[F1 ANNEX VI

METHODS OF ANALYSIS FOR THE DETERMINATION OF CONSTITUENTS OF ANIMAL ORIGIN FOR THE OFFICIAL CONTROL OF FEED

Textual Amendments

F1 Substituted by Commission Regulation (EU) No 51/2013 of 16 January 2013 amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed (Text with EEA relevance).

2. METHODS

2.2. **PCR**

2.2.1. *Principle*

Deoxyribonucleic acid (DNA) fragments of animal origin which may be present in feed materials and compound feed are detected by a genetic amplification technique through PCR, targeting species-specific DNA sequences.

The PCR method first requires a DNA extraction step. The amplification step shall be applied afterwards to the so-obtained DNA extract, in order to detect the animal species targeted by the assay.

2.2.2. *Reagents and equipment*

2.2.2.1. Reagents

2.2.2.1.1. Reagents for DNA extraction step

Only reagents approved by the EURL-AP and published on its website shall be used.

2.2.2.1.2. Reagents for genetic amplification step

2.2.2.1.2.1. Primers and probes

Only primers and probes with sequences of oligonucleotides validated by the EURL-AP shall be used(1).

2.2.2.1.2.2. Master Mix

Only Master Mix solutions which do not contain reagents susceptible to lead to false results due to presence of animal DNA shall be used(2).

2.2.2.1.2.3. Decontamination reagents

- 2.2.2.1.2.3.1. Hydrochloric acid solution (0,1 N)
- 2.2.2.1.2.3.2. Bleach (solution of sodium hypochlorite at 0,15 % of active chlorine)
- 2.2.2.1.2.3.3. Non-corrosive reagents for decontaminating costly devices like analytical balances (e.g. DNA Erase ™ of MP Biomedicals)

2.2.2.2. Equipment

2.2.2.2.1. Analytical balance with an accuracy of 0,001 g

2.2.2.2.2. Grinding equipment

2.2.2.2.3. Thermocycler enabling real-time PCR

2.2.2.2.4. Microcentrifuge for microfuge tubes

2.2.2.2.5. Set of micropipettes allowing to pipet from 1 µl up to 1 000 µl

2.2.2.2.6. Standard molecular biology plastic-ware: microfuge tubes, filtered plastic tips for micropipettes, plates suitable for the thermocycler.

2.2.2.2.7. Freezers to store samples and reagents

2.2.3. *Sampling and sample preparation*

2.2.3.1. Sampling

A representative sample, taken in accordance with the provisions laid down in Annex I, shall be used.

2.2.3.2. Sample preparation

The preparation of laboratory samples up to DNA extraction shall comply with the requirements set out in Annex II. At least 50 g of the sample shall be sub-sampled for analysis and subsequently ground.

The sample preparation shall be performed in a room different from the ones dedicated to DNA extraction and to genetic amplification reactions as described by ISO 24276.

Two test portions of at least 100 mg each shall be prepared.

2.2.4. *DNA extraction*

The DNA extraction shall be performed on each test portion prepared using the SOP established by the EURL-AP and published on its website.

Two extraction controls shall be prepared for each extraction series as described by ISO 24276.

— an extraction blank control,

— a positive DNA extraction control.

2.2.5. *Genetic amplification*

The genetic amplification shall be performed using the methods validated for each species requiring identification. These methods are laid down in the SOP established by the EURL-AP and published on its website. Each DNA extract shall be analysed at least at two different dilutions in order to evaluate inhibition.

Two amplification controls shall be prepared per species target as described by ISO 24276.

— a positive DNA target control shall be used for each plate or series of PCR assays,

— an amplification reagent control (also called no template control) shall be used for each plate or series of PCR assays.

2.2.6. *Interpretation and expression of results*

When reporting the results, the laboratory shall indicate at least the weight of the test portions used, the extraction technique used, the number of determinations carried-out and the limit of detection of the method.

Results shall not be interpreted and reported if the positive DNA extraction control and the positive DNA target controls do not provide positive results for the target under assay while the amplification reagent control is negative.

In case results from the two test portions are not consistent, at least the genetic amplification step shall be repeated. If the laboratory suspects that the DNA extracts can be the cause of the inconsistency, a new DNA extraction and a subsequent genetic amplification shall be performed before interpreting the results.

The final expression of the results shall be based on the integration and the interpretation of the results of the two test portions in accordance with the SOP established by the EURL-AP and published on its website.

2.2.6.1. Negative result

A negative result shall be reported as follows:

No DNA from X was detected in the submitted sample (with X being the animal species or group of animal species that is targeted by the assay).

2.2.6.2. Positive result

A positive result shall be reported as follows:

DNA from X was detected in the submitted sample (with X being the animal species or group of animal species that is targeted by the assay).**]**

(1) **[**F1The list of these primers and probes for each animal species targeted by the assay is available on the EURL-AP website.**]**

(2) **[**F1Examples of Master Mixes that are functional are available on the EURL-AP website.**]**

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Changes to legislation:
There are currently no known outstanding effects for the Commission Regulation (EC) No 152/2009, Division 2.2..