Methods evolution for PAP detection

Past, present, future

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Content

- Current legal framework
- Reference method**s** for the detection of PAPs
- Advantages and drawbacks of methods
- Combinations of methods
- Complementary methods in development

Introduction: use of animal proteins in feed

1986

First BSE case in UK

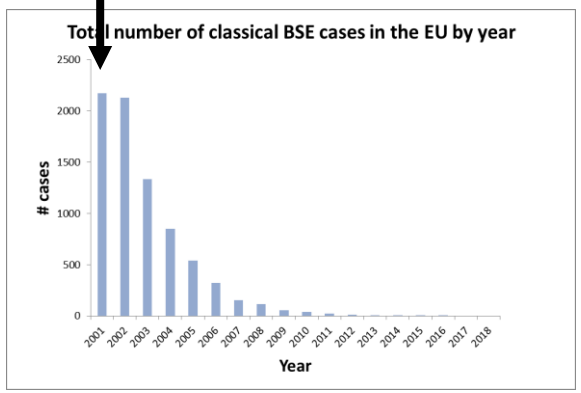
1994

Partial Feed ban: ruminant feed

2001:

Total Feed ban: farmed animals

Feed ban



2013

2017

2021

Introduction: current feed ban provisions

By-product nature:

- Species of origin
- By-product type

Animal by-products of Category 3

		Destination					
		Feed intended for farmed					
		Rum.	Pigs	Poultry	Others	Fish	Pets and fur animals
Ruminant	PAP						
	Blood meal						
	Blood products						
	Gelatine and collagen		2021				
	Hydrolysed proteins other than those derived from hides/skins						
	Hydrolysed proteins derived from hides/skins						
	Milk, milk products, colostrum						
Fish	Fishmeal	+					
Pig	PAP			2021		2013	
	Blood meal						
	Blood products						
	Gelatine and collagen						
	Hydrolysed proteins other than those derived from hides/skins						
	Hydrolysed proteins derived from hides and skins						
Poultry	PAP		2021			2013	
	Blood meal						
	Blood products						
	Egg, egg products						
	Hydrolysed proteins other than those derived from hides/skins						
	Hydrolysed proteins derived from hides/skins						
Other non-ruminant	PAP					2013	
	Blood meal						
	Blood products						
	Gelatine and collagen						
	Hydrolysed proteins other than those derived from hides/skins						
	Hydrolysed proteins derived from hides/skins						
Insect	PAP		2021			2017	
Di and tricalcium phosphate of animal protein							
Animal proteins other than those mentioned							

+except in milk replacer

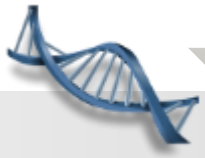
Methods of PAPs detection : back in time

Commission Directive No 88/1998 establishing guidelines for the microscopic identification and estimation of constituents of animal origin for the official control of feedingstuffs

Commission Directive No 126/2003 on the analytical method for the determination of constituents of animal origin for the official control of feedingstuffs

Commission Regulation No 152/2009 laying down the methods of sampling and analysis for the official control of feed

Commission Regulation No 51/2013 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



Methods of PAPs detection

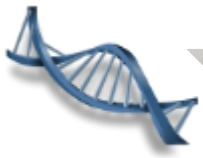
Commission Implementating Regulation No 2020/1560

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

Commission implementing Regulation No 2022/893 amending Annex VI to Regulation (EC) No 152/2009 as regards the methods of analysis for the detection of constituents of terrestrial invertebrates for the official control of feed

For a readable text :

