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Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of *Listeria monocytogenes* —

Part 1: Detection method

AMENDMENT 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data

Microbiologie des aliments — Méthode horizontale pour la recherche et le dénombrement de Listeria monocytogenes —

Partie 1: Méthode de recherche

AMENDEMENT 1: Modification des milieux d'isolement, de la recherche de l'hémolyse et introduction de données de fidélité

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Foreword

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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 11290-1:1996 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

The isolation media have been modified, as has the haemolysis test. Precision data have been added.

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Page 2, Subclause 4.3

Replace this subclause by the following:

4.3 Plating out and identification

From the cultures obtained in 4.1 and 4.2, plating out on the two selective solid media:

— Agar *Listeria* according to Ottaviani and Agosti (ALOA[1)]) (see Reference [1] and B.3);

— any other solid selective medium at the choice of the laboratory complementary to Agar *Listeria* according to Ottaviani and Agosti, such as Oxford or PALCAM.

Incubation of the Agar *Listeria* according to Ottaviani and Agosti at 37 °C ± 1 °C and examination after 24 h ± 3 h, and if necessary after a further 24 h ± 3 h, to check for the presence of characteristic colonies which are presumed to be *L. monocytogenes*.

Incubation of the 2nd selective medium at the appropriate temperature and examination after the appropriate time.

Page 2, Subclauses 5.4.1 and 5.4.2

Replace these subclauses by the following:

5.4.1 First medium: Agar *Listeria* according to Ottaviani and Agosti (ALOA[1)]) [1]

See B.3.

5.4.2 Second medium

The choice of the second medium is left to the discretion of the testing laboratory. If a commercial medium is used, the manufacturer's instructions shall be precisely followed regarding its preparation for use.

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1) ALOA is an example of a suitable medium available commercially. This information is given for the convenience of users of this part of ISO 11290 and does not constitute an endorsement by ISO of this product. The use of other media with the same formulation is allowed.

Page 4, Subclause 9.4

Replace 9.4.1 by the following:

9.4.1 From the primary enrichment culture incubated for 24 h ± 3 h at 30 °C (9.2), take, by means of a loop or glass rod (6.5), a portion of the culture and inoculate the surface of the first selective plating medium, Agar *Listeria* according to Ottaviani and Agosti (5.4.1), so that well-separated colonies are obtained.

Proceed in the same way with the second selective plating-out medium (5.4.2).

Replace 9.4.3 by the following:

9.4.3 Invert the dishes obtained in 9.4.1 and 9.4.2 and place them in an incubator set at 37 °C for Agar *Listeria* according to Ottaviani and Agosti (5.4.1) and at the appropriate temperature for the second selective medium (5.4.2). If a commercial medium is used for the second selective medium, follow the manufacturer's instructions.

Delete Note 6.

Replace 9.4.4 by the following:

9.4.4 After incubation for 24 h ± 3 h (and for an additional 24 h ± 3 h if the growth is weak or if no colony is observed after 24 h incubation) for Agar *Listeria* according to Ottaviani and Agosti or for the appropriate time (second selective agar), examine the dishes (9.4.3) for the presence of colonies presumed to be *Listeria* spp.

Replace 9.4.4.1 by the following:

9.4.4.1 Agar *Listeria* according to Ottaviani and Agosti: Consider as *L. monocytogenes* the green-blue colonies surrounded by an opaque halo (typical colonies). If growth is slight, or if no colony is observed, or if no typical colony is present after 24 h ± 3 h of incubation, re-incubate the plates for a further 24 h ± 3 h.

NOTE 1 Some strains of *L. monocytogenes* show a very weak halo (even no halo) in cases of stress, in particular acid stress.

NOTE 2 Some *L. monocytogenes* are characterized by a slow PIPLC (phosphatidyl inositol phospholipase C) activity. Such bacteria are detected when the total duration of incubation is more than, for example, 4 days. Some of these strains could be pathogenic (see Reference [2]).

Replace 9.4.4.2 by the following:

9.4.4.2 Second selective medium: Examine after the appropriate time to check for the presence of colonies which, from their characteristics, are considered to be presumptive *Listeria* spp. or *monocytogenes*, depending on the type of medium used.

Page 5, Subclause 9.6.1 Haemolysis test

Insert the subclause numbers 9.6.1.1 at the beginning of the text of 9.6.1.

