European Union Reference Laboratory for Animal Proteins in feedingstuffs

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EURL-AP Standard Operating Procedure

Operational protocol for the combination of light microscopy and PCR

Experts for edition and revision	
Version 1.0	**Last major revision**
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DISCLAIMER

This SOP includes the use of the PCR test validated for the detection of poultry DNA and the double sedimentation for the detection of terrestrial invertebrate constituents. Its full application is on the condition that poultry calibrants are available and that the PE/TCE sedimentation step is included in the light microscopy protocol laid down in Annex VI to Regulation (EC) No 152/2009.

1. SCOPE AND PURPOSE

The purpose of the SOP is to present the operational protocol combining light microscopy and PCR for the detection of constituents of animal origin in feed materials and compound feed. **This SOP is applicable in accordance with the last paragraph of point 1 of Annex VI to Commission Regulation (EC) No 152/2009 as lastly amended by Commission Implementing Regulation (EU) No 2020/1560.**

The current SOP details the operational protocol that has to be followed in order to control the application of the prohibitions laid down in Article 7 and Annex IV to Regulation (EC) N°999/2001 (feed ban). Depending on the declared and / or detected constituents of animal origin, a specific sequence of techniques and decisions has to be followed in order to avoid performing unnecessary analyses while ensuring the secure monitoring of the products.

Table 1: The SOP takes into consideration the current European Union legislation regarding the feed ban, which main restrictions are presented in Table 1. Summary of PAP and constituents of animal origin currently authorised in animal feed in the EU.

	Feed for farmed animals other than fur animals			Pets and fur animals
	Ruminants	Non ruminants (except aquaculture)	Aquaculture	
Ruminant PAP (incl. ruminant blood meal)	U	U	U	A
Ruminant blood products	U	U	U	A
Non-ruminant blood products	U	A	A	A
Pig and poultry PAP	U	A†	A	A
Non-ruminant PAP other than from pig and poultry	U	U	A	A
Pig and poultry blood meal	U	A†	A	A
Non-ruminant blood meal other than from pig and poultry	U	U	A	A
Insect PAP	U	A‡	A	A
Fishmeal	U*	A	A	A
Ruminant collagen and gelatine	U	A	A	A
Non-ruminant collagen and gelatine	A	A	A	A
Hydrolysed proteins from ruminants other than those derived from hides and skins	U	U	U	A
Hydrolysed proteins from ruminants derived from hides and skins	A	A	A	A
Hydrolysed proteins from non-ruminants	A	A	A	A
Di and tricalcium phosphate of animal origin	U	A	A	A
Eggs and egg products, milk and milk products, colostrum and derivates	A	A	A	A
Animal proteins other than those mentioned ones	U	A	A	A

U: unauthorised
A: authorised
*: fishmeal is allowed for unweaned ruminants in milk replacers
†: for use only for pigs and poultry, intraspecies recycling is prohibited
‡ : for use only for pigs and poultry

The techniques used are light microscopy and / or PCR described in Annex VI to Commission Regulation (EC) No 152/2009. The results of the analyses are expressed as detection / non detection of definite animal particles or DNA.

2. SUMMARY

This SOP details the operational protocol that has to be followed in order to control the application of the prohibitions laid down in Article 7 and Annex IV to Regulation (EC) N°999/2001 (feed ban). The operational protocol is based on the use of the light microscopy and the PCR methods, alone or in combination. Following the protocol provides information on the content of a sample in terms of animal constituents.

3. VALIDATION STATUS AND PERFORMANCE CHARACTERISTICS

NA

4. DEFINITIONS

- Feed : compound feed as defined in Article 3(1)(h) of Regulation (EC) No 767/2009
- Feed material : products of vegetable or animal origin as defined in Article 3(1)(g) of Regulation (EC) No 767/2009

Abbreviations used:

- SOP : standard operating procedure
- NA : not applicable
- PCR : polymerase chain reaction
- PAP : processed animal proteins

Reminder: By definition, PAP does not include blood products, milk, milk-based products, milk-derived products, colostrum, colostrum products, centrifuge or separator sludge from milk processing, gelatine, hydrolysed proteins and dicalcium phosphate, eggs and egg-products, including eggshells, tricalcium phosphate and collagen. However, PAP includes blood meal and fishmeal.

