European Commission

Joint Research Centre

Determination of Processed Animal Proteins in Animal Feed - Collaborative Study

www.jrc.ec.europa.eu

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Serving society
Stimulating innovation
Supporting legislation

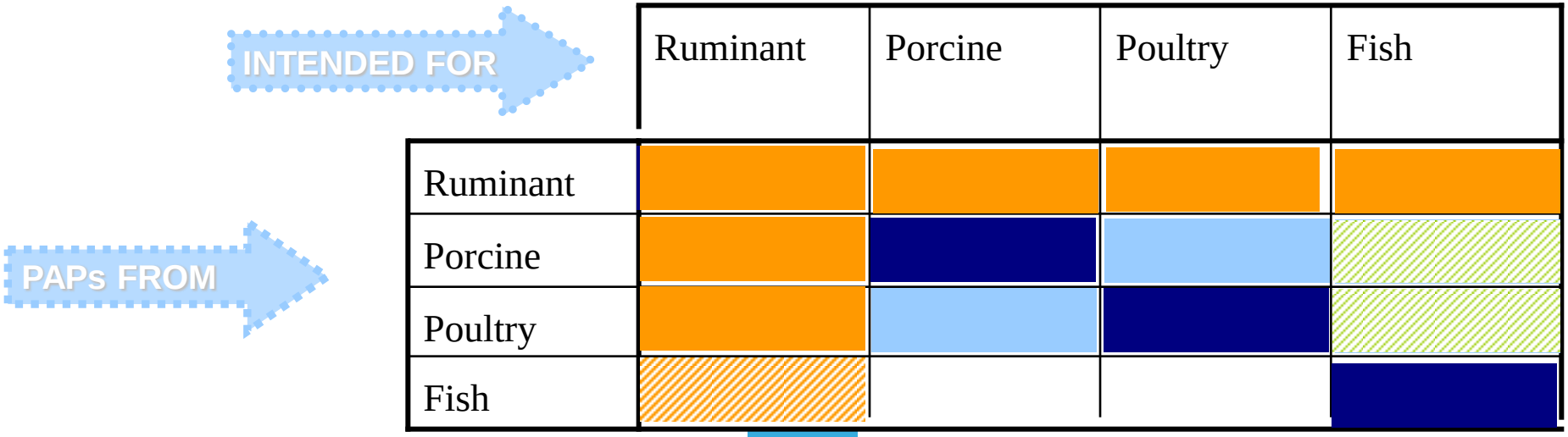
Legal frame

Prohibited by Regulation (EC) 1069/2009 **Ban of Cannibalism** — **PERMANENT BAN**

Prohibited by TSE Regulation (EC) 999/20014 — **PERMANENT BAN**

Extended Feed Ban (Reg. 1234/2003 amending TSE Regulation)

Commission Regulation (EU) 56/2013 (amending TSE Regulation)

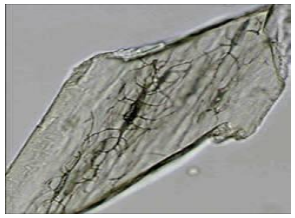


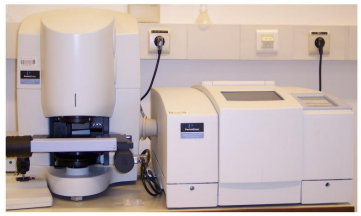
Challenge of methods

- No legal limit for PAPs in feed
- Target: 0.1% PAPs in feed
- Only qualitative detection is required: zero tolerance
- Interaction with authorised ingredients (milk, egg products…)

PAPs never measured as such….

Particles	Classical Microscopy Near Infrared Microscopy
Proteins	Immunoassays Proteomics
DNA	PCR





