



BSI Standards Publication

Molecular biomarker analysis — Detection of animal-derived materials in foodstuffs and feedstuffs by real-time PCR

Part 6: Horse DNA detection method

National foreword

This Published Document is the UK implementation of ISO/TS 20224-6:2020.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its committee manager.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

© The British Standards Institution 2020
Published by BSI Standards Limited 2020

ISBN 978 0 539 04897 1

ICS 67.050

Compliance with a British Standard cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 August 2020.

Amendments/corrigenda issued since publication

| Date | Text affected |
|------|---------------|
|------|---------------|

**Molecular biomarker analysis —
Detection of animal-derived materials
in foodstuffs and feedstuffs by real-
time PCR —**

**Part 6:
Horse DNA detection method**

*Analyse de biomarqueurs moléculaires — Détection de matériaux
d'origine animale dans les denrées alimentaires et les aliments pour
animaux par PCR en temps réel —*

Partie 6: Méthode de détection de l'ADN de cheval





COPYRIGHT PROTECTED DOCUMENT

© ISO 2020

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

| | Page |
|--|-----------|
| Foreword | iv |
| Introduction | v |
| 1 Scope | 1 |
| 2 Normative references | 1 |
| 3 Terms and definitions | 1 |
| 4 Scientific basis | 2 |
| 5 Reagents and materials | 2 |
| 5.1 General..... | 2 |
| 5.2 PCR reagents..... | 2 |
| 6 Apparatus | 3 |
| 7 Procedure | 3 |
| 7.1 Preparation of the test portion/sample..... | 3 |
| 7.2 Preparation of DNA extracts..... | 3 |
| 7.3 PCR setup..... | 3 |
| 7.3.1 Reaction mixes..... | 3 |
| 7.3.2 PCR controls..... | 4 |
| 7.3.3 Real-time PCR thermocycler plate set-up..... | 4 |
| 7.4 Temperature-time programme..... | 4 |
| 8 Accept/reject criteria | 4 |
| 8.1 General..... | 4 |
| 8.2 Identification..... | 5 |
| 9 Validation status and performance criteria | 5 |
| 9.1 General..... | 5 |
| 9.2 Robustness..... | 5 |
| 9.3 Reproducibility..... | 6 |
| 9.4 Sensitivity..... | 6 |
| 9.5 Specificity..... | 9 |
| 10 Test report | 11 |
| Annex A (informative) Blast 2.9.0 results for query of GenBank refseq genomes (452 databases) | 12 |
| Bibliography | 15 |

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

A list of all parts in the ISO 20224 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Fraudulent adulteration of meat in food and feed threatens both public safety and commerce. Adulteration can affect those adhering to ethnological dietary rules, economic development and social stability. This document provides a real-time polymerase chain reaction (real-time PCR) analytical method for the identification of meat animal species from nucleic acid present in the ingredients of food and feed.

Animal-derived biological materials in food and feed are detected and identified in the laboratory with the following successive (or simultaneous) steps: preparation of the test portion/sample, nucleic acid extraction and purification, PCR amplification and interpretation of results. This document provides guidance for PCR amplification and interpretation of results, specific to horse DNA detection.

The ISO 20224 series consists of technical specifications that describe specific applications. New species DNA detection methods established in the future will be added as independent parts.

Molecular biomarker analysis — Detection of animal-derived materials in foodstuffs and feedstuffs by real-time PCR —

Part 6: Horse DNA detection method

1 Scope

This document specifies a real-time polymerase chain reaction (real-time PCR) method for the qualitative detection of horse-specific DNA derived from food and feed. It requires the extraction of an adequate amount of PCR amplifiable DNA from the relevant matrix and can be applied to the detection of horse material derived from domestic horse (*Equus caballus*), mule (*Equus caballus* ♀ × *Equus asinus* ♂), hinny (*Equus caballus* ♂ × *Equus asinus* ♀) and zebroid (*Equus caballus* × *Equus simplicidens*). The assay also detects the species Przewalski's horse (*Equus przewalskii*) and zebra (*Equus burchellii*).

The target sequence is an *Equus caballus* isolate Twilight breed thoroughbred chromosome 28, EquCab3.0, whole genome shotgun sequence (i.e. GenBank accession number NC_009171.3)^[1], which is present as a single copy per haploid genome. The provided PCR assay for this target has an absolute limit of detection of five copies per reaction, with ≥ 95 % replicability at this concentration (LOD_{95 %}).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 16577, *Molecular biomarker analysis — Terms and definitions*

ISO 20813, *Molecular biomarker analysis — Methods of analysis for the detection and identification of animal species in foods and food products (nucleic acid-based methods) — General requirements and definitions*

ISO 21571, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*

ISO 24276, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>