5. HEALTH AND SAFETY WARNINGS

NA

6. EQUIPMENT AND MATERIALS

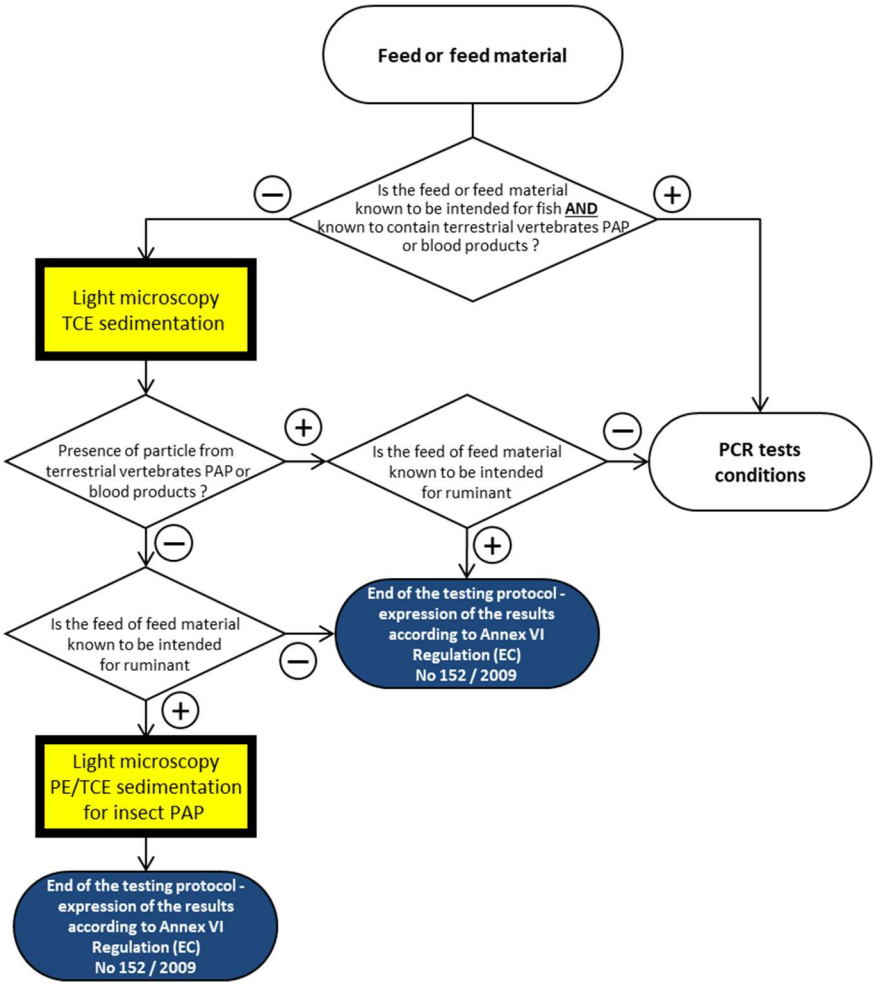
NA

7. STEP BY STEP PROCEDURE

7.1. Sample preparation

NA

7.2. Protocol for the determination of animal constituents in feed or feed material



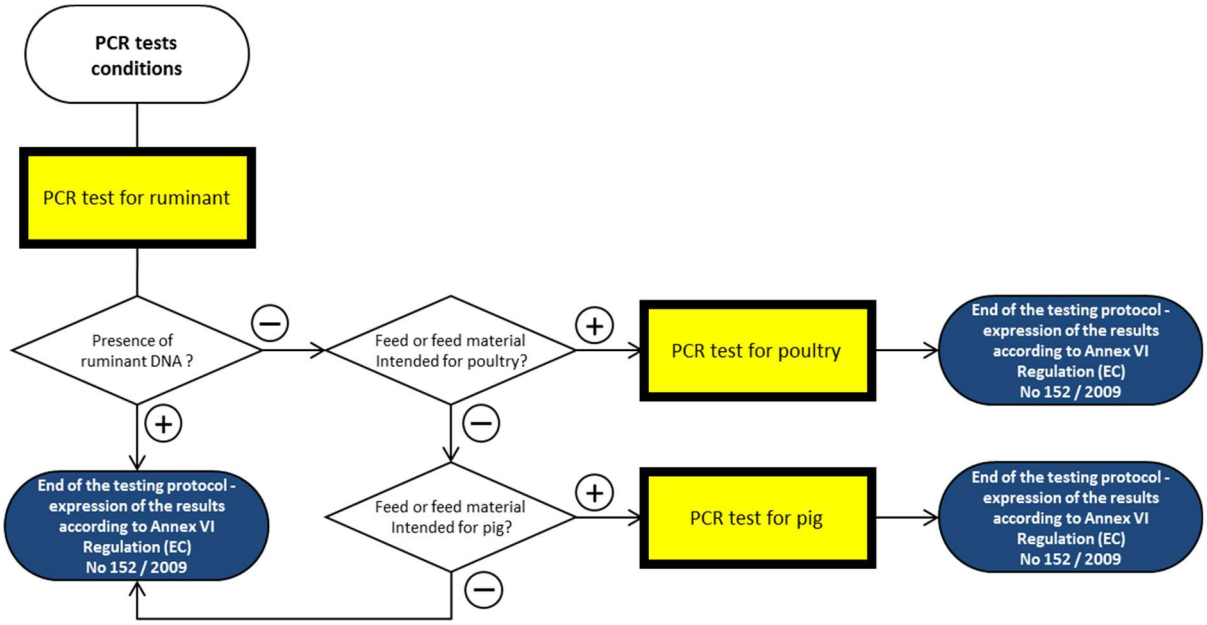


Figure 1. Operational protocol for the analysis of feed or feed material.

The results of all analyses performed shall be reported.

Light microscopy shall be applied in the first instance in all cases EXCEPT if the feed or feed material is known to be intended for fish AND is known to contain terrestrial vertebrates PAP and/or blood products, as indicated from the declaration of content or the labelling. In the latter case no microscopic analysis is necessary and the PCR methods shall be directly applied.

As soon as one particle from terrestrial vertebrates PAP and/or blood products is detected by light microscopy, or the presence of such products is indicated from the declaration of content or labelling, the PCR methods shall be applied

When the feed material is a PAP, without any information on its intended farmed animal category, it shall be analysed both by light microscopy and PCR for each target.

In absence of any information on the destination of a feed or feed material, when presence of terrestrial vertebrates' particle is detected by light microscopy it shall be analysed by PCR for target.