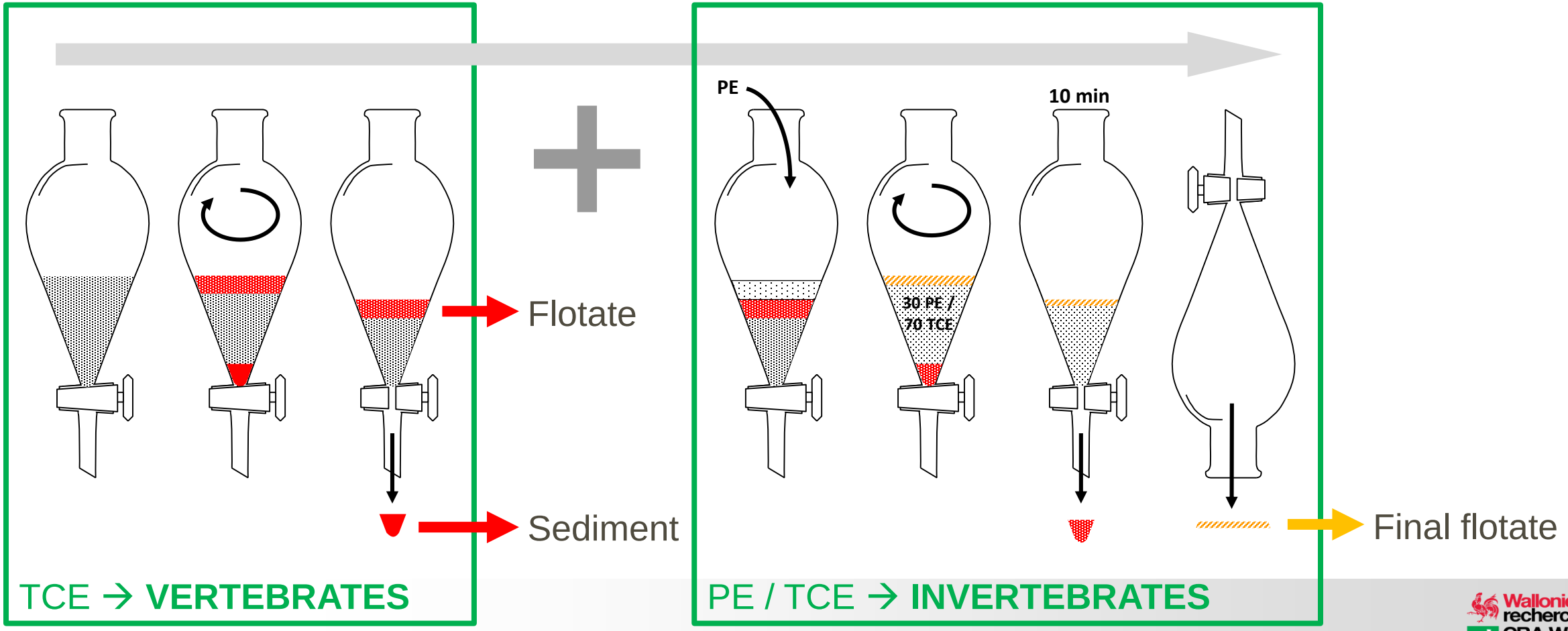
look for the consolidated version of Commission Regulation No 152/2009.



Light microscopy

Principle

- Observation of identifiable structures on # fractions obtained by sedimentation(s)



Light microscopy

Principle

- Observation of identifiable structures on # fractions obtained by sedimentation(s)

