retailer sectors as **Good practice guides**.

But it is for each manufacturing site to define relevant indicators according to established critical points, product type, processes, and history.

The application stage has also to be defined, so that the indicator takes on full significance, gives a real information.

In practice, the ideal indicator has the following features :

- it is usually **non-pathogenic**;
- it must be **systematically present** (even if only at **low level**) and can be detected;
- it must be detectable, preferably **countable** in a **simple, fast and inexpensive** way;
- it should have the same **habitat** as the target pathogenic agent.

Once chosen, it is generally regarded as good practice to maintain the same indicator organism(s) for purposes of evaluating process control.

## Types and use of Indicators

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The approach to use indicators may be different depending on the country of use. In Europe (Regulation EC 2073/2005) and in US (Legislation 9 CFR, ...), for example, common process indicators comprise the following:

→ **Total aerobic count at 30°C / Total viable count:** Indicator of the global hygiene level of the product, and its history from manufacturing to the distribution, may also be an indicator of the contamination level incoming raw material. An incubation at 30°C facilitates the growth of psychrophilic microorganisms preferring cool environments. o The technological flora, intentionally added or part of the natural flora of the product, should not be taken into account.

→ **E.coli:** understanding  $\beta$ -glucuronidase positive *E.coli*, growing at 44°C, give a more precise information about potential contamination with animal fecal material and potential presence of pathogens of fecal origin.

→ **Salmonella:** pathogen gram-negative bacteria, non-lactose fermenting, belong to *Enterobacteriaceae* family. Indicator used especially for cattle, sheep, goats, equine and poultry carcasses to assess hygiene during the slaughter. However, for US, *Salmonella* can be an indicator but is first a safety indicator : if *Salmonella* is found in a product that is linked to illnesses, then the product is not sold or must be recalled.

### Specific indicators in US :

→ **Coliforms:** Particularly those of fecal origin, gram negative bacteria, lactose fermenting, belong to *Enterobacteriaceae* family.

→ **Listeria spp:** Gram-positive rod-shaped bacteria, able to grow at refrigerated temperatures, are used as indicators for:

- the potential presence of *Listeria monocytogenes*, the pathogen species;
- the level of contamination of the food processing environment : cleanliness of equipment, surfaces...

### Specific indicators in Europe

While US continue to use coliforms as indicators, the current trend in Europe is to use the entire family *Enterobacteriaceae*.

→ **Enterobacteriaceae** : family gathering a large number of Gram negative bacteria, ubiquitous in the environment, in which take part coliforms (lactose fermenters), fecal coliforms and *E.coli*, are indicators of :

- **Fecal contamination** of human or animal origin (found in intestinal tracts);
- **Environmental contamination** (soil, dust, water, insects) and contaminated equipment (surfaces inadequately cleaned or sanitized).

This family is the indicator the most commonly used because relatively sensitive to standard treatments implemented to inactivate microorganisms in food (heat, ionizing radiation, freezing, drying, extremes of pH, and water activity) and in the environment (destroyed by most of disinfectants).

→ **Coagulase positive staphylococci**: including but not limited to *S.aureus*. Indicators of good handling procedures and contamination from workers (e.g., dirty hands, skin, nose or throat infections). It should be noted that “healthy” workers may also be carriers of staphylococci. High numbers of staphylococci in foods may indicate a potential presence of enterotoxins responsible for food poisoning. Regulation EC 2073/2005 requires in this case the detection of staphylococcal enterotoxins.

→ **Bacillus of the cereus group**<sup>3</sup>: these ubiquitous bacteria, of telluric origin (soil), are indicators for the following:

- raw material hygiene: spores are found almost in all foods and especially in dry or dehydrated products such as spices, aromatic herbs, some vegetables, cereals, flours. These raw materials component of finished products are all potential sources of contamination;
- efficiency of cleaning processes: spores of *Bacillus cereus* have strong capacities of adhesion to stainless surfaces and can accumulate in manufacturing equipment which then becomes spores reservoirs;
- insufficient thermal treatment or storage at inappropriate temperatures that allow their growth after thermal treatment : the most cases reported were for rice, potatoes, pastas, milk powder.

In sectors, some specific microorganisms can be used as spoilage indicators, such as:

- Lactic acid bacteria in deli meats
- Yeasts and molds in yogurt,
- *Pseudomonas* in fishes, poultry, dairy products
- *Alicyclobacillus* in beverages and fruit juices
- SRB (sulfate Reducing bacteria) and/or *Clostridium perfringens* in cooked products (ready-cooked dishes, cooked meats...)

These microorganisms are specific to given matrices because in some food products they take part of the normal flora of the product. For example, lactic acid bacteria are not relevant in dairy products nor in ready cooked dishes containing cheese, and fermented food (sauerkraut).