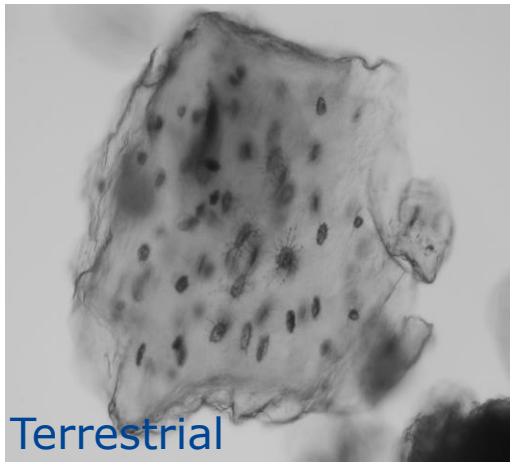
….targets are used as markers for PAPs

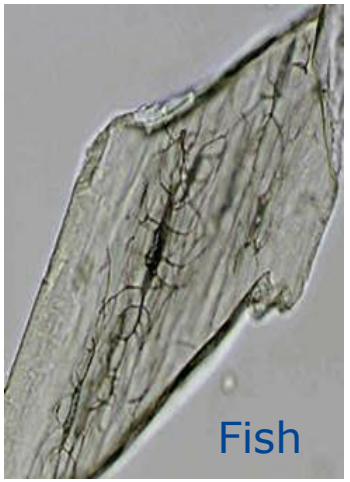
The official method

Light Microscopy: Commission Regulation (EC) 152/2009

Microscopic observation of specific morphological features

Able to discriminate between fish and terrestrial PAPs

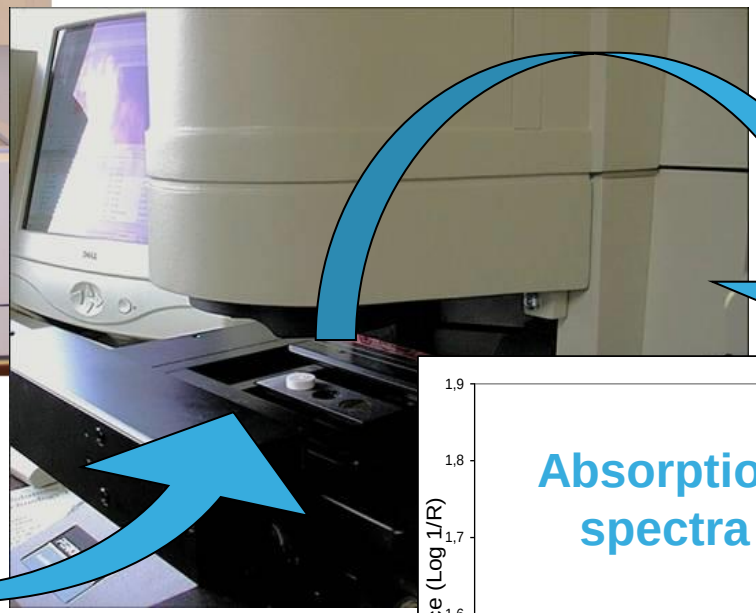


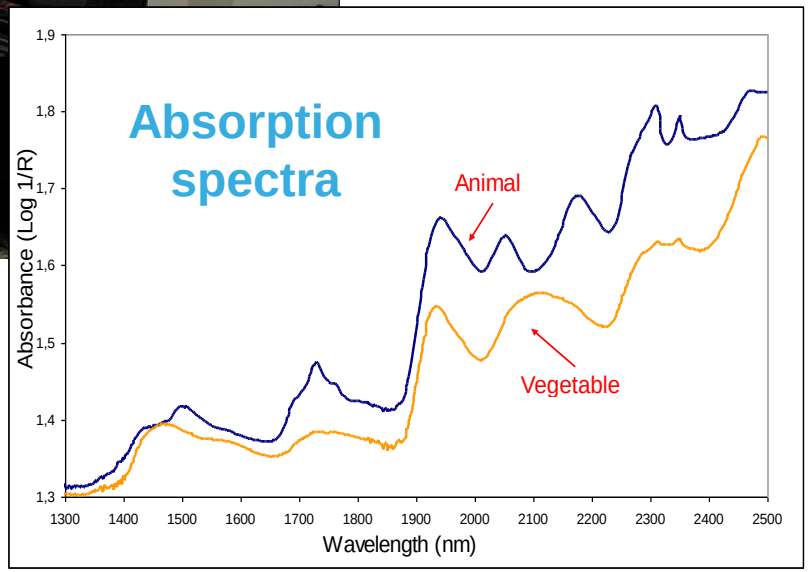


- Sensitivity regarding detection of terrestrial PAPs decreases in the presence of fish meal
- Not species-specific
- Performance of the method depends on the experience of the operator
- Not reliable for the purpose of quantification

