



## EURL-AP Standard Operating Procedure

# Operational protocols for the combination of light microscopy and PCR

| <b>Experts for edition and revision</b>  |   |
|--|---|
| <b>Version 1.0</b>   | <b>Last major revision</b>  |
| Alessandro BENEDETTO<br>Gilbert BERBEN<br>Hermann BROLL<br>Olivier FUMIÈRE<br>Geneviève FRICK<br>Christoph HALDEMANN<br>Lotte HOUGS<br>Jette MÅRTENSSON<br>Inge PARADIES-SEVERIN<br>Ingrid SCHOLTENS<br>Igor UJČIČ VRHOVNIK<br>Pascal VEYS | Aggeliki DEVARI<br>Giannos IOANNOU<br>Nelly JARDY<br>Daniela MARCHIS<br>Begoña MUÑIZ ALCARAZ<br>Pascal VEYS |

## 1. SCOPE AND PURPOSE

The purpose of the SOP is to present the operational protocols combining light microscopy and PCR for the detection of constituents of animal origin in feed materials and compound feed. **This SOP is a binding complement to point 1 of Annex VI to Commission Regulation (EC) No 152/2009 as lastly amended by Commission Regulation (EU) No 51/2013.**

The current SOP details the operational protocols that have to be followed, depending on the type of feed being analysed, 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 final destination of the compound feed or feed materials determines the operational protocol which has to be followed.

Taking into consideration the current European Union legislation regarding the feed ban, the two following protocols for the detection of constituents of animal origin shall be applied depending on the type of feed or feed material being tested.

## 2. SUMMARY

This SOP details the operational protocols that have to be followed, depending on the type of feed being analysed, 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 final destination of the feed or feed materials determines the operational protocol which has to be followed. Two operational protocols are described: one for feed or feed material intended for farmed animals others than aquaculture and fur animals , and a second for feed or feed material intended for aquaculture animals.

## 3. VALIDATION STATUS AND PERFORMANCE CHARACTERISTICS

NA

## 4. DEFINITIONS

Abbreviations used :

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

## 5. HEALTH AND SAFETY WARNINGS

NA

---

\* *Feed and feed ingredients intended for fur animals are not subjected to EU feed ban regulations. It is only referred to them as they are also farmed animals.*

**6. EQUIPMENT AND MATERIALS**

NA

**7. STEP BY STEP PROCEDURE**

**7.1. Sample preparation**

NA

**7.2. Protocol 1: for feed or feed material intended for farmed animals other than aquaculture and fur animals (Figure 1)**

For this protocol analysis by light microscopy is sufficient to detect the presence of prohibited constituents of animal origin.

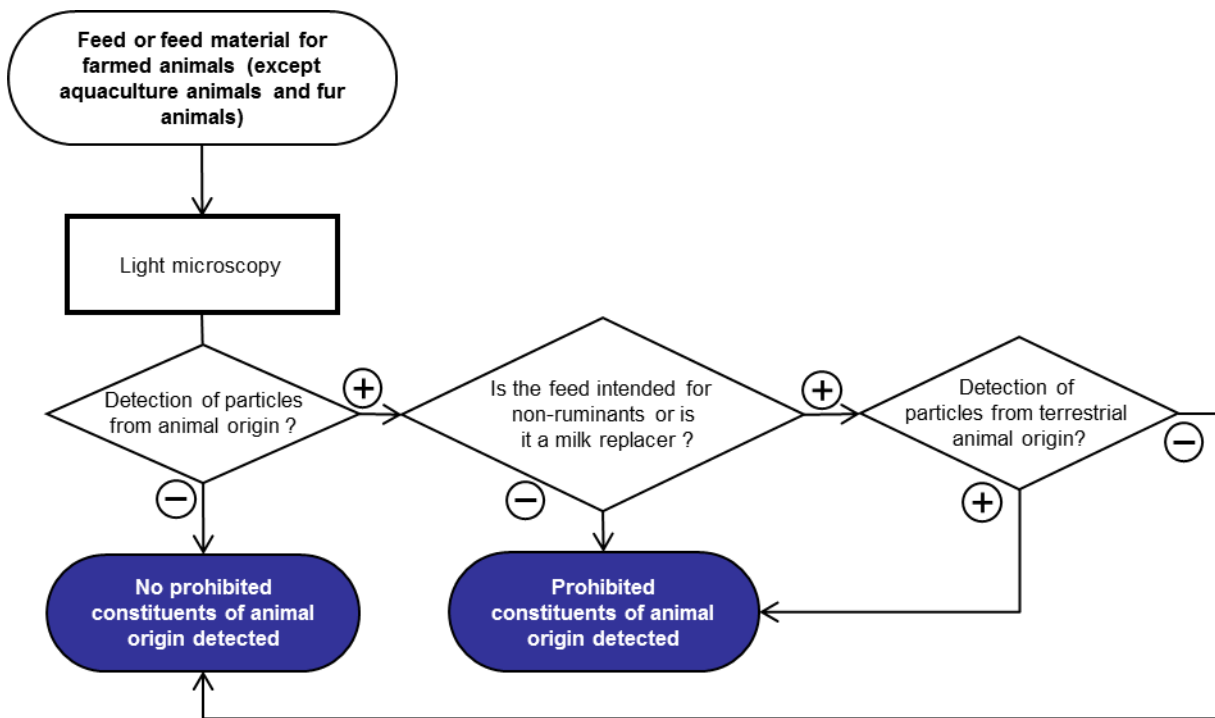


Figure 1. Operational protocol for the analysis of feed or feed material intended for farmed animals other than aquaculture animals and fur animals (e.g. feed for ruminants, pigs, poultry, horses, rabbits,...).

