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AMENDMENT 1
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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

AMENDMENT 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage

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Microbiologie des aliments — Méthode horizontale pour la recherche des Salmonella *spp.*

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AMENDEMENT 1: Annexe D: Recherche des Salmonella *spp. dans les matières fécales des animaux et dans des échantillons environnementaux au stade de la production primaire*

Reference number
ISO 6579:2002/Amd.1:2007(E)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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 6579:2002 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

AMENDMENT 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage

Page 1, Clause 2

Replace the introductory text as follows and add the two references.

2 Normative references

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The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

Page 27, after Annex C

Add the following as Annex D.

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Annex D
(normative)

Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage

D.1 Introduction

The method given in the main text of this International Standard is primarily intended for the isolation of *Salmonella* spp. from food and feeding stuffs and is not always suitable for the detection of *Salmonella* spp. from other matrices.

This annex is applicable to the detection of *Salmonella* spp. in

— animal faeces (such as from poultry, pigs, cattle), and

— environmental samples in the area of the primary production stage (such as dust).

The method in this annex is based upon Clause 9, with a different selective enrichment medium. Therefore, where possible, reference will be made to Clause 9.

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The selective enrichment medium as described in this annex (modified semi-solid Rappaport-Vassiliadis: MSRV) is intended for the detection of motile Salmonellae and is not appropriate for the detection of non-motile Salmonellae.

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NOTE The non-motile *Salmonella* biovars of *Salmonella* Gallinarum (*Salmonella* Gallinarum biovar gallinarum and *Salmonella* Gallinarum biovar pullorum do not seem to survive long in environmental samples and will therefore rarely be detected in faecal or environmental (such as dust) samples (regardless of the method). The number of other non-motile *Salmonella* serovars in faecal samples seems to be generally low. For example, in Reference [7] in which circa 1 000 faecal samples of poultry layer flocks and circa 900 faecal samples of broiler flocks were analysed, less than 1 % of the total number of samples were positive in a selective broth and at the same time negative on MSRV (and likely to be non-motile). Similar results were found in a Dutch study with circa 3 200 faecal samples of pigs (non-published data). On the other hand, in the case of the study in Reference [7], up to almost 40 % of positive samples would not have been detected (i.e. false negatives) if only a selective broth (in this case Rappaport Vassiliadis) had been used instead of a semi-solid medium.

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D.2 Principle

D.2.1 General

The detection of *Salmonella* in animal faeces and in samples of the primary production stage necessitates four stages, as described in Clause 4.

D.2.2 Pre-enrichment in non-selective liquid medium

Buffered peptone water (BPW) is inoculated at ambient temperature with the test portion, then incubated at 37 °C ± 1 °C for 18 h ± 2 h.

D.2.3 Enrichment on selective semi-solid medium

Modified semi-solid Rappaport-Vassiliadis (MSRV) agar plates are inoculated with the culture obtained in D.2.2.

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The MSRV is incubated at 41,5 °C ± 1 °C for 24 h ± 3 h. If a plate is negative after 24 h, it is incubated for a further 24 h ± 3 h.

D.2.4 Selective plating and identification

From the culture obtained in D.2.3, two selective solid media are inoculated:

— xylose lysine deoxycholate (XLD) agar;

— any other solid selective medium complementary to XLD agar (see 4.4).

The XLD agar is incubated at 37 °C ± 1 °C and examined after 24 h ± 3 h.

The second selective medium is incubated in accordance with the manufacturer's instructions.

D.2.5 Confirmation of identity

Colonies of presumptive *Salmonella* are subcultured, then plated-out as described in D.2.4, and their identity is confirmed by means of appropriate biochemical and serological tests.

D.3 Culture media, reagents and sera

D.3.1 General

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For current laboratory practice, see ISO 7218.

All media and reagents needed for this annex are described in Annex B, except for modified semi-solid Rappaport-Vassiliadis (MSRV) medium, which is described in D.3.2. Alternatively, dehydrated complete media or diluents may be used. Follow, in that respect, the manufacturer's instructions.