Near Infrared Microscopy

NIR spectra of individual particles
fingerprint based on chemical composition





- ✓ Same principle as for EU official method
- ✓ Objective
- ✓ Non-destructive

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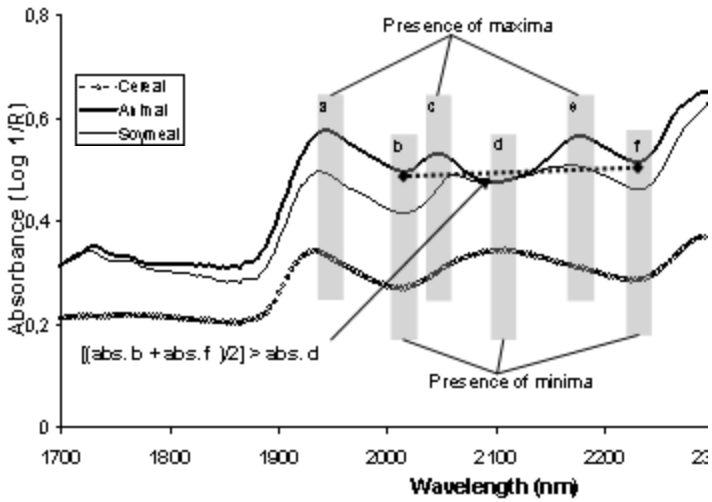
NIRM method for detection of PAPs in feed

Discrimination
animal – vegetable
based on visual observation
of the spectra



Decision criteria for specific wavelengths

Anal Bioanal Chem (2005) 382: 149–157
DOI 10.1007/s00216-005-3193-5

ORIGINAL PAPER

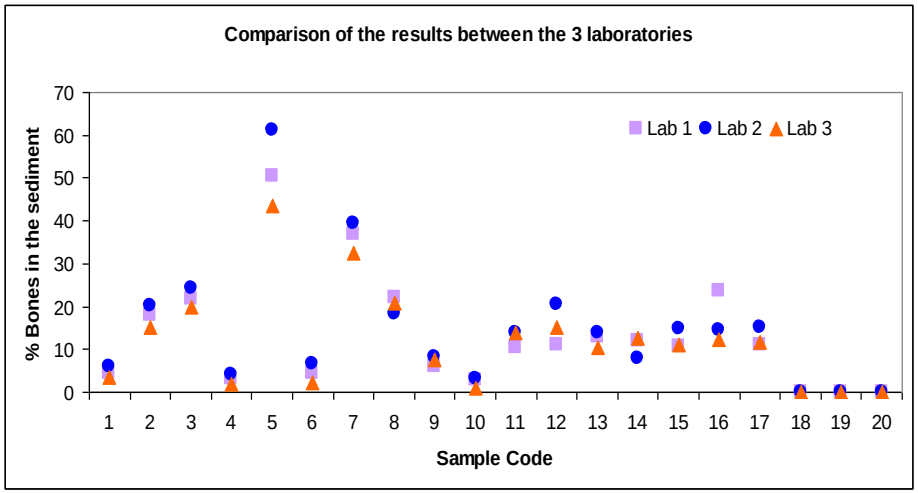
Vincent Baeten · Christoph von Holst · Ana Garrido
Jeroen Vancutsem · Antoine Michotte Renier
Pierre Dardenne

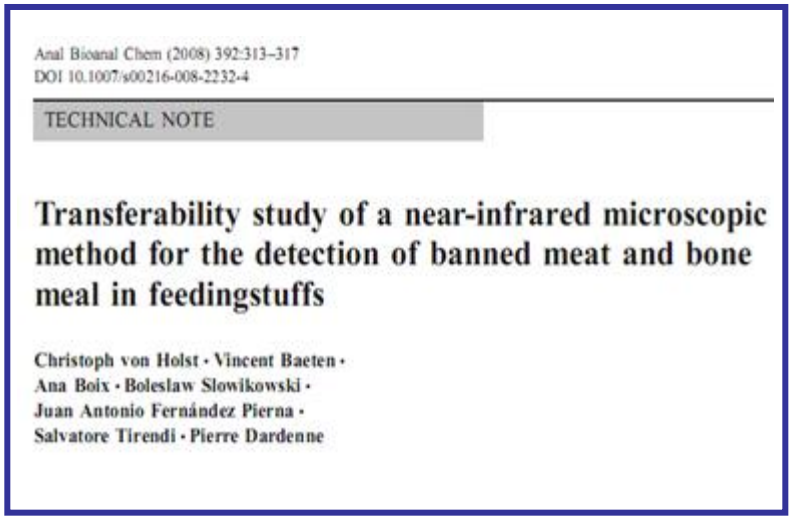
Detection of banned meat and bone meal in feedstuffs by near-infrared microscopic analysis of the dense sediment fraction