Replace Note 9 by the following subclause:

9.6.1.2 The haemolytic reaction may also be carried out as follows using sheep red blood corpuscles.

Disperse the colony in 150 µl of TSYEB (B.6); incubate at 37 °C for 2 h. Add 150 µl of a suspension of sheep red blood corpuscles (B.4 of this Amendment). Incubate at 37 °C for 15 min to 60 min, then refrigerate at 3 °C ± 2 °C for approximately 2 h. Examine for haemolytic activity. If the reaction is not definite, leave at 3 °C ± 2 °C for up to 24 h ± 3 h.

Page 7, new Clause 11

Add the following clause after Clause 10:

11 Precision of the method

11.1 General

It is not possible to express the precision of a qualitative method by using the parameters of repeatability and reproducibility which can be calculated only for quantitative methods. Thus new performance characteristics have been selected (see Reference [3]). These characteristics are: accuracy (sensitivity for positive samples, specificity for negative samples), accordance and concordance (see 11.2, 11.3 and 11.4).

The values of these characteristics have been determined by an interlaboratory test on the method organized within the framework of a European project (see Annex D). Performance characteristics were determined using three types of food contaminated at various levels and for reference materials. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and matrices other than those given in Annex D.

WARNING — The method which was tested was without this amendment, i.e. the isolation was performed on PALCAM and Oxford agars. The precision data give some general guidance to the user on the global performance of the method and these precision data are applicable in particular to this part of ISO 11290 together with this amendment when the second isolation agar is either Oxford or PALCAM.

11.2 Accuracy

11.2.1 Definition

Accuracy is the percentage of samples correctly identified.

For positive samples, the accuracy is called sensitivity and is the percentage of samples correctly identified as positives. For the purpose of this calculation, it must be assumed that all supposedly positive samples do in fact contain the organism.

For negative samples, the accuracy is called specificity and is the percentage of samples correctly identified as negatives.

11.2.2 Overall values

As a general indication of specificity (Sp), the following value may be used when testing food samples in general: Sp = 97,4 %.

As a general indication of sensitivity (Se) the following value may be used when testing food samples in general: Se = 85,2 %.

For reference materials (capsules containing 23 CFU, prepared by RIVM, Netherlands, for the trial), the following value has been obtained: Se = 89,5 %.

These values may be interpreted to mean that a sample which contains *L. monocytogenes* will be recognized as positive when analysed with the method described in this part of ISO 11290-1 in 85,2 % of cases.

11.3 Accordance

11.3.1 Definition

Accordance is the percentage chance of finding the same result (i.e. both negative or both positive) from two identical test portions analysed in the same laboratory, under repeatability conditions (i.e. one operator using the same apparatus and same reagents within the shortest feasible time interval).

The accordance is therefore the equivalent of repeatability for quantitative methods.

To calculate accordance from the results of an interlaboratory test, the probability that two samples give the same result is calculated for each participating laboratory in turn, and this probability is then averaged over all laboratories.

11.3.2 Overall values

As a general indication of accordance (Ac), the following value may be used when testing food samples in general: Ac = 88,7 %.

For reference materials (capsules containing 23 CFU, prepared by RIVM, Netherlands, for the trial), the following value may be used: Ac = 88,2 %.

These values may be interpreted to mean that if two identical test portions of a sample containing *L. monocytogenes* are analysed by the same operator in a short time and with exactly the same operating conditions, there is an 88,7 % chance of obtaining the same result (presence of *L. monocytogenes*) for the two test portions.

11.4 Concordance

11.4.1 Definition

Concordance is the percentage chance of finding the same result for two identical samples analysed in two different laboratories.

The concordance is therefore the equivalent of reproducibility for quantitative methods.

To calculate concordance from the results of an interlaboratory test, each observation in each participating laboratory is taken in turn, pairing it with the results obtained for that particular sample by all the other laboratories. The concordance is the percentage of all pairings giving the same results on all the possible pairings of data.

11.4.2 Overall values

As a general indication of concordance (Cc), the following value may be used when testing food samples in general: Cc = 84,4 %.

For reference materials (capsules containing 23 CFU, prepared by RIVM, Netherlands, for the trial), the following value can be used: Cc = 80,8 %.

These values may be interpreted to mean that if two identical test portions of a sample containing *L. monocytogenes* are analysed by two laboratories, there is an 84,4 % chance of obtaining the same result (presence of *L. monocytogenes*) for the two test portions.

Page 7

Renumber the existing Clause 11 as Clause 12.

Page 9, Annex A

Replace the exisiting Annex A by the following:

Annex A
(normative)

Diagram of procedure