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

Part 10: Duck DNA detection method

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National foreword

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

Part 10: Duck 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 10: Méthode de détection de l'ADN de canard

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Foreword

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

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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.

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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 mallard duck (*Anas platyrhynchos*) and spot-billed duck (*Anas zonorhyncha*) DNA detection. Cross detection of white-winged duck (*Asarcornis scutulata*), tufted duck (*Aythya fuligula*), muscovy duck (*Cairina moschata*) and Mandarin duck (*Aix galericulata*) is observed.

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.

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

Part 10: Duck DNA detection method

1 Scope

This document specifies a real-time polymerase chain reaction (real-time PCR) method for the qualitative detection of duck-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 duck material derived from mallard duck (*Anas platyrhynchos*) and spot-billed duck (*Anas zonorhyncha*). Cross detection of white-winged duck (*Asarcornis scutulata*), tufted duck (*Aythya fuligula*), muscovy duck (*Cairina moschata*) and Mandarin duck (*Aix galericulata*) is observed. Mallard duck and muscovy duck are domesticated poultry for food, while spot-billed duck, white-winged duck, tufted duck and Mandarin duck are wild avian.

The target sequence is a partial fragment of the *Anas platyrhynchos* breed pekin duck isolate CAU_Pekin_2.0 Chr1, whole genome shotgun sequence (i.e. GenBank accession number JACEUM010000001.1),[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 % confidence 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 — Vocabulary for molecular biomarker analytical methods in agriculture and food production*

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 terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

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4 Scientific basis

DNA is extracted from the test portion by applying a suitable method (see ISO 21571:2005, A.1). The DNA analysis consists of two parts:

— verification of the quality and amplifiability of the extracted DNA using a PCR assay specific for eukaryotes (i.e. 18S rRNA gene) or mammals and poultry (i.e. myostatin gene);

— detection of the duck species-specific DNA sequence of the single-copy *Anas platyrhynchos* breed pekin duck isolate CAU_Pekin_2.0 Chr1, whole genome shotgun sequence (i.e. GenBank accession number JACEUM010000001.1) in a real-time PCR.

NOTE The copy number of the eukaryotic ribosomal 18S RNA (18S rRNA) gene in a cell varies from several hundred to several thousand, while the specific target sequence in the duck genome and myostatin gene in mammals and poultry genome are single copy. The copy number of the specific target sequence in *Anas platyrhynchos* genome was confirmed by bioinformatics analysis at the whole genome scale (see Annex A) and digital PCR for absolute quantification.

5 Reagents and materials

5.1 General

For this document, only reagents and water of recognized analytical grade, appropriate for molecular biology, shall be used. Unless stated otherwise, solutions should be prepared by dissolving the corresponding reagents in water followed by autoclave sterilization. For all operations in which gloves are used, gloves shall be powder free. The use of aerosol protected pipette tips (protection against cross-contamination) is recommended.

5.2 PCR reagents

5.2.1 PCR master mix.

In general, real-time PCR master mix contains thermostable DNA polymerase, dNTPs, $MgCl_2$, KCl, and buffer as a dilutable concentrated mixture, that is ready to use.

NOTE The commercial real-time PCR master mix can be used.

5.2.2 Oligonucleotides.

The quality of the oligonucleotides shall be sufficient for their use as primers and probes. See Table 1.

Table 1 — Oligonucleotides

Name	DNA sequence of the oligonucleotide	Final concentration in PCR
Specific sequence in *Anas platyrhynchos* breed pekin duck isolate CAU_Pekin_2.0 Chr1, whole genome shotgun sequence (i.e. GenBank accession number JACEUM010000001.1)[a]		
Duck-105bp-F	5′-TCTTCACAAGCAGGGTCTAATGG-3′	400 nmol/l
Duck-105bp-R	5′-CTTGGCAGAAGGTCCAGAGG-3′	400 nmol/l
Duck-105bp-P	5′- [FAM]-AGGCACAGCACGCATCTCACCACA-[TAMRA][b]-3′	200 nmol/l

[a] PCR product = 200 064 152 - TCTTCACAAG CAGGGTCTAA TGGAAGACTT GCTGGCCTGC TCTCTACTGG TGATGATGTG GTGAGATGCG TGCTGTGCCT CTCTTCCTCT GGACCTTCTG CCAAG - 200 064 048 - JACEUM010000001.1

[b] FAM: 6-carboxyfluorescein, TAMRA: 6-carboxytetramethylrhodamine.

Duck-105bp-F is base pairs 200 064 152 – 200 064 130, Duck-105bp-R is base pairs 200 064 048 – 200 064 067 and Duck-105bp-P is 200 064 073 – 200 064 096 of JACEUM010000001.1, *Anas platyrhynchos* breed pekin duck isolate CAU_Pekin_2.0 Chr1, whole genome shotgun sequence. Equivalent reporter dyes and/or quencher dyes can be used if they yield the same or better results.

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