VERYfinder

Food Frauds Investigation Real-Time PCR kits

GENERON
Food Safety in our Genes

What is a food fraud?

Food fraud is a collective term used to encompass the deliberate and intentional substitution, addition, tampering, or misrepresentation of food or food ingredients, for economic gain.

Adulteration

Refers to the addition of one or more undeclared ingredient(s), to improve the appearance of the product or to increase its weight fictitiously. For example, adding cow to goat milk for cheese production.

Substitution

Involves replacement of one ingredient by a similar or cheaper one. For example, using pollack in place of cod.

Misdescription

Refers to the method of production, the geographical origin of the food or the amounts of key ingredients used. For example, including animal derived ingredients in vegetarian meals.

There is a clear link

and

How can DNA help?

Nowadays, the analysis of DNA is widely used in the determination of food authenticity and Real-Time PCR is the most sensitive method for the detection and quantification of DNA sequences of different contaminating species. The method, combined with an appropriate nucleic acid extraction system, allows the analysis of raw materials, semi-finished and finished products as well.

DNA testing allows an efficient and sensitive identification of plant and animal derivatives, easily detecting accidental contaminations or potential frauds.

between **food fraud**

food safety.

What challenges?

Real-Time is powerful but it is paramount to understand the applicability of the technology

I. Understanding the **fitness** of Real-Time PCR for answering the relevant analytical question

II. Ensuring the marker has the **specificity** required to avoid false positives

III. **Compatibility** with a variety of matrices

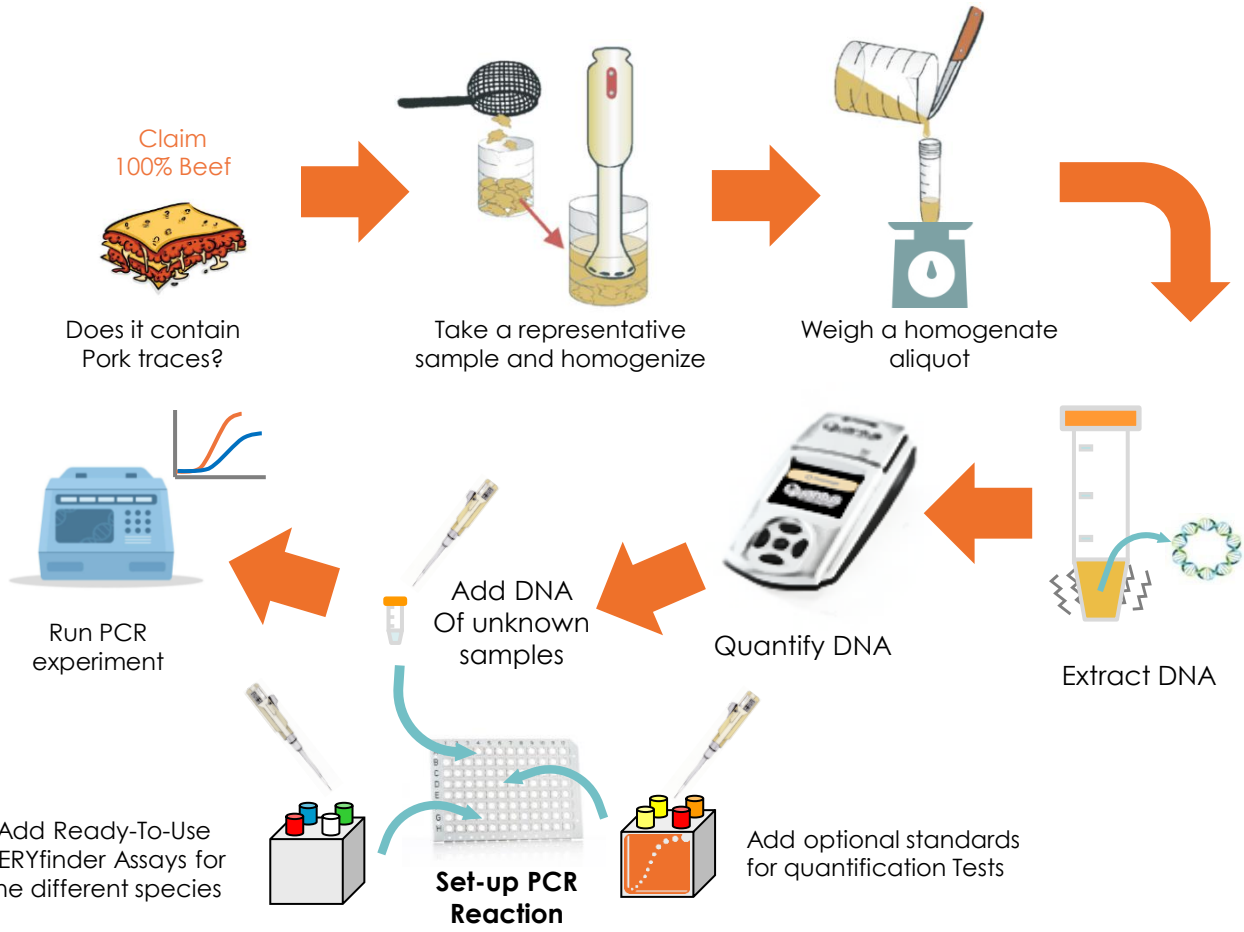
IV. **Conformity** to standards, guidance or legal requirements