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NOTE The composition of MSRV, as described in Reference [8], contained 20 mg/l of novobiocin. However, from a scientific point of view, 10 mg/l novobiocin is preferred. In studies performed at the CRL-*Salmonella*, more *Salmonella*-positive results were found in pig faeces samples when tested with MSRV containing 10 mg/l than with MSRV containing 20 mg/l novobiocin (see Reference [9]). Furthermore, when testing different animal faeces (pigs, chicken, cattle) and naturally contaminated dust, the migration zones on MSRV containing 10 mg/l novobiocin were (much) larger than on MSRV containing 20 mg/l novobiocin (Reference [9]). The influence of novobiocin on bacterial motility was earlier described in Reference [10].

For the preparation of the selective plating agar media (see B.4, XLD-agar), standard size Petri dishes may be used (90 mm or 100 mm) instead of large size Petri dishes (140 mm).

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D.3.2 Modified semi-solid Rappaport-Vassiliadis medium (MSRV)

D.3.2.1 Base medium

D.3.2.1.1 Composition

Enzymatic digest of animal and plant tissue	4,6	g
Acid hydrolysate of casein	4,6	g
Sodium chloride (NaCl)	7,3	g
Potassium dihydrogenphosphate (KH_2PO_4)	1,5	g
Magnesium chloride anhydrous ($MgCl_2$)	10,9	g
Malachite green oxalate	0,04	g
Agar	2,7	g
Water	1 000	ml

D.3.2.1.2 Preparation

Suspend the ingredients into the water.

Heat to boiling with agitation. **Do not autoclave.**

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Do not hold the medium at high temperatures longer than necessary.

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Cool the medium to 47-50 °C.

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D.3.2.2 Novobiocin solution

D.3.2.2.1 Composition

Novobiocin sodium salt	0,05	g
Water	10	ml

D.3.2.2.2 Preparation

Dissolve the novobiocin sodium salt in the water.

Sterilize by filtration through a filter with a pore size of 0,22 µm.

The solution may be stored for up to 4 weeks at 5 °C ± 3 °C or in small portions (e.g. of 2 ml) at –20 °C for up to one year.

D.3.2.3 Complete medium

D.3.2.3.1 Composition

Base medium (D.3.2.1)	1 000	ml
Novobiocin solution (D.3.2.2)	2	ml

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D.3.2.3.2 Preparation

Aseptically add 2 ml of the novobiocin solution (D.3.2.2) to 1 000 ml of base medium (D.3.2.1) at 47 °C to 50 °C. Mix carefully.

The final pH shall be 5,2 (5,1 to 5,4) at 20 °C to 25 °C.

Pour into plates up to a volume of 15 ml to 20 ml in Petri dishes with a diameter of 90 mm.

Allow the medium to solidify before moving and handle with care.

Store the plates, **with surface upwards**, for up to 2 weeks at 5 °C ± 3 °C in the dark.

Do not invert the plates, as the semi-solid agar is too liquid to do so.

Any plates in which the semi-solid agar has liquefied or fragmented shall not be used.

Immediately before use, and only if necessary, dry the surface of the agar plates carefully, for example by placing them with the lids off and the agar surface **upwards** in a laminar air flow cabinet. Take care not to overdry the medium.

D.4 Apparatus and glassware

Use the apparatus listed in Clause 6, and the following.

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D.4.1 Sterile loops, of 1 µl.

D.5 Sampling

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See Clause 7.

D.6 Preparation of test sample

See Clause 8.

In general, an amount of sample is added to a quantity of BPW to yield a 1/10 dilution (e.g. 25 g of sample added to 225 ml of BPW). However, for some type of samples it may be necessary to use another ratio.

D.7 Procedure

D.7.1 Non-selective pre-enrichment

Pre-warm the BPW to room temperature before use.

Mix samples well by the most suitable means for the sample type.

Weigh the sample and add it to the appropriate quantity of BPW (see D.6). Incubate the jars at 37 °C ± 1 °C for 18 h ± 2 h.

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