

CRL-AP & SAFEED-PAP : two European initiatives in the framework of PAP detection

Dr Vincent Baeten

Walloon Agricultural Research Centre (CRA-W, Belgium),
Department of Agricultural Products Quality

baeten@cra.wallonie.be



Community Reference
Laboratory Detection of
animal proteins in
feedingstuffs **CRL-AP**
DG-SANCO



Detection of presence of
species-specific processed
animal proteins in animal
feed **SAFEED-PAP**
DG-RESEARCH





CRL-AP activities

Activity 1 : Scientific advice and support to the European Commission

Activity 2 : Coordination of activities of NRL network

Activity 3 : Interlaboratory studies and quality assurance

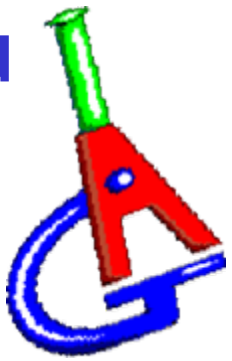
Activity 4 : Development of analytical methods and tools

Activity 5 : Workshops/trainings

Activity 1 : Scientific advice and support to the EC



- ❖ Scientific and technical assistance
- ❖ International fora/committees (EFSA, WHO/FAO, JRC, etc)
- ❖ Standardisation of analytical methods and their implementation (CEMA, ISO/CEN, OIE, IAG, etc)
- ❖ To perform analyses on samples with disputed results



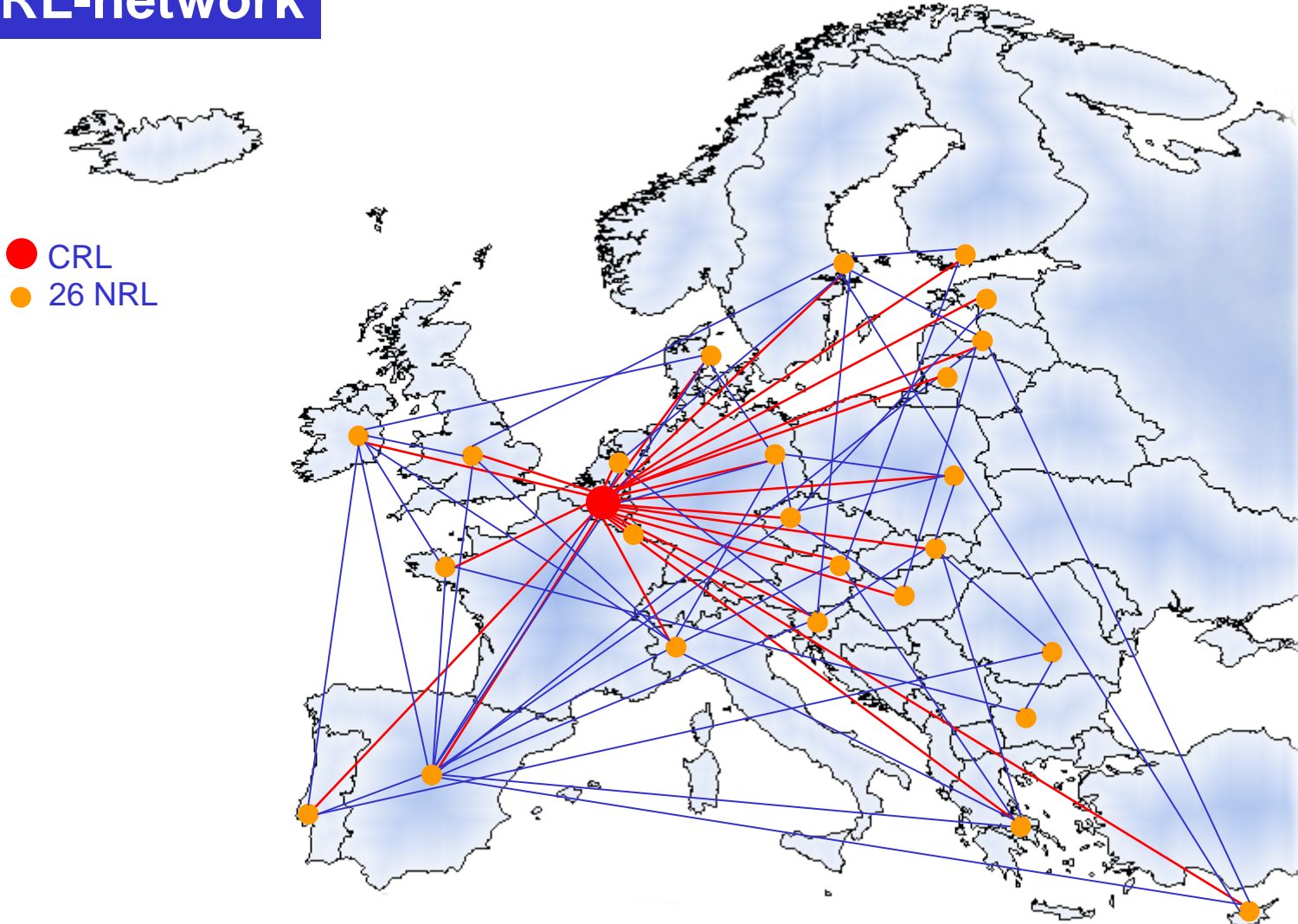
Activity 2 : Coordination of activities of NRL network

