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ISO 6888-2

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AMENDMENT 1
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Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) —

Part 2:
Technique using rabbit plasma fibrinogen agar medium

AMENDMENT 1: Inclusion of precision data

*Microbiologie des aliments — Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus *et autres espèces) —*

Partie 2: Technique utilisant le milieu gélosé au plasma de lapin et au fibrinogène

AMENDEMENT 1: Inclusion des données de fidélité

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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

Amendment 1 to ISO 6888-2:1999 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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Page iv

Introduction, Subclause 0.2

Replace part of the second paragraph by the following text.

"Both parts of ISO 6888 are given equivalent status. Nevertheless, it is recommended to use the procedure described in ISO 6888-2 for the foods (such as cheeses made from raw milk and certain raw meat products) likely to be contaminated by:"

Page 6

Replace Clause 11 by the following text.

11 Precision

11.1 General

The precision of quantitative methods can be expressed in terms of repeatability and reproducibility, as defined in ISO 5725-2. However, the method of calculation used in ISO 5725-2, based on the mean, is not always appropriate for microbiological analyses, which do not always show a normal (Gaussian) distribution. Therefore ISO 16140, which has been especially developed for microbiological methods and which uses robust estimators for calculating repeatability and reproducibility, has been followed. These statistics have the advantage of being less sensitive to extreme values, thus permitting outliers by statistical tests to be retained. These estimators are therefore used in this part of ISO 6888.

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given. Precision data were determined using three types of food contaminated at various levels and for reference materials. Factors such as the strain considered, the competitive flora and the physiological status of target and competitors have an influence on the precision values.

11.2 Repeatability

11.2.1 Repeatability limit

The absolute difference between two single ($\log_{10}$-transformed) test results (number of coagulase-positive staphylococci per gram or per millilitre) or the ratio of the higher to the lower of the two test results on the normal scale, obtained using the same method on identical test material by the same operator using the same apparatus within the shortest feasible time interval, will in not more than 5 % of cases be greater than the repeatability limit (r).

11.2.2 Overall values

As a general indication of the repeatability limit (r), the following values can be used when testing food samples in general. These values of r are general means for all matrices considered:

$r =$ 0,22 (expressed as an absolute difference between $\log_{10}$-transformed test results), or

$r =$ 1,7 (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

For reference materials (capsules containing approximately 5 000 CFU, see Annex A), the following values can be used:

$r =$ 0,17 (expressed as an absolute difference between $\log_{10}$-transformed test results), or

$r =$ 1,5 (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

EXAMPLE A first test result of 10 000 or $1,0 \times 10^4$ of coagulase-positive staphylococci per gram of food was observed. Under repeatability conditions, the ratio of the higher to the lower test result should not be greater than 1,7. Therefore the second result should be between 5 882 (= 10 000/1,7) and 17 000 (10 000 × 1,7) coagulase-positive staphylococci per gram.

11.3 Reproducibility

11.3.1 Reproducibility limit

The absolute difference between two single ($\log_{10}$-transformed) test results (number of coagulase-positive staphylococci per gram or per millilitre) or the ratio of the higher to the lower of the two test results on the normal scale, obtained using the same method on identical test material in different laboratories with different operators using different apparatus, will in not more than 5 % of cases be greater than the reproducibility limit (R).

11.3.2 Overall values

As a general indication of the reproducibility limit (R), the following values can be used when testing food samples in general. These values of R are general means for all matrices considered:

$R =$ 0,33 (expressed as a difference between $\log_{10}$-transformed test results), or

$R =$ 2,2 (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

For reference materials (capsules containing approximately 5 000 CFU, see Annex A), the following values can be used:

$R =$ 0,31 (expressed as a difference between $\log_{10}$-transformed test results), or

$R =$ 2,0 (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

EXAMPLE 1 A test result of $1,0 \times 10^4$ coagulase-positive staphylococci per gram of food product was obtained by a first laboratory. Under reproducibility conditions, the ratio of test results of the first and second laboratory should not be greater than 2,2. Therefore the result of the second laboratory should be between $4,5 \times 10^3$ ($= 1,0 \times 10^4/2,2$) and $2,2 \times 10^4$ ($= 1,0 \times 10^4 \times 2,2$) coagulase-positive staphylococci per gram.

