



# EURL-AP Activities

Joint Workshop EURL-TSE/AP - Rome, Italy, 14-15 May 2024

European Union Reference Laboratory for Animal Proteins in feedingstuffs

Walloon Agricultural Research Centre

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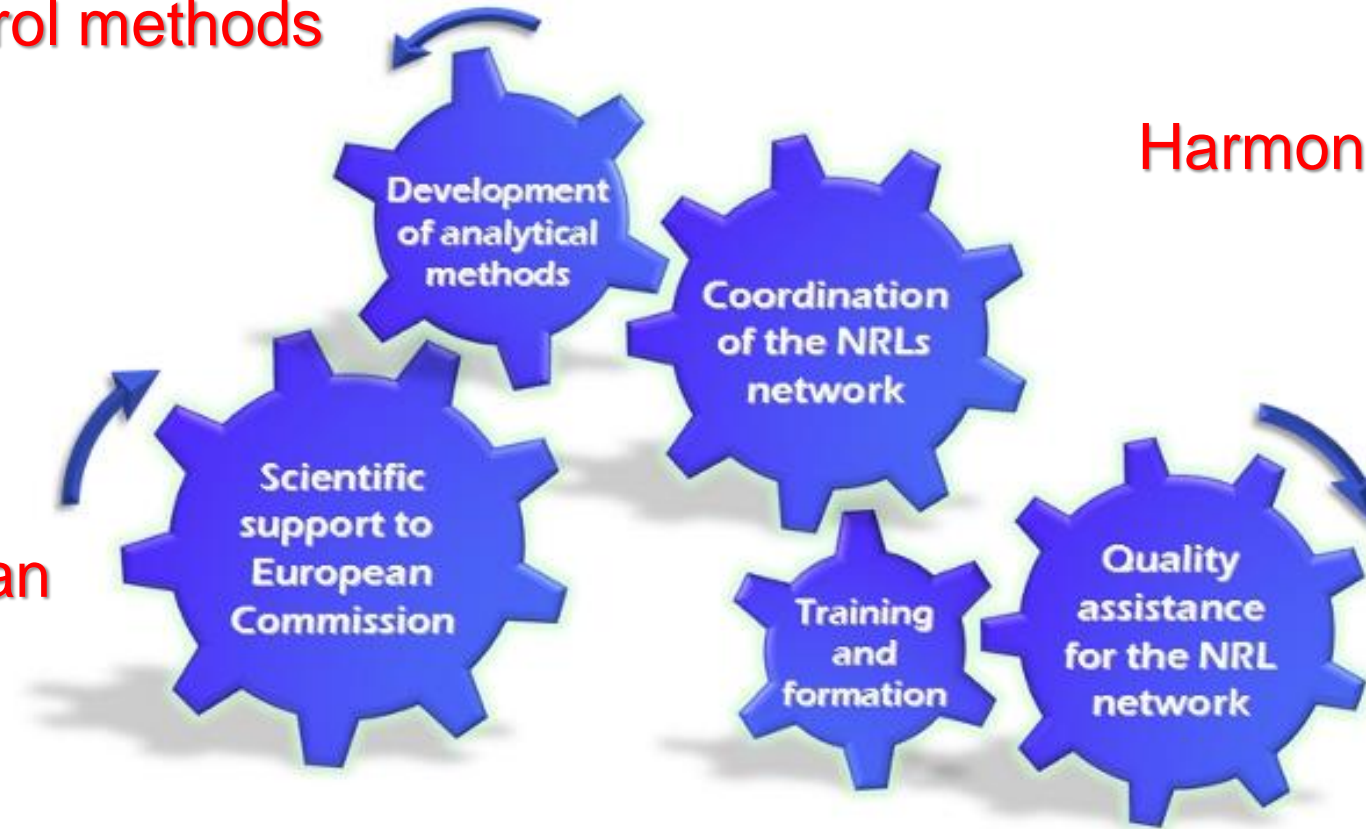
secretary@eurl.craw.eu – website: <https://eurl.craw.eu>