- **Detection of 0.05% PAPs in feed**
- **NIRM vs CM equivalent results**

NIRM Transferability

Sample Code	MBM (%)	CRA-W		IHCP		IRMM	
		Nb of analysed particles	*Nb of positive particles*	*Nb of analysed particles*	*Nb of positive particles*	*Nb of analysed particles*	*Nb of positive particles*
35	0,5	157	7	201	12	337	12
37	1	172	31	154	31	275	42
33	1,5	180	39	153	37	251	50
34	2	164	3	208	4	286	2
42	8	161	81	154	94	310	135
44	0,5	162	7	200	13	314	7
66	7	189	70	152	60	313	102
58	7,5	191	42	152	28	317	66
63	8	168	10	206	17	316	24
01	0,5	185	5	200	6	324	3
17	1	165	17	158	22	201	28
11	1,5	183	20	152	31	250	38
13	2	155	20	152	21	249	26
08	4	175	21	151	12	235	30
24	3	205	22	156	23	255	28
22	2,5	177	42	202	29	282	35
46	8	193	21	152	23	333	39
16	0	150	0	206	0	275	0
19	0	150	0	202	0	262	0
12	0	150	0	202	0	241	0




Anal Bioanal Chem (2008) 392:313–317
DOI 10.1007/s00216-008-2232-4

TECHNICAL NOTE

Transferability study of a near-infrared microscopic method for the detection of banned meat and bone meal in feedingstuffs

Christoph von Holst · Vincent Baeten · Ana Boix · Boleslaw Slowikowski · Juan Antonio Fernández Pierna · Salvatore Tirendi · Pierre Dardenne

Correct classification of blind samples demonstrated transferability of the method between laboratories

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NIRM validation: outline

Aim: to establish the **performance characteristics** of a NIRM method when apply to the detection of animal products in feed.

- ►Discrimination of animal and vegetal feed ingredients based on the evaluation of NIR spectra obtained from individual particles.
- ►Method supported by decision rules for the absorbances at specific wavelengths
- ►Method successfully transferred to 2 independent laboratories.

is the method

fit for the purpose ?

NIRM validation: outline

7 EU laboratories and 1 lab from China

All laboratories

- Apply strictly the **same method protocol**

  Target: Animal Particles ➡ presence/absence

- Need to know and to apply correctly the protocol ➡ IRMM workshop

- Work on a set of **identical samples**

  compound feeds containing MBM at different concentration levels: sedimented and not ➡ sedimentation not included in the validation

- Send the **results back** to the organiser for data assessment

  Spectra classified as positive/negative by applying the protocol

  Report on positive spectra out of the total analysed

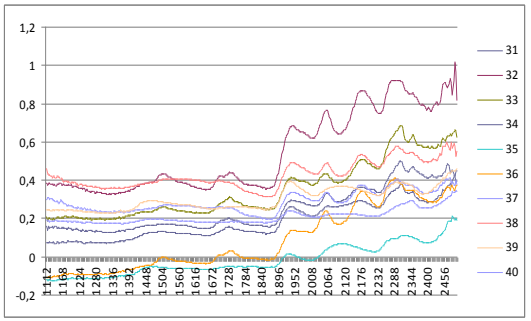
NIRM validation: Training period

STEP 1: Standard cell

12 different pure materials

10 spectra per material - 2 consecutive days

STEP 2: Evaluation Spectra



To test the ability of applying the decision rules specified in the protocol.

Classify 50 spectra of unknown origin as animal or not

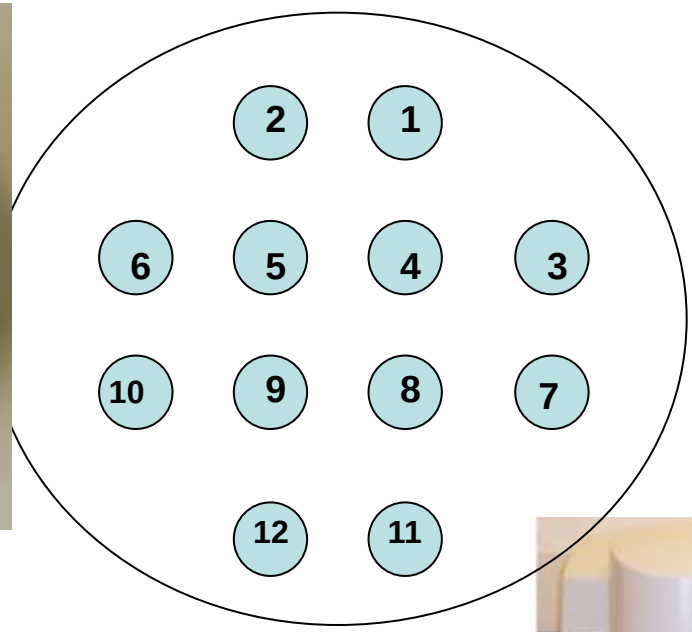
STEP 3: Blind samples

3 sediments and 3 not sedimented samples.