EXAMPLE 2 A laboratory wants to know the maximum level it may find that is still in compliance with a pre-set limit (for example a limit of 10^5 or 5 in $\log_{10}$). For this the R value (on the log scale) has to be multiplied by a factor of 0,59. This value is 0,19 ($0,33 \times 0,59$) as a difference between $\log_{10}$-transformed test results or $10^{0,19}$ as a ratio between test results. Therefore results up to $\log_{10}$ 5,19 ($\log_{10} 5 + \log_{10} 0,19$) or $1,55 \times 10^5$ do not indicate non-compliance with the limit. The factor 0,59 reflects the fact that a test with a one-sided 95 % interval is used to test whether the limit is exceeded. The factor 0,59 is obtained from the following formula:

$$0,59 = \frac{1,64}{1,96 \times \sqrt{2}}$$

Page 6

Add the following Annex A after Clause 12.

Annex A
(informative)

Results of the interlaboratory study

An international interlaboratory study was organized by the Laboratory for Study and Research on Hygiene and Quality of Food (LERHQA) of the French Food Safety Agency (AFSSA) in 1999, in the frame of the European project SMT CT 96 2098 (see reference [7]). This study involved 24 laboratories in 16 countries in Europe and was carried out on cheese, meat, egg powder and a reference material. The food samples were each tested at three different levels of contamination with coagulase-positive *Staphylococcus*, plus a negative control.

The precision data derived from this interlaboratory study are shown with respect to each sample type in Tables A.1 to A.4. They have been calculated using robust statistics, as described in ISO 16140. Data obtained by some laboratories have been excluded from the calculations when it was known that they deviated from the specified study protocol.

Table A.1 — Results of data analysis obtained with cheese samples

Sample/level of contamination	Cheese low level	Cheese medium level	Cheese high level
Number of laboratories having returned results	19	19	19
Number of samples per laboratory	2	2	2
Number of laboratories retained after eliminating outliers	18	18	18
Number of accepted samples	36	36	36
Median value (in $\log_{10}$ CFU/g)	3,25	5,03	6,00
Repeatability standard deviation, s_r (in $\log_{10}$ CFU/g)	0,09	0,04	0,06
Repeatability relative standard deviation (%)	2,74	0,88	0,98
Repeatability limit (r), as difference on $\log_{10}$ scale (in $\log_{10}$ CFU/g)	0,25	0,13	0,17
Reproducibility standard deviation, s_R (in $\log_{10}$ CFU/g)	0,09	0,12	0,11
Reproducibility relative standard deviation (%)	2,94	2,33	1,91
Reproducibility limit (R), as difference on $\log_{10}$ scale (in $\log_{10}$ CFU/g)	0,27	0,33	0,32

Table A.2 — Results of data analysis obtained with meat samples

Sample/level of contamination	Meat low level	Meat medium level	Meat high level
Number of laboratories having returned results	20	20	20
Number of samples per laboratory	2	2	2
Number of laboratories retained after eliminating outliers	17	17	17
Number of accepted samples	34	34	34
Median value (in $\log_{10}$ CFU/g)	3,16	4,13	6,08
Repeatability standard deviation, s_r (in $\log_{10}$ CFU/g)	0,09	0,07	0,07
Repeatability relative standard deviation (%)	2,85	1,79	1,22
Repeatability limit (r), as difference on $\log_{10}$ scale (in $\log_{10}$ CFU/g)	0,25	0,21	0,21
Reproducibility standard deviation, s_R (in $\log_{10}$ CFU/g)	0,11	0,10	0,12
Reproducibility relative standard deviation (%)	3,66	2,38	1,96
Reproducibility limit (R), as difference on $\log_{10}$ scale (in $\log_{10}$ CFU/g)	0,32	0,28	0,33

Table A.3 — Results of data analysis obtained with egg powder samples

Sample/level of contamination	Egg powder low level	Egg powder medium level	Egg powder high level
Number of laboratories having returned results	20	20	20
Number of samples per laboratory	2	2	2
Number of laboratories retained after eliminating outliers	19	19	19
Number of accepted samples	38	38	38
Median value (in $\log_{10}$ CFU/g)	3,25	4,20	5,32
Repeatability standard deviation, s_r (in $\log_{10}$ CFU/g)	0,12	0,06	0,09
Repeatability relative standard deviation (%)	3,66	1,41	1,68
Repeatability limit (r), as difference on $\log_{10}$ scale (in $\log_{10}$ CFU/g)	0,33	0,17	0,25
Reproducibility standard deviation, s_R (in $\log_{10}$ CFU/g)	0,14	0,11	0,14
Reproducibility relative standard deviation (%)	4,20	2,74	2,64
Reproducibility limit (R), as difference on $\log_{10}$ scale (in $\log_{10}$ CFU/g)	0,38	0,32	0,39