Official control methods

Harmonization

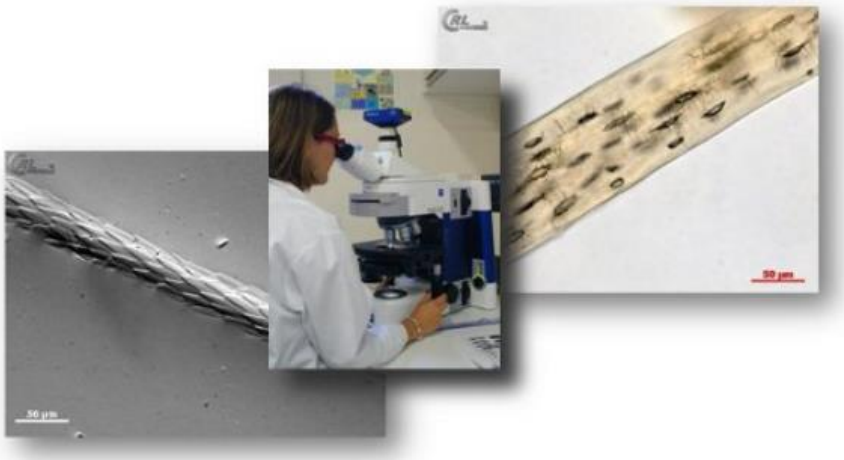
Scientific support



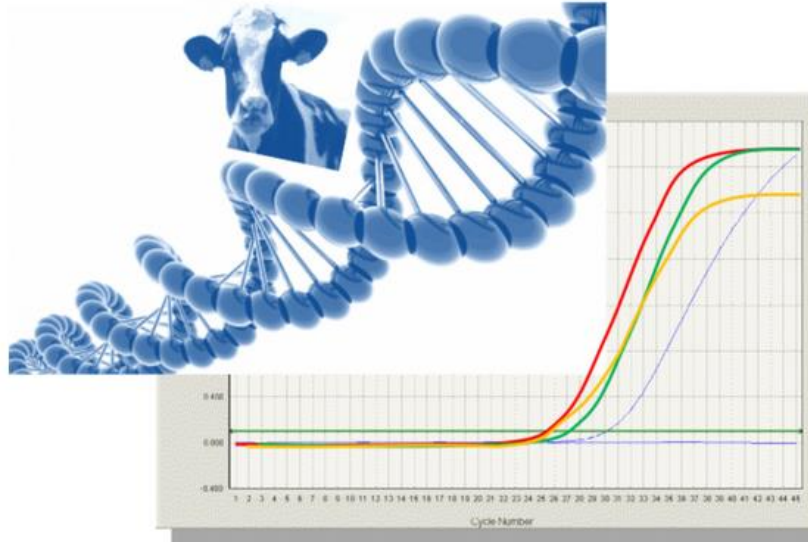
Continuous training

Lifting of the Feed ban

## Light microscopy



## Real-time PCR



## Mass spectrometry UHPLC-MS/MS



**Challenge for the EURL-AP and the NRLs :  
To become experts of very different techniques**

# Specificity of EURL-AP



2<sup>nd</sup> challenge :  
sample and legislation complexity

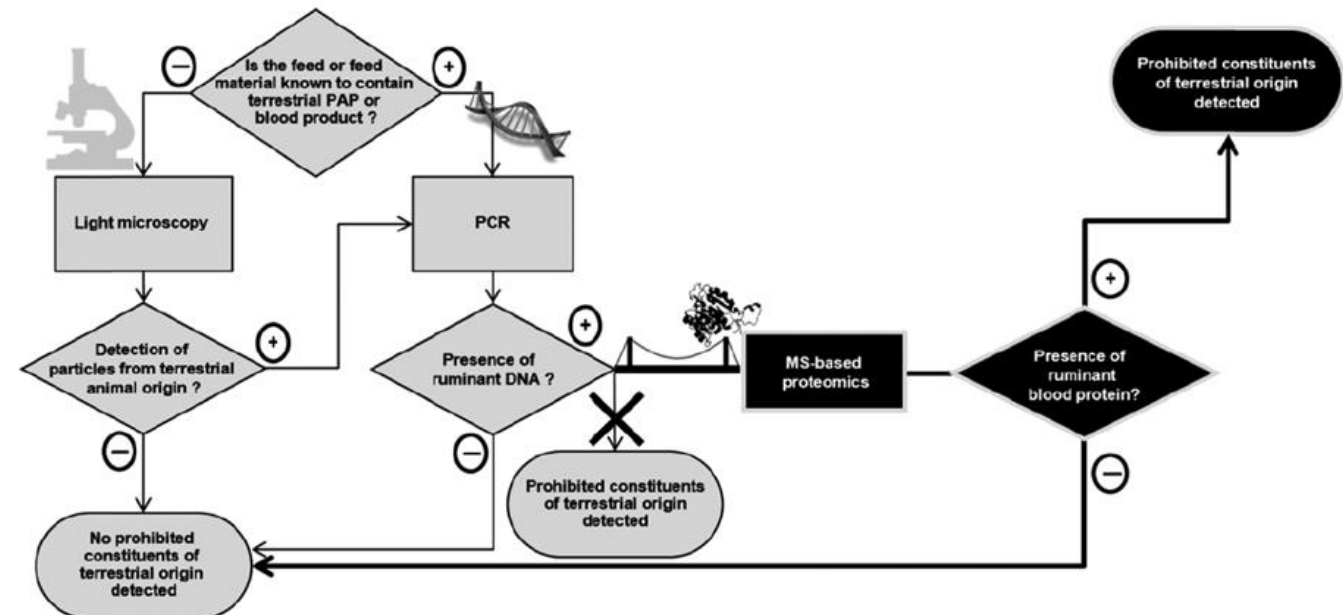
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adequate combination of techniques

