NEW PUBLICATION OF THE EN ISO 7218 STANDARD IN OCTOBER 2008

ROUND-UP OF THE CHANGES OBSERVED IN RELATION TO THE LAST VERSION OF 1996 AND ITS AMENDMENT OF 2001

The new version of the ISO 7218 standard, which is considered as the basic document in food microbiology, was published at the end of 2007. This standard was elaborated by an ISO work group, for which Alexandre LECLERCQ (Pasteur Institute Paris) is the project manager.
The aim of this article is to underline the most important modifications operated during this revision, the principal change being the possibility, for reference methods, to use one dish per dilution.
However, upon publication of this standard, it appeared that modifications were necessary. An amendment is thus being prepared to provide precisions, notably concerning the conditions of use of one dish per dilution in reference methods and in the calculation and the expression of the results.

ISO 7218: 1996	CHAPTERS (ISO 7218 2007)	ISO 7218: 2007
Food microbiology – **General rules for microbiological examinations.** Many rules were required and others recommended, but their distinction was not formal.	**TITER**	Food microbiology – **General requirements and recommendations** **Recommendations and requirements are clearly expressed:** - The recommendations are in « Times New Roman » font and begin by: « it is recommended » - The requirements are in « Arial » font
1 Application field No details	**1 Application field**	Specify particularly: - Three main uses: the implementation of standards, good laboratory practices and laboratory accreditation . - **Examination of yeasts and moulds**. - The standard applies to food microbiology, animal feeding stuffs, the food production environment and **the primary production environment** (new).
2 Standard reference Only one reference: ISO 6887 (dilutions)	**2 Standard references**	9 standard references: ISO 835, ISO 8199, ISO 8261, ISO 8655-1, ISO/TS 11133 (all the parts), ISO 16140, ISO/TS 19036 and ISO 22174 were added.
3 Premises	**3 Premises**	Were added: - The requirement **to separate sample examination from the primary production** (especially for the reception and preparation of the samples) - **Safety considerations:** Laboratories are classified in risk categories from 1 to 4 according to the risk for the technician and the collectivity. It is specified in this standard that the layout of the premises corresponds to the examination of the microorganims belonging to the risk categories 1, 2 and 3. *It must be noted that the parts describing the layout of the premises, the cleaning and the disinfection are recommendations.*
5 Personnel Mouth pipetting was proscribed only in the case of handling pathogenic germs (chap. 9.1)	**4 Personnel**	Practically the same requirements are requested for the hygiene of the personnel, an important point was added for the technician: **« mouth pipetting is to be proscribed »**

4 Material and equipment **17 instruments or types of material are described**	**5 Apparatus and material**	- In the generalities, it is specified that the frequency of calibration and verification must be determined by each laboratory. - **29 instruments or types of material are described**: for each type of instrument or material, the « Maintenance and control » section was divided into 2 parts : « Cleaning and disinfection » and **« verification »** this last part is more detailed than in the 1996 version.
6 Material preparation	**6 Glassware and material preparation**	Few modifications: a few more details and a **« waste control »** section was added. In the preamble, it is noted that: « The correct elimination of the contaminated materials does not have direct consequences on the quality of sample analysis, but is a part of good laboratory management. »
7 Preparation and sterilisation of culture media and reagents Part widely developed	**7 Preparation and sterilisation of culture media**	In this chapter, only **ISO/TS 11133-1 and ISO /TS 11133-2 standards** are indicated.
8 Samples for the laboratory	**8 Samples for the laboratory**	Few modifications in relation to the 1996 version. *It can be noted that the transport and storage temperatures, and the time limits for analysis are recommendations and not requirements.*
9 Examination techniques and expression of the results	**9 Examination**	
9.1 Sanitary precautions during analysis	**9.1 Sanitary precautions during analysis**	This part was slightly rearranged with less details and a few minor precautions added concerning for example the manipulation of powder products or the sterility of the instruments.
9.2 Preparation of the first suspension and the dilutions	**9.2 Preparation of the first suspension and the dilutions**	A part **« concentration »**, divided into a first part « centrifugation or filtration on membrane » and a second part « immunoseparation », was added.
9 Examination techniques and expression of the results	**10 Enumeration**	A part **« generality »** was added. It specifies that numerous enumeration methods exist but **this standard only deals with the enumeration on solid and in liquid media.**
9.3 Enumeration using solid medium **- For superfusion: water bath at 47°C +/-2°C**	**10. 2 Enumeration on solid medium**	**- Addition of part 10.2.2: Number of Petri dishes per dilution: « A dish per dilution must be used with at least two successive dilutions for the laboratories working under quality assurance according to ISO 17025 principles.** If only one dilution is performed or if the laboratory do not work under quality assurance, two dishes must be used according to ISO 8199 standard. » *It can be noted that the ISO 8199 standard is a standard on the quality of water.* **- For superfusion: water bath at 44 - 47°C** *To take into account IDF standards (45 +/- 1°C)* **- Addition of part 10.4.3: spiral methods**