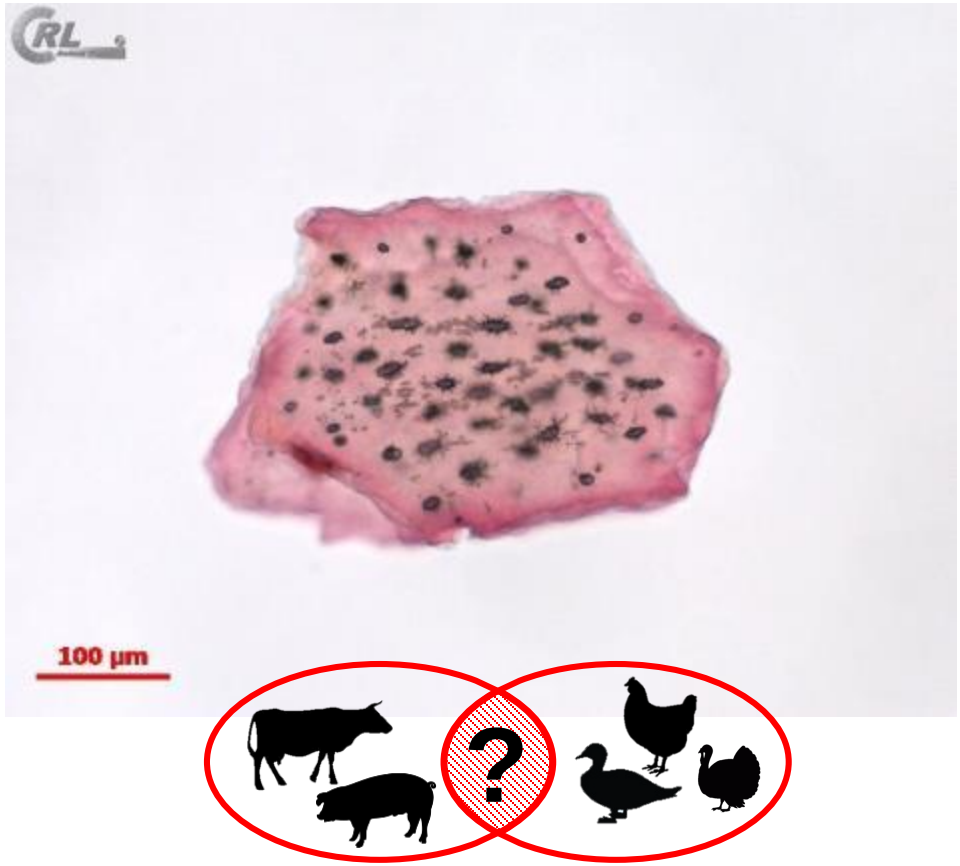
Staining can be used :

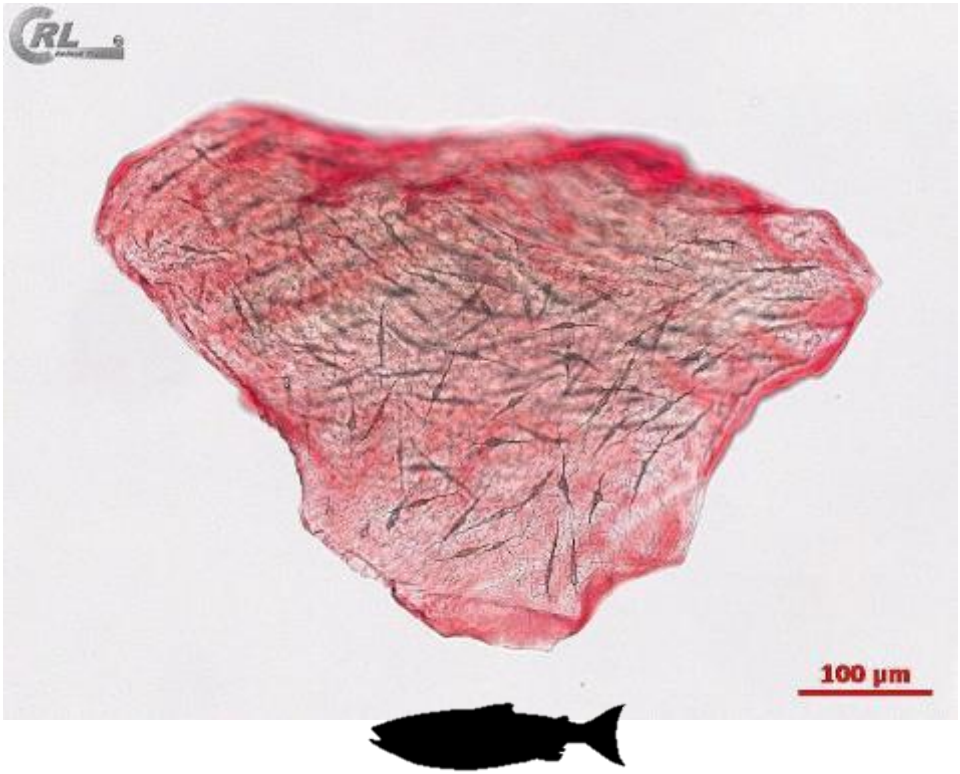
- Alizarin Red → bones, scales
- Fehling → Muscle fibres
- Cystine reagent → hairs, feathers
- ….