### **Sources and to learn more :**

1. AFSSA (ANSES) – Avis de l’Agence française de sécurité sanitaire des aliments relative à la demande de création de documents de référence concernant des flores microbiennes utilisables en tant qu’indicateurs d’hygiène des procédés. 2007
2. AFSSA (ANSES) – Recommandations pour l’élaboration de critères microbiologiques d’hygiène des procédés. 2008
3. ANSES – Fiches de description de danger transmissible par les aliments – *Bacillus cereus* – Septembre 2011
4. EFSA – Technical specifications on harmonized epidemiological indicators for biological hazards to be covered by meat inspection of poultry. 2012
5. Règlement CE 2073/2005 de la Commission du 15 Novembre 2005 concernant les critères microbiologiques applicables aux denrées alimentaires. <http://eur-lex.europa.eu/homepage.html>
6. DGAI (Direction Générale de l’Alimentation) – Critères d’hygiène des procédés - <http://agriculture.gouv.fr/Criteres-microbiologiques-hygiene-des-procedes>

7. FDA (Food and Drug Administration) - Grade A Pasteurized milk ordinance – <http://www.fda.gov/downloads/Food/GuidanceRegulation>
8. Code of Federal Regulations - 9 CFR 310.25 – Contamination with microorganisms; Process control verification criteria and testing; pathogen reduction standards. <http://www.gpo.gov/>
9. <http://www.foodsafetymagazine.com/magazine-archive1/februarymarch-2011/indicator-organism-assays-chaos-confusion-and-criteria/>

Rapid methods BIOMERIEUX certified according to ISO 16140 standard :

**(Non-exhaustive list of solutions dedicated to Hygiene indicators)**

**BIOMERIEUX – BACARA™ (Bacillus cereus Rapid Agar) - AFNOR Certification n°AES 10/10 – 07/10 –**  
Chromogenic and selective medium for the enumeration of presumptive *Bacillus cereus* in all human food and animal feeding stuffs (end of validity: 02/07/2014)

**BIOMERIEUX – chromID Coli agar - AFNOR Certification n°BIO 12/20 – 12/06 –** Chromogenic and selective medium for the enumeration of coliforms and  $\beta$  Glucuronidase positive *E.coli* from food samples - Validated for the enumeration of Coliforms at 37°C (end of validity: 14/12/2014)

**BIOMERIEUX – chromID Coli agar - AFNOR Certification n°BIO 12/19 – 12/06 –** Chromogenic and selective medium for the enumeration of coliforms and  $\beta$  Glucuronidase positive *E.coli* from food samples - Validated for the enumeration of  $\beta$  Glucuronidase positive *E.coli* at 37°C (end of validity: 14/12/2014)

**BIOMERIEUX – chromID Coli agar - AFNOR Certification n°BIO 12/5 – 01/99 –** Chromogenic and selective medium for the enumeration of coliforms and  $\beta$  Glucuronidase positive *E.coli* from food samples - Validated for the enumeration of  $\beta$  Glucuronidase positive *E.coli* at 44°C (end of validity: 14/12/2014)

**BIOMERIEUX – REBECCA™ BASE OU/OR REBECCA™ + EB - AFNOR Certification n°AES 10/06-01/08 –**  
Chromogenic and selective medium for the enumeration of *E.coli* from all human and animal food products – Renewal 01/12/2011 (end of validity: 17/01/2016)

**BIOMERIEUX – REBECCA™ + EB - AFNOR Certification n°AES 10/07-01/08 –** Chromogenic and selective medium for the enumeration of *Enterobacteriaceae* from all human and animal food products (end of validity: 17/01/2016)

**BIOMERIEUX – ALOA® One day – AFNOR Certification n°BIO 10/03-09/00 –** Chromogenic and selective medium for the detection of *Listeria spp* and *Listeria monocytogenes* from all human food products and environmental samples (end of validity: 27/09/2016)

**BIOMERIEUX – VIDAS® *Listeria* (LIS) – AFNOR Certification n°BIO 12/2-06/94 –** Detection of *Listeria spp* from all human food products and environmental samples. Immuno-enzymatic test. (end of validity: 09/06/2018)

**BIOMERIEUX – VIDAS® *Listeria* species Xpress (LSX) – AFNOR Certification n°BIO 12/12-07/04 –** Detection of *Listeria spp* from milk products, meat products, vegetable products and environmental samples. Immuno-enzymatic test. (end of validity: 01/07/2016)

**BIOMERIEUX – VIDAS® *Listeria* LDUO – AFNOR Certification n°BIO 12/18-03/06 –** Detection of *Listeria spp* and *Listeria monocytogenes* from all human food products and environmental samples. Immuno-enzymatic test. (end of validity: 09/03/2018)

**BIOMERIEUX – VIDAS® UP LISTERIA – AFNOR Certification n°BIO 12/33-05/12 –** Detection of *Listeria spp* from all human food products and production environmental samples. Immuno-enzymatic test. (end of validity: 10/05/2016)

**BIOMERIEUX – TEMPO® EC – AFNOR Certification n°BIO 12/13-02/05** – Enumeration of *E.coli* from all human food and pet food products, with the exception of beverages and cattle feed products. (end of validity: 04/02/2017)

**BIOMERIEUX – TEMPO® EB – AFNOR Certification n°BIO 12/21-12/06** – Enumeration of Enterobacteriaceae from all human food and animal food products except beverages and cattle food. (end of validity: 15/12/2014)

**BIOMERIEUX – TEMPO® AC – AFNOR Certification n°BIO 12/35-05/13** – Enumeration of *mesophilic aerobic flora* from all human food and pet foods and environmental samples. (end of validity: 23/05/2017)

**BIOMERIEUX – TEMPO® TVC – AFNOR Certification n°BIO 12/15-09/05** – Enumeration of *total viable count* from all human and animal food products (except beverages and cattle feed) (end of validity: 19/09/2017)

**BIOMERIEUX – TEMPO® TC - AFNOR Certification n°BIO 12/17 – 12/05** – Enumeration of total *Coliforms* in food and pet food products, with the exception of beverages and cattle feed (end of validity: 09/12/2017)

**BIOMERIEUX – TEMPO® STA - AFNOR Certification n°BIO 12/28 – 04/10** – Enumeration of *coagulase positive staphylococci* in all human food products and animal feeding (end of validity: 01/04/2018)

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