- ❖ **CRL website (internet/intranet)**
- ❖ **Four-months newsletter for NRLs**
- ❖ **Annual NRL meeting/workshop**
- ❖ **Supply information, scientific advices and protocols to NRLs**
- ❖ **Annual CRL Directors co-ordination meeting**
- ❖ **Reports of activities**

<http://crl.cra.wallonie.be/>

Actors getting connected

NRL-network



CRL-AP Annual workshops

- First workshop was organised in Belgium in April 2007

Community Reference Laboratory for Animal Proteins in Feedstuffs

Walloon Agricultural Research Centre, Quality of Agricultural Products Department
Chaussée de Namur 24, B - 5030 GEMBLoux

Tel: +32 (0) 81 62 35 74 +32 (0) 81 62 35 99
Email: pascal.veys@warcra.be vince.baeten@warcra.be

1st CRL-AP Workshop – Final Agenda
16-17-18 April 2007 Gembloux, Belgium

16th April 2007

Time	No.	Item	
14:00	0	Participants arrival - Welcome at the « Hotel 3 clés»	20 min
14:20	1	CRL work and the importance of ring trials (Koen Van Dyck, DG-Sanco, EC)	20 min
14:40	2	CRL-AP presentation (Vincent Baeten, CRL-AP)	30 min
15:10	3	NRL-AP presentation (Vincent Baeten, CRL-AP)	30 min
<i>14:40 - Coffee break -</i>			
16:00	4	Organization of the NRL-AP network (Vincent Baeten and Pascal Veyts, CRL-AP)	20 min
16:20	5	JAG presentation (Inge Parodi, LUFPA)	20 min
16:40	6	Visit of the CRL-AP facilities	30 min
<i>- Reception drink offered by CRA-W -</i>			

17th April 2007

Time	No.	Item	
9:00	7	Presentation of the CRL-AP interlaboratory study 2006 (Pascal Veyts, CRL-AP)	30 min
9:30	8	Results: qualitative part (Pascal Veyts, CRL-AP)	30 min
10:00	9	Results: quantitative part (Pascal Veyts, CRL-AP)	30 min
<i>10:30 - Coffee break -</i>			
10:50	10	Discussion of the CRL-AP interlaboratory study 2006 (NRL comments)	45 min
11:35	11	Examples of quantitative protocols (Erik Nordkvist – Sweden-NRL, Genevieve Flick – Luxembourg-NRL)	25 min
<i>12:00 - Lunch at the 'Hotel 3 clés' sponsored by the CRL-AP -</i>			
13:30	12	Quantitative analysis by classical microscopy: which improvements? (Pascal Veyts, CRL-AP + NRLs) : Correction factors, Others	30 min
15:00	13	Presentation and demonstration of APRES (Leo van Raamsdonk, RIKILT)	120 min
<i>19:30 - Meeting Dinner (post-40K participants) -</i>			

18th April 2007

Time	No.	Item	
9:00	14	CRL-AP requirements (Vincent Baeten, CRL-AP)	45 min
9:45	15	NRL-AP requirements and expectations (Vincent Baeten, CRL-AP + NRLs)	45 min
<i>10:30 - Coffee break -</i>			
10:50	16	Sample need: reference material (Vincent Baeten, CRL-AP + NRLs)	30 min
11:20	17	Next interlaboratory studies (Pascal Veyts, CRL-AP)	15 min
11:35	18	Development of alternative methods (Gilbert Berben and Olivier Fumière, CRL-AP) : status at European and international level, NRLs' initiatives	40 min
12:25	19	Conclusion	10 min
<i>12:35 - Lunch at the 'Hotel 3 clés' sponsored by the CRL-AP -</i>			



Activity 3 : Interlaboratory studies and quality assurance

- ❖ **Coordinate the preparation, reception, storage, maintenance and distribution to national reference laboratories (NRL) of samples**
- ❖ **Organize interlaboratory studies**
- ❖ **At NRLs level : help to keep the highest standard of technical skill and quality management**

