
Irish Standard
I.S. EN 16936:2017

# Animal feeding stuffs: Methods of sampling and analysis - Screening on the antibiotics tylosin, virginiamycin, spiramycin, bacitracin-zinc and avoparcin at sub-additive levels in compound feed by a microbiological plate test

**I.S. EN 16936:2017**

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## National Foreword

I.S. EN 16936:2017 is the adopted Irish version of the European Document EN 16936:2017, Animal feeding stuffs: Methods of sampling and analysis - Screening on the antibiotics tylosin, virginiamycin, spiramycin, bacitracin-zinc and avoparcin at sub-additive levels in compound feed by a microbiological plate test

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EUROPEAN STANDARD

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# Animal feeding stuffs: Methods of sampling and analysis - Screening on the antibiotics tylosin, virginiamycin, spiramycin, bacitracin-zinc and avoparcin at sub-additive levels in compound feed by a microbiological plate test

Aliments pour animaux : Méthodes d'échantillonnage et d'analyse - Dépistage des antibiotiques tylosine, virginiamycine, spiramycine, bacitracine-zinc et avoparcine à des niveaux sous-additifs dans les aliments composés par essai sur plaque microbiologique

Futtermittel - Probenahme- und Untersuchungsverfahren - Screening auf die Antibiotika Tylosin, Virginiamycin, Spiramycin, Bacitracin-Zink und Avoparcin in Konzentrationen unterhalb von Zusatzstoffen in Mischfuttermitteln mittels mikrobiologischem Plattentest

This European Standard was approved by CEN on 6 February 2017.

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**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

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## European foreword

This document (EN 16936:2017) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs: Methods of sampling and analysis”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2017, and conflicting national standards shall be withdrawn at the latest by November 2017.

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# 1 Scope

This European Standard presents a method describing the screening on the antibiotics tylosin, virginiamycin, spiramycin, bacitracin-zinc and avoparcin at sub-additive levels in complete feeding stuffs and milk replacers by a microbiological 3-plate test.

The limit of detection of the method is 1 mg/kg for avoparcin, tylosin, spiramycin and virginiamycin, and 5 mg/kg for zinc bacitracin. The presence of other (veterinary) antibiotics may interfere with the method.

Furthermore, high concentrations of metals (Cu, Zn) may interfere. The method should be used as a qualitative screening method. Positive results can be analysed further by TLC; for confirmatory purposes LC-MS is required [1].

A lower limit of detection for zinc bacitracin (3 mg/kg) is achievable (see Table 2), but should be established with an in house validation first.

# 2 Normative references

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

EN ISO 13969, *Milk and milk products - Guidelines for a standardized description of microbial inhibitor tests (ISO 13969)*

# 3 Principle

The feed sample is extracted with a mixture of acetone, hydrochloric acid (HCl) and water. Neutralized extract is dispensed into wells in three different test plates. Each of these test plates holds a different composition with respect to culture medium, indicator bacterium and/or pH. After a 16-18 h incubation period, the presence of antibiotic residues is indicated by the appearance of a zone of growth inhibition around the sample. Comparison of the inhibition pattern with a reference set (Table 1) may yield a presumptive identification of the antibiotic.

# 4 Reagents and materials

**WARNING** — The use of this protocol involves hazardous materials, operations and equipment, This protocol does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

## 4.1 Test organisms:

*Kocuria rhizophila* ATCC 9341 (formerly: *Micrococcus luteus*)

*Micrococcus luteus* ATCC 10240

*Bacillus megaterium* ATCC 10778

See Annex A for the preparation of the bacterial suspensions.

## 4.2 Culture media:

In order to improve the reproducibility of the method, it is recommended to use dehydrated basic components or dehydrated complete media for the preparation of culture media. Follow the manufacturers' instructions.