The PCR test for ruminant should NOT be applied in the following specific cases, as it will not provide any useful additional information: if milk or dairy products is detected by microscopy or indicated from the declaration of content or labelling, as such products will often trigger a positive response for ruminant DNA by PCR.

8. INTERPRETATION OF RESULTS

The conclusion to be drown by the competent authority from the results obtained following the protocol combining light microscopy and PCR should take the declaration of content and / or the destination of the feed or feed material into consideration in order to take a decision about compliance or non-compliance of the product.

9. REFERENCES

Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Official Journal of the European Union, L147, 31.5.2001, p 1–40.

Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union, L54, 26.2.2009, p 1–130.

Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. Official Journal of the European Union, L229, 1.9.2009, p 1–28.

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. Official Journal of the European Union, L20, 23.1.2013, p 33-43.

Commission Implementing Regulation (EU) 2020/1560 of 26 October 2020 amending Annex VI to Regulation (EC) No 152/2009 laying down the methods of analysis for the determination of constituents of animal origin for the official control of feed. Official Journal of the European Union, L357, 27.10.2020, p 17–23.

Version	Publication date	Application date	Repeal date
5.0	10.09.2021	15.09.2021	
4.1	20.05.2021	25.05.2021	10.09.2021
4.0	10.12.2020	15.12.2020	25.05.2021
3.0	13.05.2015	18.05.2015	15.12.2020
2.0	29.04.2013	04.05.2013	18.05.2015
1.0	03.04.2013	03.05.2013	04.05.2013