Microscopy



CRL-AP Interlaboratory Study 2006

Results qualitative analysis

Sample	Material	n	AC	
			Terrestrial	Fish
A	blank	66	1,000	0,879 (8)
B	0,1% MBM	66	0,985 (1)	0,909 (6)
C	0,5% Fish III	44	1,000	1,000
D	0,25% Fish III	44	1,000	1,000
E	1,5% Fish III	44	0,955 (2)	1,000
F	1% Fish III	44	1,000	1,000
G	0,1% MBM + 5% Fish I	66	0,879 (8)	1,000
H	1% Fish I	44	0,955 (2)	1,000

ACTIONS

- Organisation of training at CRL-AP for the underperforming labs
 - Small interlaboratory study in June-July 2007
- Need of continuous training

Global results expressed as accuracy (AC) for the 8 samples (accuracy = sensitivity in case of FN and specificity in case of FP) in brackets the nr of FN or FP

CRL-AP Interlaboratory Study 2006

Results quantitative analysis

Lab ID	0,25% Fish III	
	D1	D2
1	0,09%	0,07%
3	2,73%	2,69%
4	0,04%	0,03%
5	1,00%	0,90%
6	0,50%	0,50%
7	0,54%	0,34%
10	0,01%	0,03%
14	0,80%	1,10%
18	0,21%	0,17%
19	0,14%	0,13%
21	0,23%	0,23%
22	1,60%	1,70%
23	0,40%	0,70%
29	0,20%	0,20%
32	0,10%	0,13%
33	0,09%	0,14%
35	0,20%	0,10%

- Part of NRLs are unable to realise quantitative analysis

- Poor reproducibility → establishment of a more detailed protocol for quantitative purpose

n	34
mean	0,53%
STD	0,70%
min	0,01%
max	2,73%
median	0,21%

Activity 4 : *Development of analytical methods and tools*

- ❖ New and complementary methods of analysis and improvement of existing methods of analysis.

- ❖ Evaluation studies on alternative methods

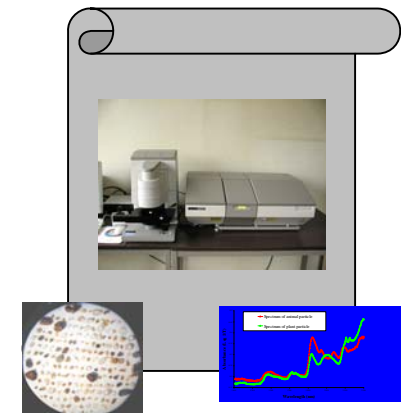
- ❖ Construction and extension of CRL-AP sample bank

 - production of microscopic images

PCR

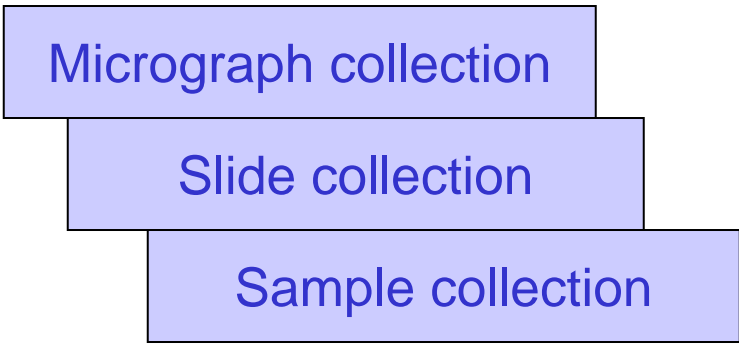


NIR

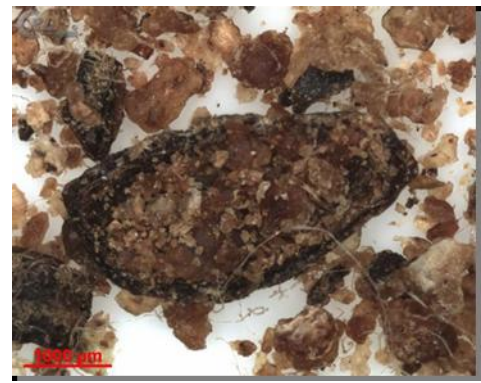
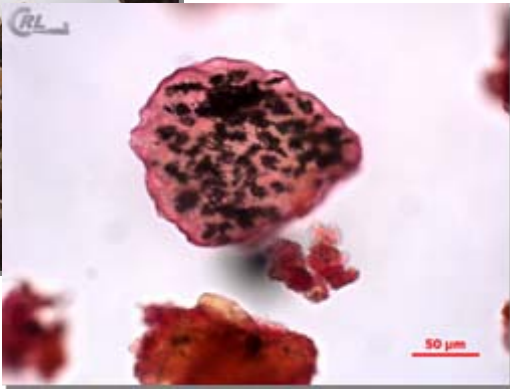
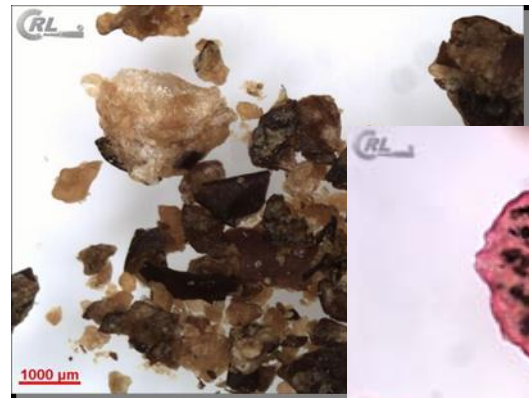