- The storage was at maximum 24 h.		**- Addition of the possibility to refrigerate the dishes inoculated for a maximum of 24 h before incubation**, ensuring that this practice has no incidence on the results. **- Possibility to store the dishes for a maximum of 48 h in a fridge before reading.**
9.3.4 and 9.3.5 REPLACED BY AMENDMENT 1 2001 **- At least one dish containing at least <u>15 colonies.</u>** **- When the dish contains less than 15 colonies: indicate « number estimated »** - This chapter did not exist 9.3.6 Confidence Limits	**10.3 Calculation and expression of the results on solid medium**	- The amendment 1 is the basis of this part but **the expression of the results in the case of two dishes was completely eluded.** **- At least one dish containing at least <u>10 colonies</u> is necessary.** *This limit is used in the IDF standards.* **- Two cases:** **1) When the dish contains less than 10 colonies: to indicate « number estimated »** **2) If the number of colonies is between 1 and 3: to indicate « the microorganism is present, but < 4/d per g or ml »** where d= dilution rate. *« 4 x d » is notified in the standard but it is an error.* **- New part: 10.3.2.6 Measure of the uncertainty**, which refers to ISO/TS 19036 standard. The part « Confidence Limits » was not integrated in this new version.
This part did not exist	**10.4 Enumeration of yeasts and moulds**	This part contains generalities on the handling of yeasts and moulds.
9.4 Enumeration by use of a liquid medium	**10.5 Enumeration in liquid medium**	This part was developed notably concerning the choice of the inoculation system to be implemented and by the addition of mathematical expressions to calculate the NPP values and for the estimation of the accuracy.
9.5 Detection method	**11 Detection method**	Part almost identical.
9.6 Basic identification techniques - Possibility to use commercialised biochemical galleries	**12 Confirmation method**	- The example of an isolation technique is no longer given. - The GRAM coloration is still described, but the catalase and oxydase tests are not anymore. - It is possible to use commercialised biochemical galleries for identification, **nucleic borers** and **serological methods of antigen-antibody agglutination or latex tests.**
	13 Test report **14 Validation** **15 Quality assurance**	New chapters developed little. In part 13, all the elements necessary for a test report are specified. Part 14 refers to the validations of the alternative (ISO 16140), reference and internal methods (reflection in process within ISO). Part 15 gives information on the internal control, the reference strains, and the external evaluation of the quality (proficiency test).

BIBLIOGRAPHY	BIBLIOGRAPHY	
Annex A: Limit of the confidence interval for the estimation of small numbers (normative) Annex deleted	**Annex A: Properties of certain disinfectants** (informative)	5 types of disinfectant, the types of organism against which they are active, which substances can inactivate them and their toxicity for humans are presented in the table of this new part.
Annex B: MPN tables (normative) Table given only for 3 tubes per dilution for 3 successive dilutions.	**Annex B: Determination of the most probable number (MPN)** (normative)	MPN tables given for 10, 15, 20, 25 tubes of 1 dilution; and for 3 tubes and 5 tubes per dilution for 3 successive dilutions.

AMENDMENT 1: 2001	AMENDMENT	AMENDMENT IN PROCESS:
Many parts are reviewed to give precisions and examples: **9.3.4: Counting of colonies** **9.3.5: Expression of the results**		A work group of the commission V08B was created to help laboratories in interpreting the standard. This group, animated by M. ETIEVANT (LDA 58), met in January 2008 and worked more particularly on the following parts: **10.2.2: Number of Petri dishes per dilution**: to clarify the cases of using one or two dishes per dilution. **10.3: Calculation and expression of the results on solid media**: to rectify many errors and specify the calculations for one or two dishes per dilution and for the case of small numbers. Certain parts of the ISO 7218 standard 1996 version, which were put aside, will probably be added.: - Formula of part 9.3.6.1 « confidence limits » - Table in annex A « Limit of the confidence interval for the estimation of small numbers »

In italics: author's notes

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