Compound feeds containing MBM at different concentration

100 spectra per sample

Training period: standard cell – STEP 1



CRA-W :	1112 : 4 : 2500 nm
JRC:	7696 : 8 : 4000 cm-1
IZSTO:	1306 : 2 : 2500 nm
SERIDA:	1160 : 4 : 2500 nm
UCO:	1112 : 4 : 2500 nm
VLA:	7776 : 2 : 4000 cm-1
BRUKER:	1000 : 1 : 5563 nm
CAU:	7774 : 2 : 4000 cm-1

1 – Polystyrene
2 – Teflon
3 – Pig meal
4 – Sheep meal
5 – Feather meal
6 – Soya meal
7 – Chicken meal
8 – Corn
9 – Fish meal
10 – Blank
11 – Bovine meal
12 – Blood (Pig)

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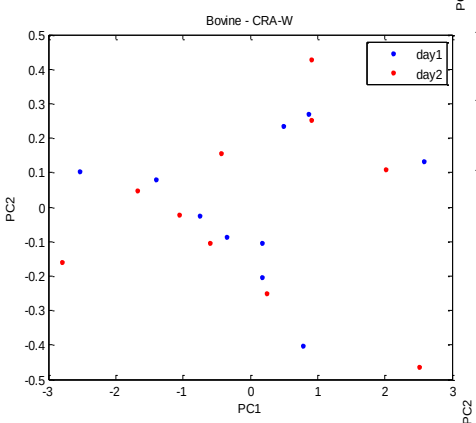
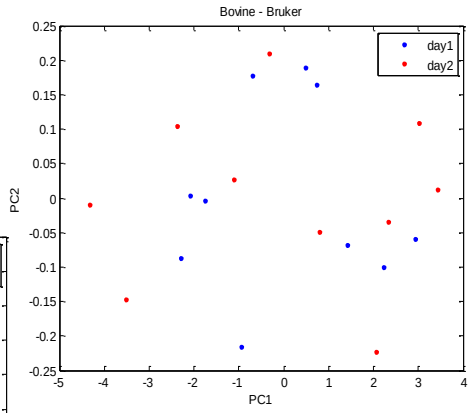
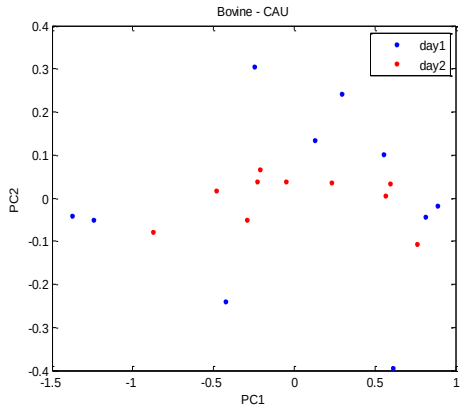
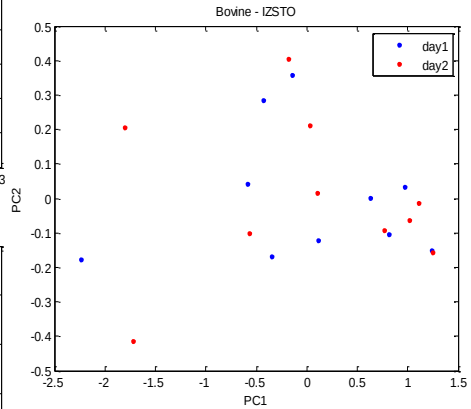
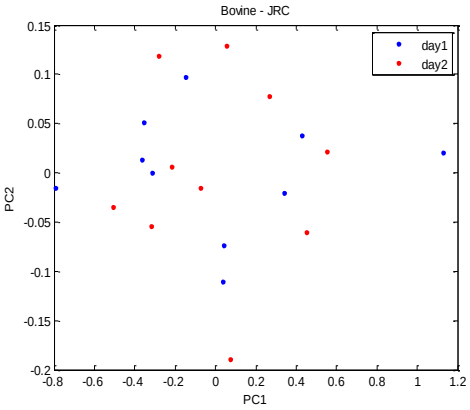
Training period: standard cell – STEP 1