Database generation

- CRL-AP at service of NRLs
- Currently developed:

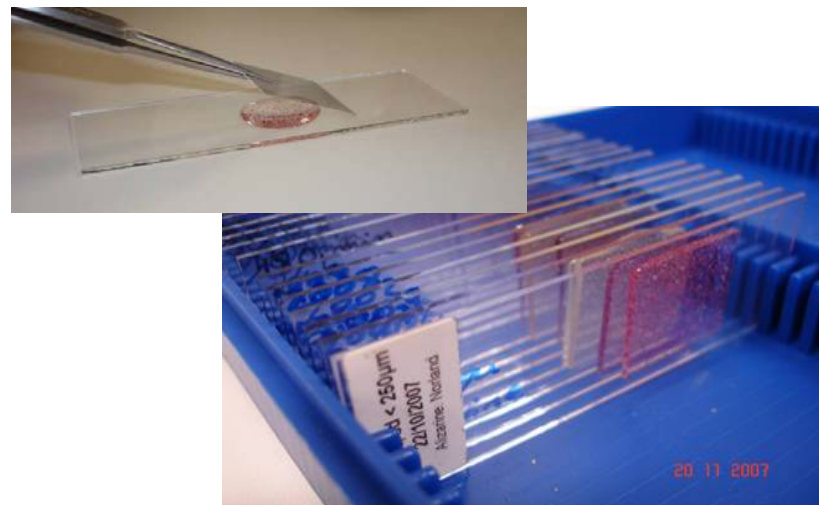


In order to help NRLs !!!

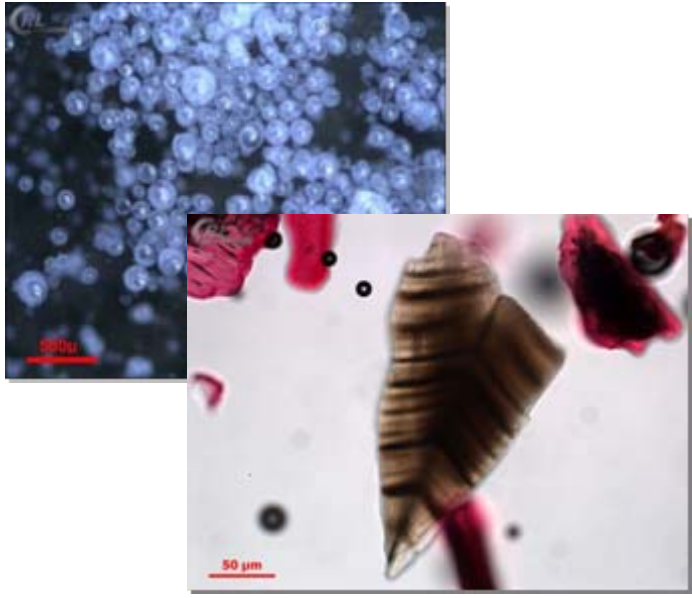


CRL-AP Sample & Slide collection

- Over 2000 samples of different meals are recorded :
 - feeds
 - ingredients
- Some information will be published on CRL-AP intranet for NRLs
 - stock availability,
 - origin,
 - cession possibilities ...
- Permanent slides
- Total traceability and storage
- Allows to reiterate analysis
- Permanent labelling