V. Requirement for analytical **standards**

DNA measurements used in quantification of species are however hardly correlated to meat content (w/w) for several reasons

While detection of undeclared meat is straightforward quantification is not. There is a tendency to focus on meat content expressed as weight/weight (w/w). DNA measurements used in quantification of species are however hardly correlated to meat content (w/w) for several reasons: differences in species genome size, tissue cell size (i.e., cell number per gram meat) and mitochondrial distribution, DNA extractability, DNA degradation, water content. Hence, the results obtained using PCR shall be intended as percentages of DNA copies and not percentages in meat weight, unless an appropriate standard is set.

How does it work?



ISO 20813:2019 - 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/TS 20224-(1/7):2020 - Molecular biomarker analysis — Detection of animal-derived materials in foodstuffs and feedstuffs by real-time PCR — Part 1: Bovine DNA detection method

Relevant ISO norms for the detection and identification of animal DNA in food were recently defined.

Rules for PAP in feed

EU reference laboratory for animal proteins in feed validated three qPCR diagnostic methods detecting any ruminant, pork and poultry material in feed.

In the 1990s, due to BSE risks control, the use of animal meal (PAPs) in zootechnical nutrition was strictly prohibited. This measure was very effective and BSE in cattle was eradicated. Hence, in 2013 the European Commission decided to allow again the use of PAPs, with the exclusion of those obtained from ruminants (cattle and sheep), in the preparation of feed. In June 2021 amendment, the regulation on the use of PAPs allowed new uses but banned "intra-species recycling". To enforce these rules the EU reference laboratory for animal proteins in feed (EURL-AP) validated three qPCR diagnostic protocols detecting any ruminant, pork and poultry material in feed.

Instructions for ordering – Detection kits

DNA extraction kits

EXD001	ION Force FAST DNA Extraction Kit (100 Ext.)*
EXD018K	ION Spin K DNA Extraction Kit (50 Ext.)*
EXD020	FASTfood DNA Extraction Reagent (100 ml)
EXD912	Promega Wizard Magnetic DNA Purification kit (200 Ext.)**

Ancillary reagents and equipment

ACC2911	ION Force luer-lock reservoirs (100)
EXD010-P	DNA Extraction manifold
EXD910	Promega MagneSphere Stand for 1.5 ml tubes (2 slots)
EXD011	Proteinase K (100 mg/vial)
EXD010	Proteinase K resuspension Buffer

2-Plex VERYfinder meat traces Semi-Q qPCR kits with Internal Amplification Control

Each kit box contains target specific reagents (mastermix, primers and probes) for 50 reactions, negative control, 2 vials of positive control (120 µL Target DNA at a concentration 8 ng/µL; 1% target DNA – 99% bovine/swine DNA) extracted from raw material or heat treated material respectively. Heat treatment performed in autoclave at 121°C for 20'.

PMA01S-50	VERYfinder PORK	PMA02S-50	VERYfinder BOVINE	PMA03S-50	VERYfinder TURKEY
PMA04S-50	VERYfinder CHICKEN	PMA05S-50	VERYfinder SHEEP	PMA06S-50	VERYfinder GOAT
PMA07S-50	VERYfinder BUFFALO	PMA08S-50	VERYfinder EQUINE	PMA12S-50	VERYfinder RABBIT
PMA13S-50	VERYfinder DUCK	PMA14S-50	VERYfinder GOOSE	PMA31S-50	VERYfinder COW milk***
PMA96S-50	VERYfinder CAT	PMA97S-50	VERYfinder DOG	PMA99S-50	VERYfinder VEGGIE ****

Singleplex VERYfinder meat traces detection qPCR kits according to ISO 20224 (without IAC)

Each kit box contains target specific reagents (mastermix, primers and FAM labelled probes) for 50 reactions, negative control, positive control.

PMA01ISO-50	PORK (ISO 20224-3)	PMA02ISO-50	BOVINE (ISO 20224-1)	PMA04ISO-50	CHICKEN (ISO 20224-4)
PMA05ISO-50	OVINE (ISO 20224-2)	PMA06ISO-50	GOAT (ISO 20224-5)	PMA08ISO-50	HORSE (ISO 20224-6)
PMA11ISO-50	DONKEY (ISO 20224-7)	PMA99ISO-50	Amplifiability Control (acc. to ISO 20224)		

Singleplex VETfinder qPCR kits for PAP detection in feed according to EURL-AP method (without IAC)

Each kit box contains target specific reagents (approved mastermix, primers and FAM labelled probes) for 200 reactions, negative control, positive control.