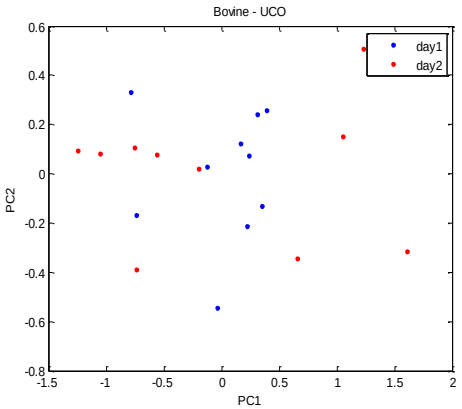








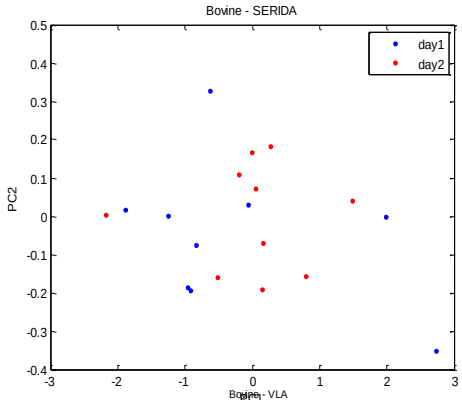


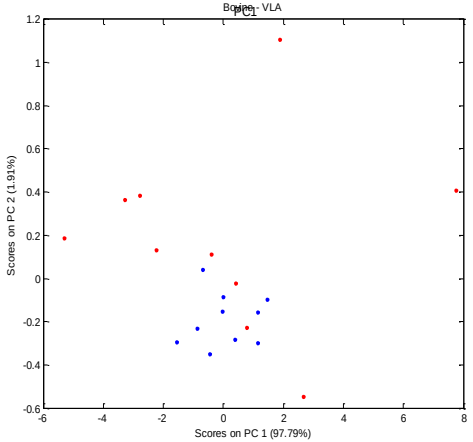


Bovine

Day 1

Day 2

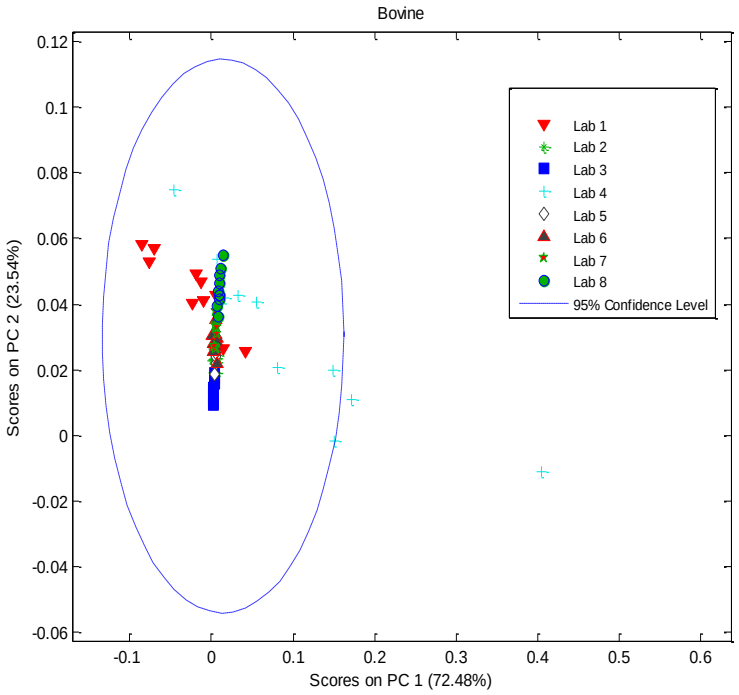




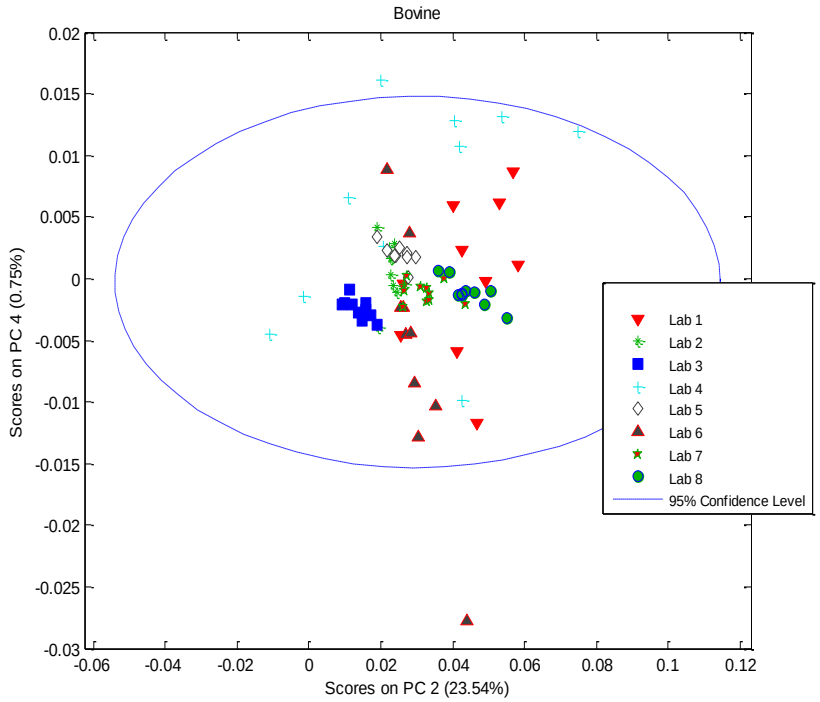
No differences by day

Training period: standard cell – STEP 1