CRL-AP Micrograph collection



Database generation of typical micrographs of each type of meal

- High quality photographs

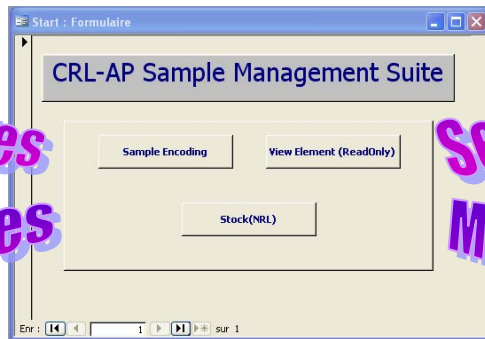
microscope

stereoscope

- Taken by qualified personal
- Copyright
- Scale bars

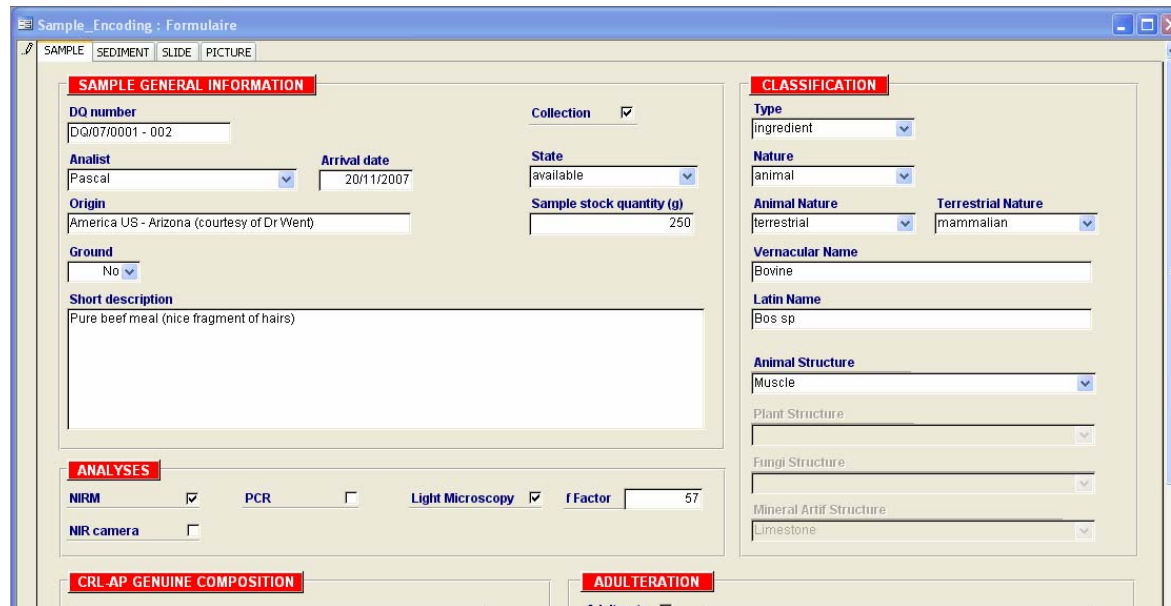
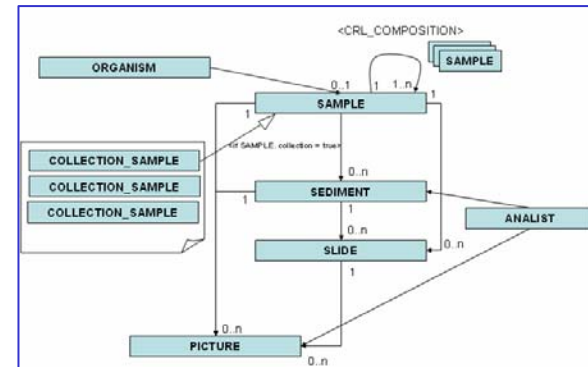
CRL-AP Management system

- Dedicated and **integrated collection management system** (V.1 yet in use)



*Samples
Slides*

*Sediments
Micrographs*





Activity 5 : Workshops/trainings

- ❖ **Workshop for the benefit of NRLs for**
 - 126/2003/EC directive
 - new validated method

- ❖ **Provide training through dissemination tools like CD's or DVD's. Development of analytical support and libraries**

Training course in microscopy

Polarisation

- Technical
- Micrographs
 - Bright field
 - Polarised

Bones – Detection 6

Other terrestrial structures :

Avian egg shell

Granular surface (colored...)

Hairs – Detection 2

Macroscopic and microscopic characteristics

In Europe : mainly avoiding misidentification with

- Cereals epidermal trichomes (Oats,...)

polarized +++
straight structure

Blood meal

Processing ways impact on appearances of blood meal

- Drum-dried (cold treated)
= dark red/black irregular powder with broken pieces

- Intended for NRL network
- Theoretical and practical approach
- Successful return
- Modular approach