Distinction of PAPs from terrestrial vertebrates, fish and terrestrial invertebrates

Light microscopy

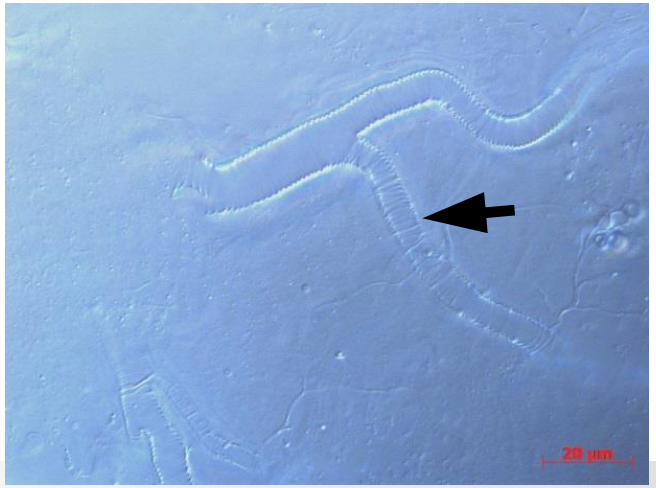
Identification : terrestrial vertebrates ⟷ fish





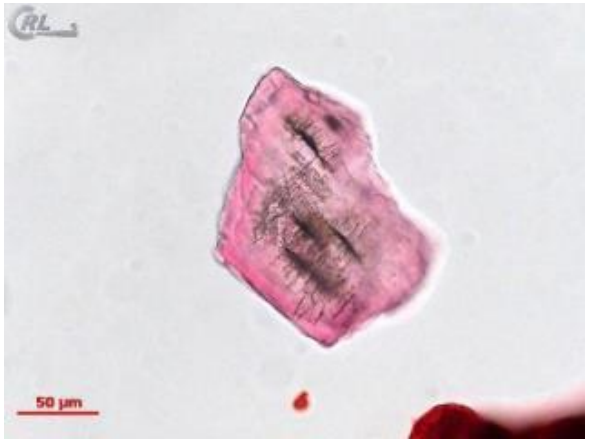
Light microscopy

Identification : terrestrial invertebrates



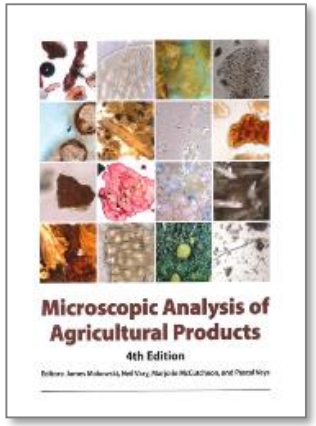
Light microscopy

Expertise... (plant or animal ?)