↓

relevant conclusion

Animal by-products of Category 3		Feed intended for farmed					
		Rum.	Pigs	Poultry	Others	Fish	Pets and fur animals
Ruminant	PAP						
	Blood meal						
	Blood products						
	Gelatine and collagen						
	Hydrolysed proteins other than those derived from hides/skins						
Fish	Hydrolysed proteins derived from hides/skins						
	Milk, milk products, colostrum						
	Fishmeal						
	PAP						
	Blood meal						
Pig	Blood products						
	Gelatine and collagen						
	Hydrolysed proteins other than those derived from hides/skins						
	Hydrolysed proteins derived from hides/skins						
	PAP						
Poultry	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						
	Blood meal						
	Blood products						
	Gelatine and collagen						
	Hydrolysed proteins other than those derived from hides/skins						
Insect	Hydrolysed proteins derived from hides/skins						
	PAP						
	Di and tricalcium phosphate of animal protein						
Animal proteins other than those mentioned							



# Combined proficiency tests

- Organisation of annual PT combining microscopic and PCR analyses
- Under ISO17043
- Participation of 3<sup>rd</sup> country official labs  
Iceland, Serbia, Norway  
China, Peru, Argentina, Australia,  
South Africa

Samples = challenge to produce  
Combination of methods not anymore  
under evaluation

The report summary has to be printed, signed, scanned and sent by email simultaneously to the sending of the Excel file.

Report summary Proficiency Test Microscopy-PCR 2023/01

Laboratory identification code : 1

Responsibility agreement was : not signed

LIGHT MICROSCOPY ANALYSES:

Sample N°	Terrestrial vert. part.	Details of terrestrial vert. part.	Terrestrial invert. part.	Details of terrestrial invert. Part.	Fish part.	Details of fish part.	Sedimentation(s)	Number of determinations
1								
2								
3								
4								
5								
6								

Free comment

PCR ANALYSES:

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies:				cycles
Copy number at the cut-off:				copies
Master mix used:				

Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Free comment
1				
2				
3				
4				
5				
6				

Date : ..... Name : ..... Signature : .....  
Firstname : .....



- Light microscopy
- PCR
- Mass spectroscopy
- Hydrolysed proteins
- GTH

Expertise and support to :

- Choice of strategy to follow (e.g. PCR targets)
- Redaction of protocols (SOPs see after)



# Standard Operating Procedure (SOPs)



- Complements to the regulations
- Technical support,
- explanations to the Annex VI
- Better flexibility in case of changes in the protocols



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## EURL-AP Standard Operating Procedure Detection of ruminant DNA in feed using real-time PCR

Method development:  
TNO Triskelion bv  
Method assessment and validation:  
EURL-AP

Experts for edition and revision	
Version 1.0	Last major revision
Alessandro BENEDETTO Gilbert BERBEN Hermann BROLL Olivier FUMIERE Christoph HALDEMANN Lotte HOUJS Aline MARIEN Ingrid SCHOLTENS	EURL-AP team