CRL-AP Organigram

Dr Koen Van Dyck
DG-Sanco, EC

Dr P. DARDENNE
Head of Department

Dr V. BAETEN
Director

Dr G. BERBEN
Deputy of Director

Ir P. VERMEULEN
D. BONSIGNORI
ICT Team – Intranet
DB management

Dr P. VEYS
Secretary coordination
K. DOMS
Secretary

Chemical analyses

Dr G. SINNAEVE
Head of team

F. RWAGASORE

Molecular biology analyses

Ir O. FUMIERE
Head of team

J. HULIN

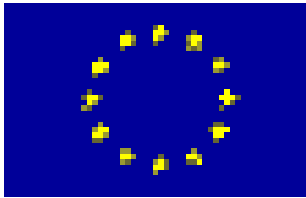
Microscopy Analyses and sample preparation

Dr P. VEYS
Head of team

Ir J. VANCUTSEM



I. FISSIAUX
C. TELLER
C. BELLINCHON



Integrating and strengthening the European Research Area Specific Targeted Project

SAFEED-PAP

Detection of presence of species-specific processed
animal proteins in animal feed

(FOOD-CT-2006-036221)

Scientific Officer : Hallgeir HERIKSTAD



SAFEED-PAP



- Duration 36 months
- Total budget: +- 1,75 M€ from EU
- 7 work packages
- 13 partners
- Start date: 1-12-2006
- Coordinator : Dr Vincent Baeten, CRA-W
Secretariat : Dr Juan Antonio Fernandez Pierna

<http://safeedpap.feedsafety.org/>



Central Science Laboratory



RIKILT
INSTITUTE OF FOOD SAFETY
WAGENINGEN UR



RIKILT
CCL
The Netherlands



PDIR
Denmark



NVL
Lithuania



NATIONAL
VETERINARY
LABORATORY

VLA
CSL
Great Britain

SAFEED-PAP



CRA-W (coordinator)
IRMM-JRC
FLVVT
DGO
Belgium



中国动物卫生与流行病学中心
China Animal Health and Epidemiology Center

CAHEC
China

UCO
Spain



UMIL
Italy



EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Advisory Board

Dr Jacob de Jong (CEN/TC 327 Animal feeding stuffs)



European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Mrs Inge Paradise (IAG Feedstuff Analysis – Section Feedstuff Microscopy)



Mr Steve Woodgate (EFPPRA)



Dr Dragan Moncilovic (USA, Food and Drug Administration, *FDA*)



Dr Kazutoshi Mizuno (Japan, Agency Fertilizer and Feed Inspection Services)

Dr Christoph von Holst (IRMM - JRC):
observer status.