Light microscopy: advantages and limitations

- Ease of use
- Cheap
- Very sensitive (<0.01%)
- References

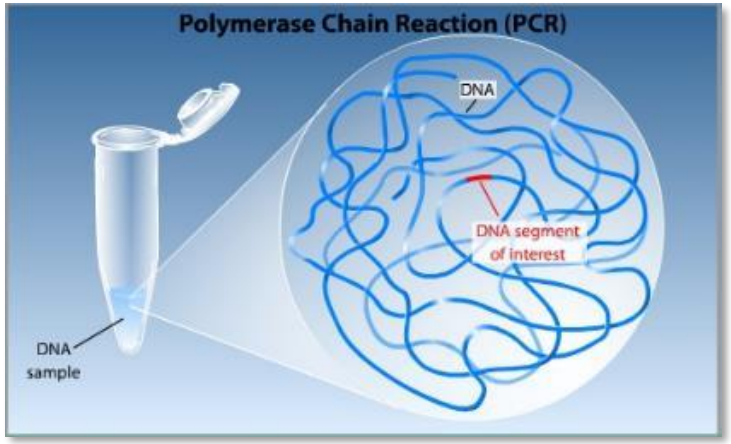


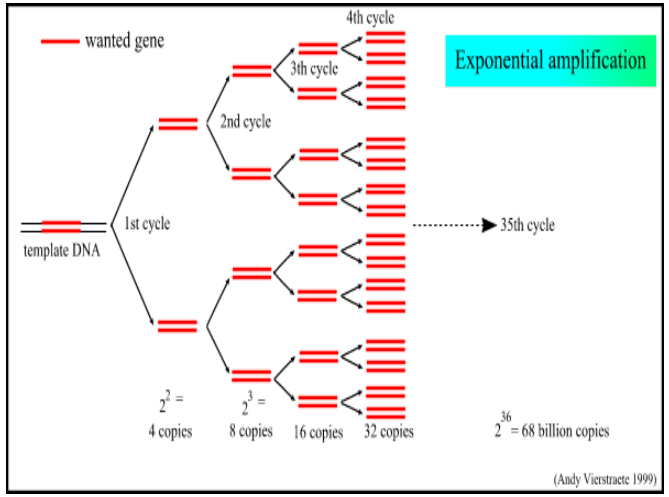
- Skilled people, real microscopists
  - Continuous training to keep skills at the top
  - new feed compounds and by products
- No species identification
- Based on particle detection only, some ingredients are not always visible
- Only qualitative…!

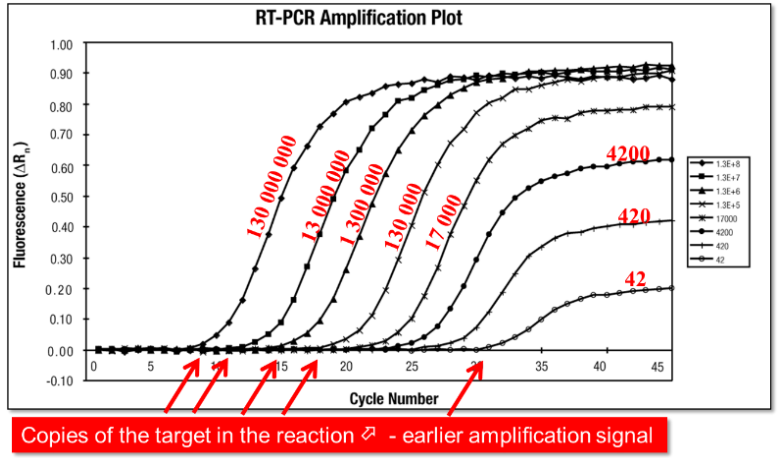
Real time Polymerase Chain Reaction

Principle

- Monitoring of the amplification of a targeted DNA molecule
- The greater the quantity of targeted DNA in the material, the earlier the fluorescent signal is above the fluorescence threshold

