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English Version

Microbiology of food and animal feed - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 2: Enumeration by a miniaturized most probable number technique (ISO/TS 6579-2:2012)

Microbiologie des aliments - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des salmonella - Partie 2: Dénombrement par une technique miniaturisée du nombre le plus probable (ISO/TS 6579-2:2012)

Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren zum Nachweis, zur Zählung und zur Serotypisierung von Salmonellen - Teil 2: Zählung unter Anwendung eines miniaturisierten Verfahrens der wahrscheinlichsten Keimzahl (ISO/TS 6579-2:2012)

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Foreword

This document (CEN ISO/TS 6579-2:2012) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 “Food analysis - Horizontal methods” the secretariat of which is held by DIN.

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Endorsement notice

The text of ISO/TS 6579-2:2012 has been approved by CEN as a CEN/TS ISO 6579-2:2012 without any modification.

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Introduction

The procedure described is based on the method reported in Reference [1].

The enumeration procedure described here concerns a miniaturized most probable number (MPN) technique. For this mini-MSRV (modified semi-solid Rappaport–Vassiliadis) MPN technique, the volume of primary dilution tested is less than the volume in the detection method specified in ISO 6579:2002 + Cor.1:2004 + Amd.1:2007.[5] For this reason, the sensitivity of the mini-MSRV technique is lower than in these detection methods (Reference [1]). The detection limit of the mini-MSRV method is approximately 1 cfu/g, but can vary according to *Salmonella* serovar and per matrix. For the previously mentioned detection methods, this is typically 1 cfu/25 g (0,04 cfu/g). For samples with (very) low numbers of *Salmonella* spp. (<1 cfu/g), it is possible that the mini-MSRV procedure is not sufficiently sensitive. If a quantitative result is requested for samples likely to contain such low numbers (and tested negative with this mini-MSRV technique, for example), it is advisable to enumerate with a “conventional” MPN technique (not miniaturized). For other samples, the mini-MSRV method can have an advantage over the conventional MPN technique because the performance of the miniaturized MPN technique can take less time and need fewer resources (due to small amounts).

Microbiology of food and animal feed — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 2: Enumeration by a miniaturized most probable number technique

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Salmonella*, are only undertaken in properly equipped laboratories, under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials.
Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This part of ISO 6579 gives a method for the enumeration of *Salmonella* spp. present in:

— products intended for human consumption and for the feeding of animals;

— environmental samples in the area of food production and food handling;

— animal faeces;

— environmental samples from the primary production stage;

by calculation of the most probable number (MPN).

The method is based on miniaturization of the dilution, pre-enrichment and selective enrichment steps. The selective enrichment medium, 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.

It is possible that the method is less appropriate to enumerate *Salmonella* ser. Typhi and *Salmonella* ser. Paratyphi.

The method is not appropriate for the enumeration of *Salmonella* spp. in (very) low contaminated samples (<1 cfu/g).

NOTE The number of non-motile salmonellae is generally low in most of the matrices relevant for this method. In this note, examples are given for samples from primary production. 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 are therefore rarely 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 [4] in which approximately 1 000 faecal samples of poultry layer flocks and approximately 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 medium (and likely to be non-motile). Similar results were found in a Dutch study with ca 3 200 faecal samples of pigs (unpublished data). On the other hand, in the case of the study reported in Reference [4], 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.

2 Normative references

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.

ISO 6887 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*