### 7.3. Protocol 2: for feed or feed material intended for aquaculture animals (Figure 2)

Reminders:

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

*The following ruminant materials are prohibited in feed for aquaculture animals: PAP, blood meal, blood products, gelatine and collagen, hydrolysed proteins other than those derived from hides and skins.*

For feed or feed material intended for aquaculture animals (Figure 2 next page), either the light microscopy or the PCR method may be performed in the first instance depending on the composition of the feed.

Light microscopy shall be applied in the first instance when the composition of the feed is unknown or when the feed is not supposed to contain PAP or blood product.

If the feed is known to contain PAP, as indicated for instance from the declaration or the labelling, the PCR method shall be applied at first.

If the feed is known to contain blood products, as indicated for instance from the declaration or the labelling, the PCR method shall only be applied in the first instance at a frequency of one in ten samples (1/10) chosen on a random basis (the random character of this selection must be recorded so that it could be proven). However, in case of suspicion of irregularity or previous PCR tests have given positive results, the PCR method shall be applied in the first instance on all feed or feed material samples from the same origin.

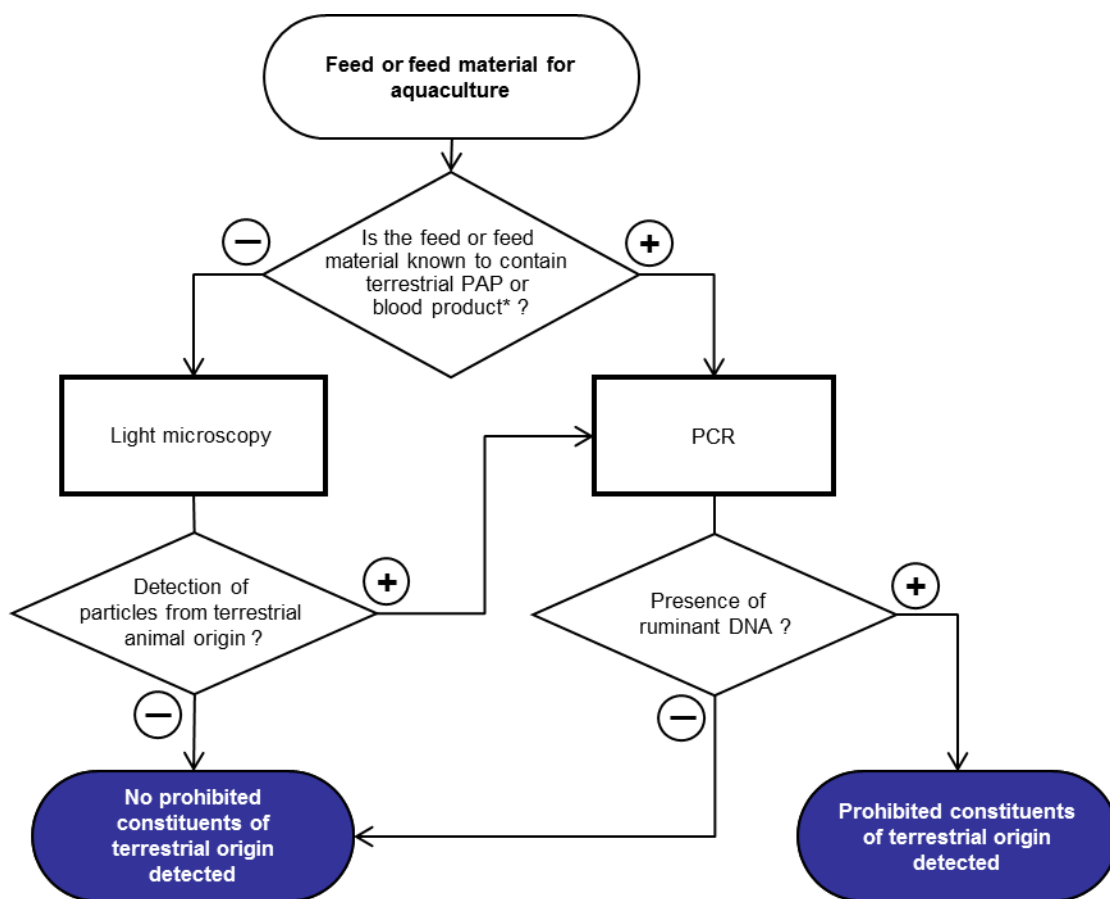


Figure 2. Operational protocol for the analysis of feed or feed material intended for aquaculture animals. (\* in case of blood product cf. last paragraph of section 7.3. for details)

### 8. INTERPRETATION OF RESULTS

NA

### 9. REFERENCES

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, 33-43.

| Version | Publication date | Application date | Repeal date |
|---------|------------------|------------------|-------------|
| 3.0     | 13.05.2015       | 18.05.2015       |             |
| 2.0     | 29.04.2013       | 04.05.2013       | 18.05.2015  |
| 1.0     | 03.04.2013       | 03.05.2013       | 04.05.2013  |