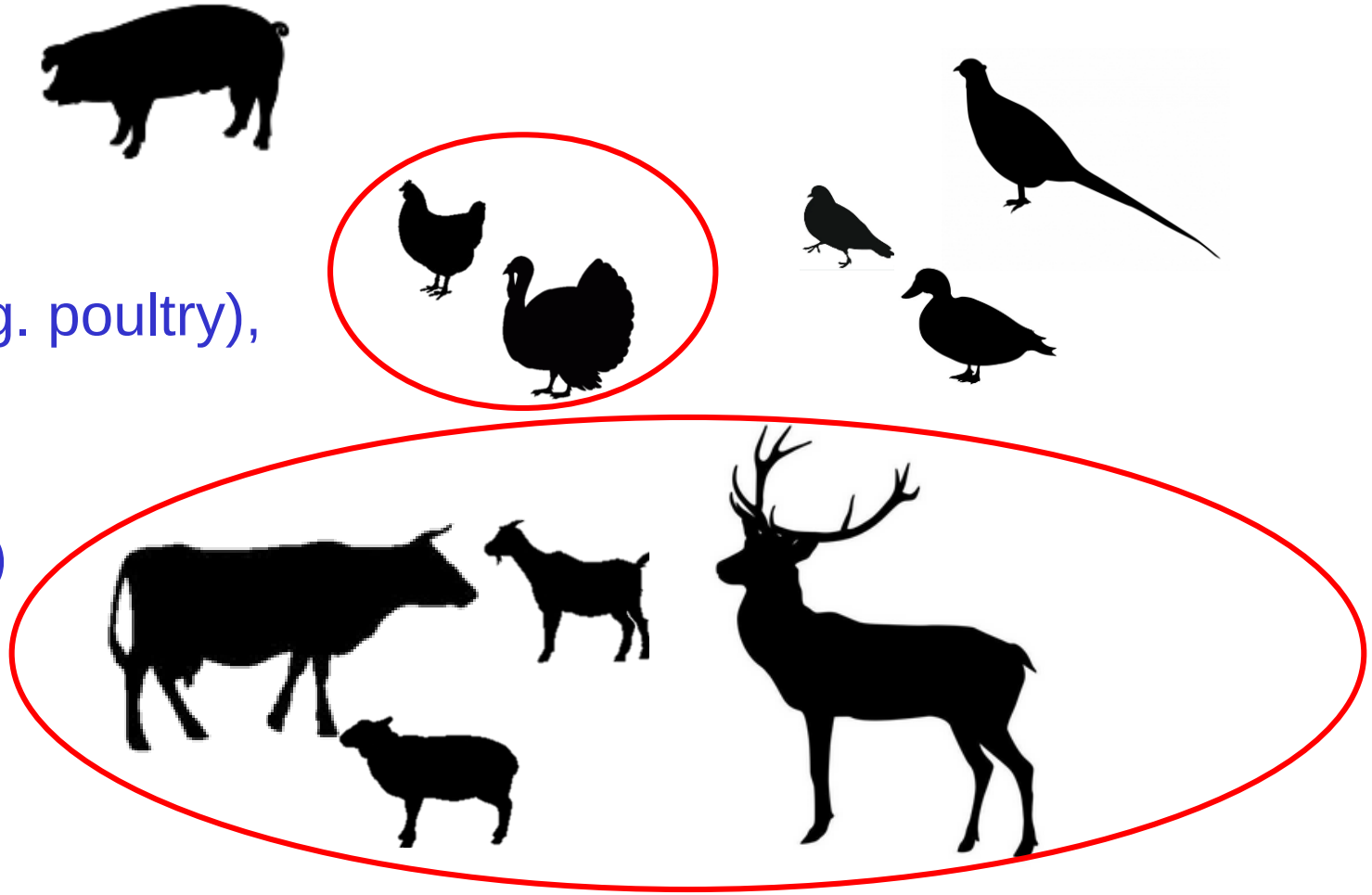






Real time Polymerase Chain Reaction

- Targeted DNA is specific to
  - ❑ a species (e.g. pig),
  - ❑ a group of species (e.g. poultry),
  - ❑ a taxon (e.g. ruminant)