Dr Koen Van Dyck (DG-SANCO)
observer status

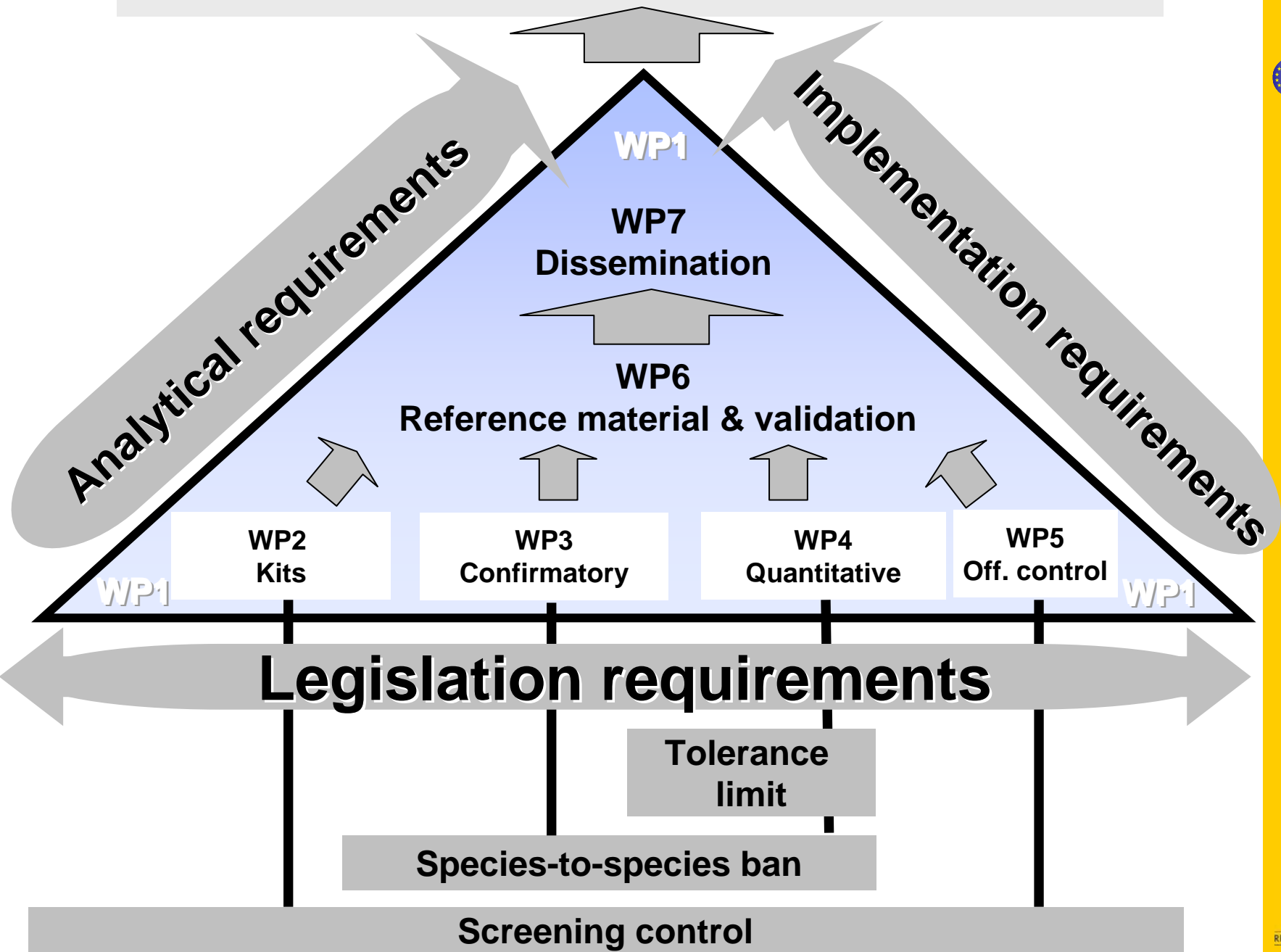


Main objectives

The *SAFEED-PAP* project has three main objectives that should lead to solve the problematic of the species specific detection of MBM in compound feeds:

- (i) Development of suitable validated methods for the species specific detection and quantification of animal protein in compound feed in order to allow the amendment of the extended total ban
- (ii) Development of tools and analytical kits for the correct implementation of the methods in the labs
- (iii) To set up the appropriate environment for the optimum application of the methods

SPECIES SPECIFIC DETECTION



WP 2 - Improvement and validation of test kits

WP leader : Gilbert Berben

Olivier Fumière



diagenode



Scott Reaney

Didier Allaer

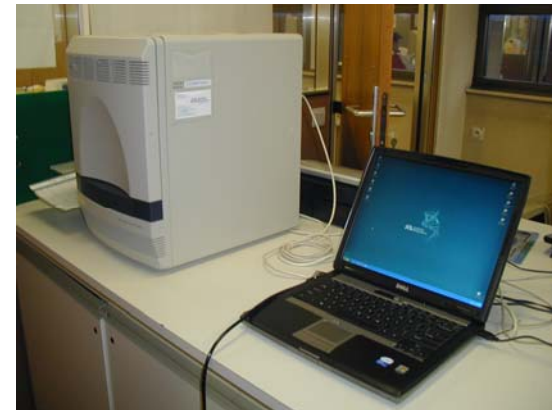
Henk Aerts

Jiansan Wu

→ Improvement of a dipstick method for the detection of ruminant animal by-products at the industrial level



→ Fine-tuning of a PCR kit for the routine detection of species-specific DNA targets at the official laboratories level



WP 3 - Development of a confirmatory method using a Proteomics approach based on the isolation and identification of protein/peptide of animal origin using capillary HPLC and mass spectrometry (MS/MS)

WP leader : Scott Reaney



Hubert Chasseigne

Monique Bremer

Paul Reece

WP 3 - Proteomics approach - protein/peptide - HPLC and mass spectrometry (MS/MS)

→ **Identification and isolation of species specific proteins from meat and bone meal samples and compound feed**

→ **Development of a confirmatory method to detect and identify the selected targets using HPLC and MS/MS**



Figure : Observed differences in the amino acid sequence of the first 60 residues of fast skeletal muscle troponin I in 6 animal species (sequence information from NCBI)