PMA95V	VETfinder MBM RuminantsFREE
PMA97V	VETfinder MBM PorkFREE
PMA98V	VETfinder MBM PoultryFREE

VERYfinder Total meat content quantification kit

Each box contains target specific reagents (enzyme, primers and FAM labelled probes) for 50 reactions, negative control. The assay needs to be used along with a QuantiScale or QuantiCust product

PMA99A-50	VERYfinder Total Meat Assay

Other food frauds detection kit

PFA01A-50	Authenticity testing: *Gadus morhua* (works on fillets)
PSV01A-50	VERYfinder Corn detection kit
PSV02Q	VERYfinder Soft/Durum Wheat Contamination Quantitative kit
PSV02S-50	VERYfinder Soft/Durum Wheat Contamination SemiQ kit
PSV09A-50	VERYfinder Potato detection kit

* Works on gelatine and cosmetics

** Mandatory use for EURL-AP method application

*** Validated on buffalo with no-DNA extraction

**** Detects all meats and fish, does not detect molluscs and crustaceans (use SPECIALfinder allergens kit)

Instructions for ordering – Reference materials

VERYfinder Pure DNA Extracts

Each box includes a vial containing 1200 ng of ION-Force purified target DNA at a fluorimetry quantified concentration 8 ng/µl extracted from, at choice, raw meat or heat-treated (HT) meat (muscle) respectively. Heat treatment performed in autoclave at 121°C for 20'. PMA99R contains 60 ng of DNA from each meat kind and bovine DNA to bring total DNA amount to 1200 ng.

VERYfinder DigiCount DNA Extracts

Each box includes a vial containing ION-Force purified target DNA with a defined genomic unit count calculated using dPCR.

VERYfinder QuantiScale

These calibrators sets allow for the quantification of the % content in the relevant meat DNA with PCR technology. The DNA content is titrated via fluorimetric testing; hence, this set can be used with any PCR kit for meat detection. Each set includes 4 vials containing 120 µl of the relevant meat DNA dispersed at scalar percentages (from 100% down to 0.1%) in a diverse meat DNA background at a total concentration of 8 ng/ul. The DNA is extracted from raw meats, but when ordering PMAxxW_HT the DNA is extracted from meats heat treated in autoclave at 121°C for 20' to simulate the DNA fragmentation occurring during meats processing

VERYfinder QuantiCust

Each vial contain 300 µl of DNA (custom composition, (e.g., 5% equine in 95% bovine) at a fixed concentration of 8 ng/µl. Target DNA can be extracted from raw meats or heat-treated meats respectively (Heat treatment performed in autoclave at 121 °C for 20') to simulate the DNA fragmentation occurring during meats processing. The DNA content is titrated via fluorimetric testing hence this set can be used with any PCR kit for bovine meat detection.

	Pure DNA extracts		QuantiScale		QuantiCust	DigiCount
VERYfinder Swine	PMA01R	PMA01R_HT	PMA01W	PMA01W_HT	PMA01X	PMA01D
VERYfinder Bovine	PMA02R	PMA02R_HT	PMA02W	PMA02W_HT	PMA02X	PMA02D
VERYfinder Turkey	PMA03R	PMA03R_HT	PMA03W	PMA03W_HT	PMA03X	
VERYfinder Poultry	PMA04R	PMA04R_HT	PMA04W	PMA04W_HT	PMA04X	PMA04D
VERYfinder Sheep	PMA05R	PMA05R_HT	PMA05W	PMA05W_HT	PMA05X	PMA05D
VERYfinder Goat	PMA06R	PMA06R_HT	PMA06W	PMA06W_HT	PMA06X	PMA06D
VERYfinder Buffalo	PMA07R	PMA07R_HT	PMA07W	PMA07W_HT	PMA07X	
VERYfinder Horse	PMA08R	PMA08R_HT	PMA08W	PMA08W_HT	PMA08X	PMA08D
VERYfinder Donkey	PMA11R	PMA11R_HT				PMA11D
VERYfinder Rabbit	PMA12R	PMA12R_HT	PMA12W	PMA12W_HT	PMA12X	
VERYfinder Duck	PMA13R	PMA13R_HT	PMA13W	PMA13W_HT	PMA13X	
VERYfinder Goose	PMA14R	PMA14R_HT	PMA14W	PMA14W_HT	PMA14X	
VERYfinder Total Meat	PMA99R	PMA99R_HT	PMA99W	PMA99W_HT	PMA99X	

Plasmid kit calibrants for PAP detection in feed according to EURL-AP method

ERM-AD482 ERM Ruminant pDNA Calibrant

ERM-AD483 ERM Porcine pDNA Calibrant

ERM-AD484K ERM Poultry pDNA Calibrant

Matrix reference materials

Each tube contain 5 grams of heat-treated (HT) pure meat powder at known weight amount dispersed in HT pure pig/chicken meat powder. The reference material was prepared by a stepwise solid/solid dispersion of HT meats according to the indications of ISO 17034: 2017.

PMA94F VETfinder heat treated meat powder 0.1% chicken and 0.1% bovine in 99.8% pig

PMA95F VETfinder heat treated meat powder 0.1% chicken in 99.9% pig

PMA97F VETfinder heat treated meat powder 0.1% bovine in 99.9% pig

PMA98F VETfinder heat treated meat powder 0.1% pig in 99.9% chicken

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