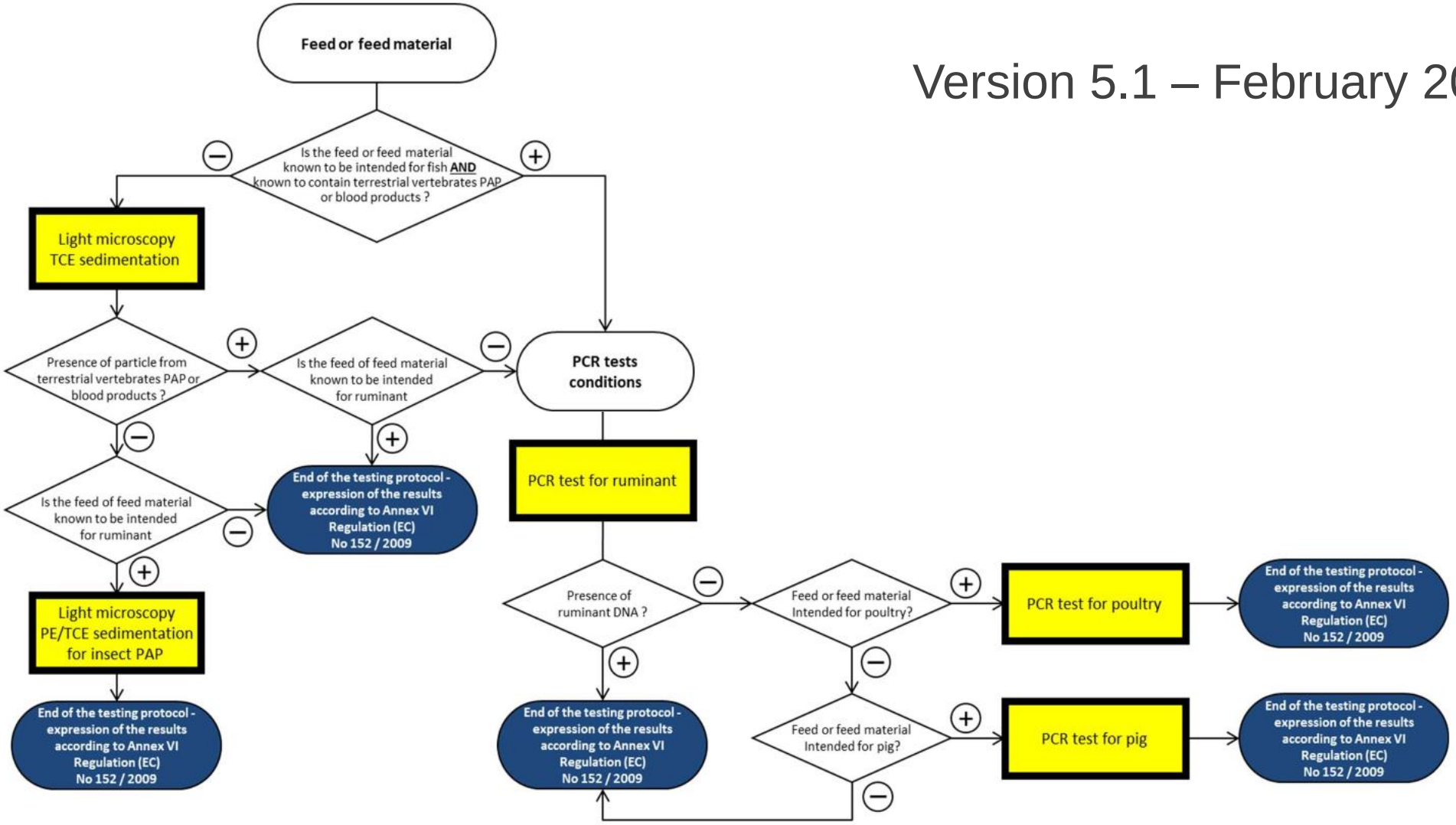


PCR: advantages and limitations

- Species or taxa identification (e.g. ruminant, pig,…)
- Very sensitive (~ 0.1%)
- Common technique
- Able to detect DNA degraded by heating processes

- Not able to determine the source of the DNA (e.g. milk vs bovine PAP)
- Trained people
- Specific and costly equipment
- Only qualitative…!

SOP on combination of methods



Version 5.1 – February 2022

SOP on combination of methods

First LM

- Exception : if feed or feed material known to be intended for fish AND known to contain terrestrial vert. PAP or blood products
    - No LM → directly PCR

Then PCR

- Conditions :
    - Terrestrial vert. PAP and/or blood product detected by LM
    - If feed material intended to non-ruminant

No PCR if dairy product is present !