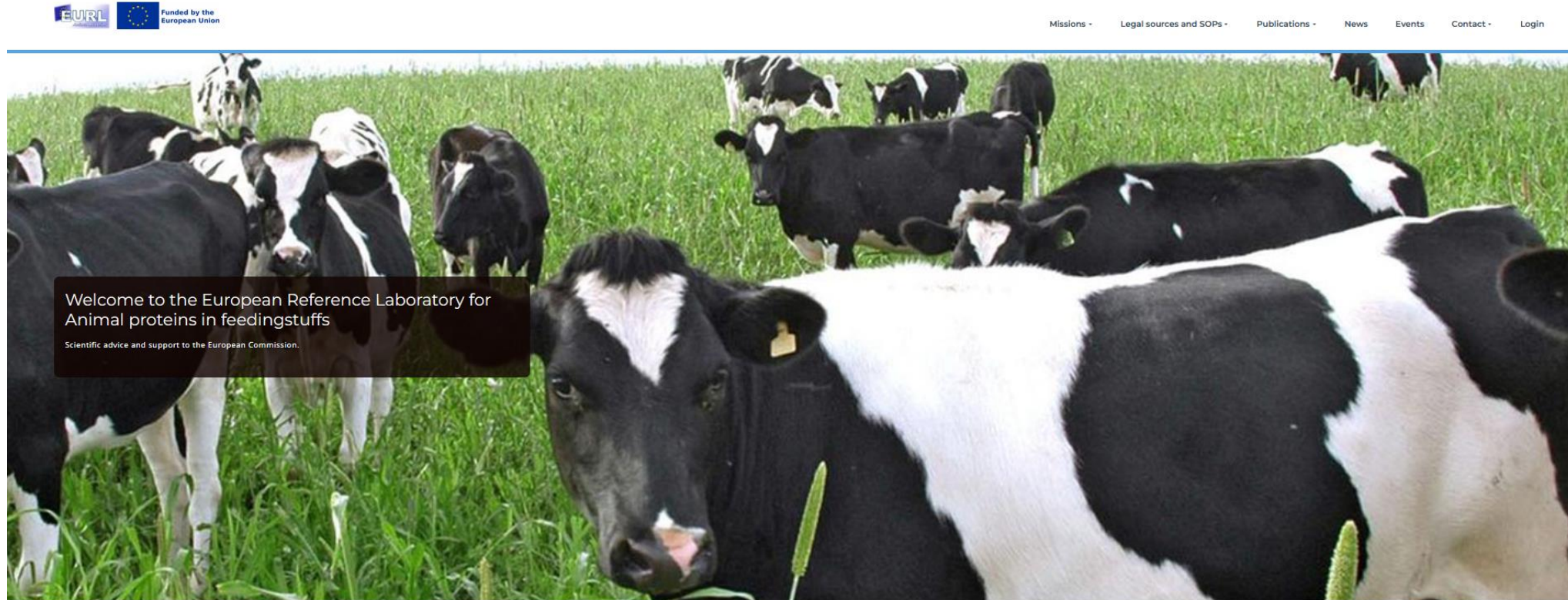
Version 1.3

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Publication date 20.05.2021  
Applicable on 25.05.2021

# Standard Operating Procedure (SOPs)

<https://www.eurl.craw.eu/>



## Missions

Our mission, in the global framework of the eradication of transmissible spongiform encephalopathies (TSE), focuses on the detection and identification of prohibited animal proteins in feed.



## Legal sources and SOPs

In Europe a very strict legislation has been set up over the years. This resulted in the "total feed ban" or the total ban of PAPs in feed. Each member state of the European Union needs to strictly apply this legislation.



## Publications

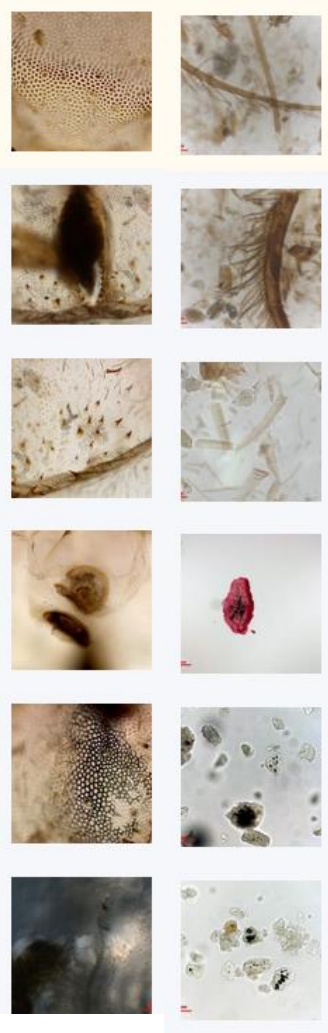
Linked to its activities, the EURL-AP team is publishing numerous papers and reports towards the scientific community or more accessible information dedicated to the general public.



## Micrograph Collection

Access here to the EURL-AP micrograph collection, the largest on-line repository of reference pictures for animal remains identification, a tool supporting the feed ban control.





Picture of Black Soldier Fly (*Hermetia illucens*) larvae cuticle

- Started in 2007;
- Basically intended to help operators;
- Over 900 high quality records;
- Public tool intended for everyone and available on the website ;
- Free use meanwhile a courtesy mention.

## On-line training



## Microscopy training

- Access to a virtual microscope that allows the participants to observe slides named according to their composition

## Production of calibrants for the PCR methods



ERM-AD482 RUMINANT pDNA CALIBRANT



ERM-AD483 PORCINE pDNA CALIBRANT



ERM-AD484k POULTRY pDNA CALIBRANT



Support to the EU Commission in the Horse meat crisis

- Not in the scope of the EURL-AP but
- EURL-AP sea mammalian PCR test not fully specific  
→ development of a horse PCR test
- Horse PCR test at disposal of the control labs on request of the Commission
- Redaction of protocols
- Production of “reference” material for semi-quantitation





# Acknowledgements to



The EURL-AP team...



...and its NRLs network