PCA based on all the bovine samples



PC1 vs PC2



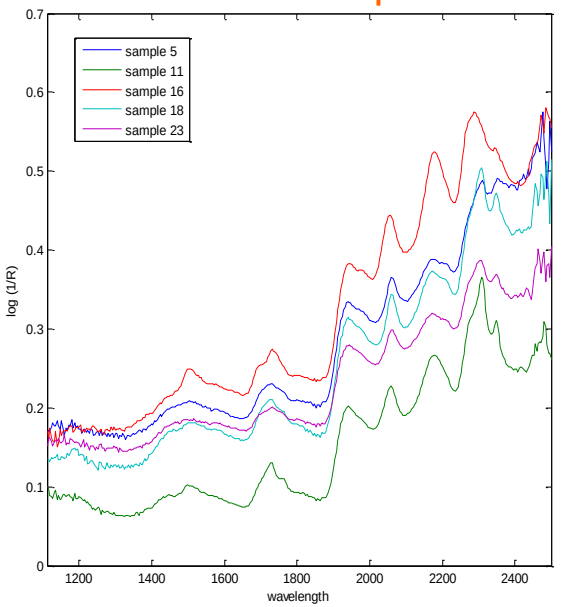
PC2 vs PC4

Training period: evaluation spectra – STEP 2

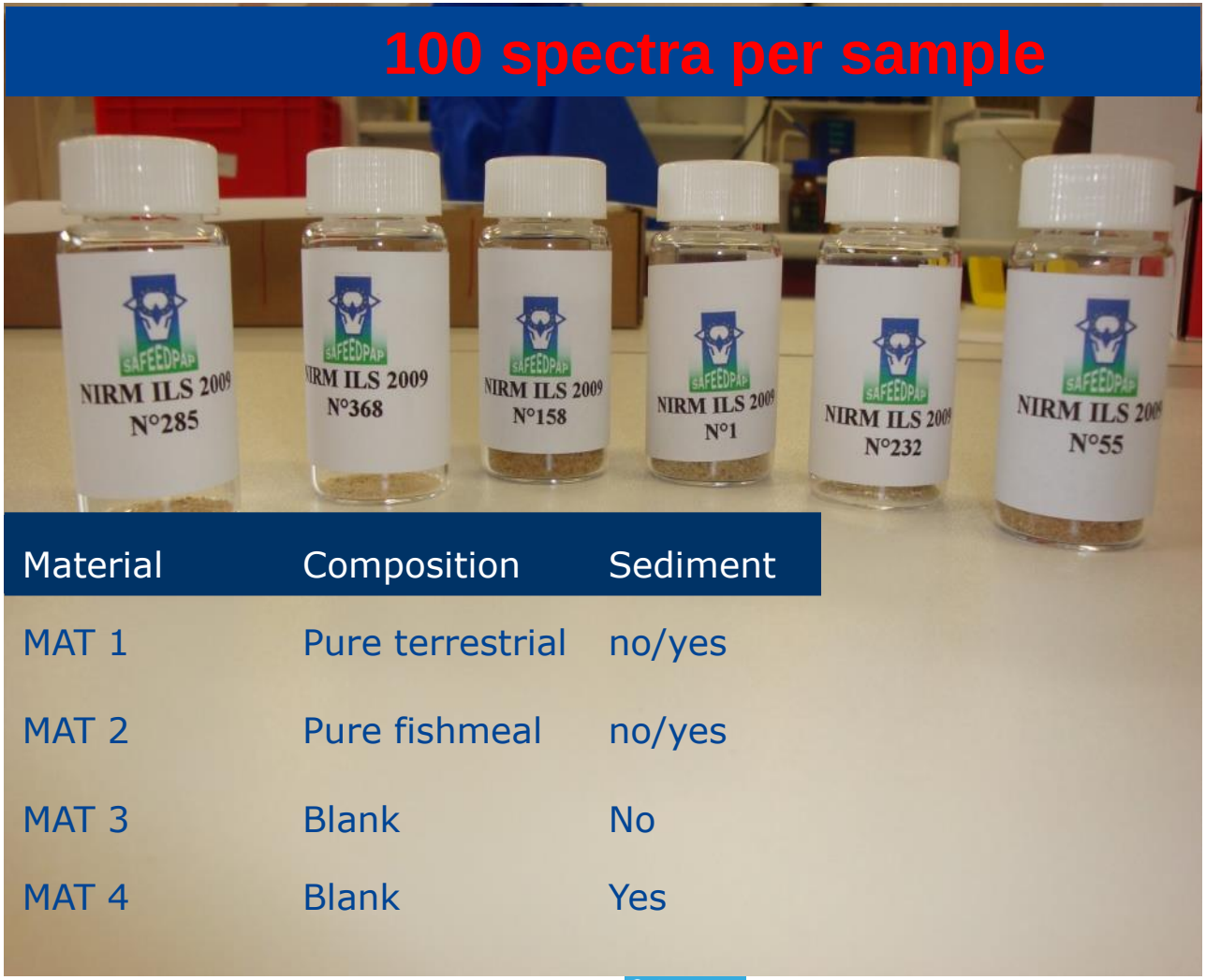
To test the ability of applying the decision rules specified in the protocol