PAPs without information on use = LM and PCR

SOP on combination of methods : example

+ Bovine PAP & Porcine plasma powder

+ Porcine PAP & Milk powder

Complementary method

NON-OFFICIAL

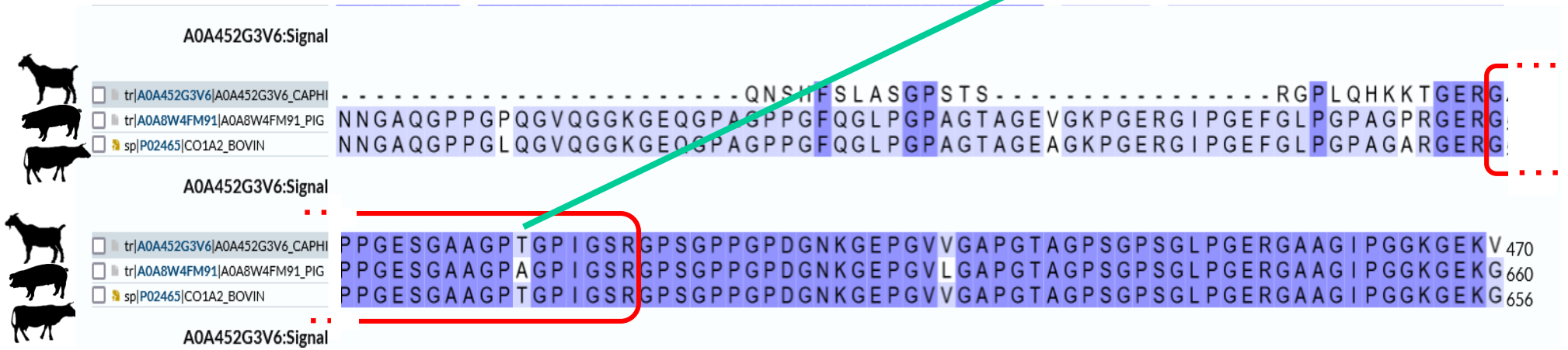
⇨ Mass spectrometry (MS)-based proteomics

Principle:

- **Targets = Proteins**, peptide (Amino Acid sequences) (>< genomics for DNA sequences)
- **MS**: identification of ionised peptides according to their mass-to-charge ratio (m/z)
- Provides information about the tissue and species of origin

Average residue mass (Da):
- Alanine (A) = 71.08
- Threonine (T) = 101.11

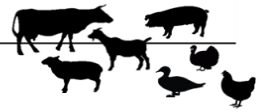
Amino acid alignment for Collagen alpha-2(I) chain



Complementary method

NON-OFFICIAL

⇨ Mass spectrometry (MS)-based proteomics

Targeted by-products	Proteins:	Targeted species
Milk	Casein	
	Beta-lactoglobulin	
Blood, PAP	Hemoglobin	
Plasma	Serotransferrin	
	Apolipoprotein	
PAP, Gelatine	Collagen	

+ Bovine PAP & Porcine plasma powder

✗ ⇨ Bovine collagen & Bovine hemoglobin

+ Porcine PAP & Milk powder

✓ ⇨ Bovine casein & Beta-lactoglobuline

Take-home messages

1. **Each method has its pros and cons.**

   Combination of methods ☺ **BUT** Higher complexity for fraud detection ☹

   e.g. technical skills, LOD, …

2. **Today : Is everything under control ? Can all fraud be detected?**

   …No ☹

Ex : porcine blood products vs blood meal

Animal by-products of Category 3		Rum.	Pigs
Ruminant	PAP		
	Blood meal		
	Blood products		
	Gelatine and collagen		
	Hydrolysed proteins other than those derived from hides/skins		
	Hydrolysed proteins derived from hides/skins		
	Milk, milk products, colostrum		
Fish	Fishmeal	+	
Pig	PAP		
	Blood meal		
	Blood products		
	Gelatine and collagen		

3. **Tomorrow?** New relaxation of the feed ban???

Ex : bovine blood products vs blood meal

=> Need of techniques to anticipate policy regulation in the matter?

Questions ?