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mgdeekrnra itarrqhlks vmlqiaatel ekeesrreae kqnylaehcp plhipgsmse MAN
mgdeekrnra itarrqhlks vmlqiaaqel ekeesrrese kqnylsehcp plhlpgsmse DOG
mssdeekkrrar atarrqhlks amlqlavtel ekeaaaakeve kqnylaehcp plslpgsmqe CHICKEN
mgdeekrhra itarrqhlks vmlqiaatel ekevgrrese kqnylsehcp plhlpgsmse PIG
mgdeekrnra itarrqhlks vmlqiaatel ekeesrrese kenylsehcp plhipgsmse MOUSE
mgdeekrhra itarrqhlks vmlqiaatel ekeegrreae kqnylsehcp plhlpgsmse BOVINE
```

WP 4 - Development of methods for the quantitative detection of animal particles and for the species-specific quantitative identification of animal particle by near-infrared microscopy and PCR techniques

WP leader : Ana Garrido



Dolores Pérez Marín



Ana Boix

Juan Antonio Fernández Pierna



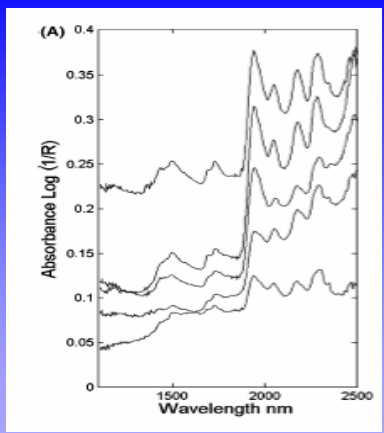
WP 4 - quantitative detection - species-specific quantitative – NIRM and NIRM/PCR

→ *Development of a quantitative method based on NIR microscopic techniques for animal particles detection*

→ *Development of a quantitative method based on the combination of NIR microscopic method and PCR for the species-specific detection of animal particles*



Sediment fraction



Terrestrial bones

WP 5 - Improvement of the official method and development of a screening method for the control laboratories

WP leader : Leo van Raamsdonk



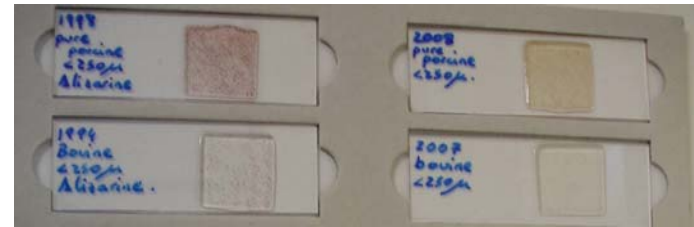
Jan Sten Jorgensen
Luciano Pinotti
Gediminas Pridotkas



WP 5 - official method - screening method - control laboratories

→ Improvement of the performance and selection as well as validation of new species-specific markers for the routine control by advanced microscopy

→ Development of a method combining advanced microscopy (AM) with immunoassays



WP 6 - Validation of methods and feasibility study for the production of certified reference materials

WP leader : Ana Boix



Hakan Emteborg

Jeroen Vancutsem

Rob Magry

Vincent Baeten

→ **Production of test materials for the development and validation of the methods developed in the course of the project**

→ **Feasibility study to specify the pre-requisite for the production of a candidate certified reference material (CRM)**

→ **Validation of the methods developed in the project**

WP 7 - Transfer, utilisation and dissemination of knowledge

WP leader : Jan Sten Jorgensen



Ministeriet for Fødevarer, Landbrug og Fiskeri
Plantedirektoratet



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RIKILT
INSTITUTE OF FOOD SAFETY
WAGENINGEN UR



中国动物卫生与流行病学中心
China Animal Health and Epidemiology Center

Philippe Vermeulen

Gediminas Pridotkas

Leo van Raamsdonk

Jiansan Wu

WP 7 – Transfer - dissemination

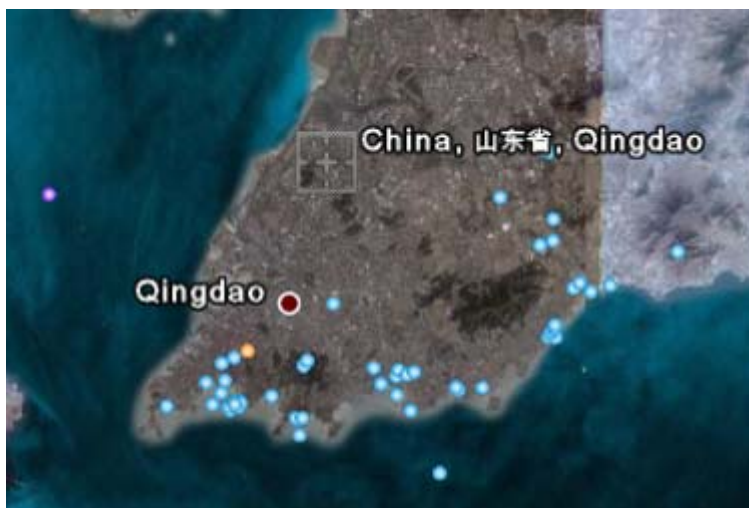
<http://safeedpap.feedsafety.org/>

→ Construction and maintenance of the public internet web site

→ Organization of international conferences

→ Organization of 2 workshops

→ Publication of a multi-authors technical book



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Detection of presence of
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