Classify 50 spectra of unknown origin as animal or not

"borderline" spectra



Training period: blind samples – STEP 3



Material	Composition	Sediment
MAT 1	Pure terrestrial	no/yes
MAT 2	Pure fishmeal	no/yes
MAT 3	Blank	No
MAT 4	Blank	Yes

Training period: results

LAB Code	Sample Code	Fraction	Material	Results
1	25	sed	Pure fish	+
1	79	raw	pure terrestrial	+
1	81	raw	blank	-
1	102	sed	pure terrestrial	+
1	132	raw	Pure fish	+
1	238	sed	blank	+
2	38	sed	Pure fish	+
2	41	raw	Pure fish	+
2	167	sed	pure terrestrial	+
2	172	raw	blank	-
2	183	raw	pure terrestrial	+
2	329	sed	blank	-
3	37	sed	pure terrestrial	+
3	40	raw	pure terrestrial	+
3	68	raw	blank	-
3	82	sed	blank	-
3	197	raw	Pure fish	+
3	220	sed	Pure fish	
4	1	raw	Pure terrestrial	+
4	55	raw	blank	-
4	158	raw	Pure fish	+
4	232	sed	pure terrestrial	+
4	285	sed	Pure fish	+
4	368	sed	blank	-

LAB Code	Sample Code	Fraction	Material	Results
5	3	raw	blank	-
5	12	sed	Pure fish	+
5	15	raw	Pure fish	+
5	66	raw	pure terrestrial	+
5	154	sed	pure terrestrial	+
5	173	sed	blank	-
6	115	sed	pure terrestrial	+
6	144	raw	pure terrestrial	+
6	145	raw	Pure fish	+
6	146	raw	blank	-
6	168	sed	Pure fish	+
6	381	sed	blank	-
7	29	raw	blank	-
7	50	sed	pure terrestrial	+
7	131	raw	pure terrestrial	+
7	155	sed	Pure fish	+
7	171	raw	Pure fish	+
7	225	sed	blank	-
8	2	raw	Pure fish	+
8	14	raw	pure terrestrial	+
8	24	sed	pure terrestrial	+
8	94	raw	blank	-
8	142	sed	Pure fish	+
8	355	sed	blank	-

- With low animal content

Validation phase

- **Blind samples:**

600 spectra per sample

- **Report on:**

- number of particles analysed
- number of spectra classified as animal per sample
- Conclusion for the sample (+ or -)

Material	Composition	Sediment
MAT 5	0.5 % terrestrial	yes
MAT 6 (X2)	0.1 % terrestrial	yes
MAT 7	Blank	yes
MAT 8	2 % (1%T+1%F)	no
MAT 9 (X2)	1% terrestrial	no
MAT 10	Blank	no

Validation phase: results

MAT 5 0,5% Terrestrial PAP - sediment		
Spectra	**Positive spectra**	**Conclusion**
610	183	+
640	301	+
864	212	+
600	**102**	**+**
636	340	+
661	180	+
600	149	+

MAT 6 0,1% Terrestrial PAP - sediment		
Spectra	**Positive spectra**	**Conclusion**
610	20	+
610	12	+
644	40	+
645	36	+
928	9	+
928	10	+
600	25	+
600	37	+
624	31	+
611	25	+
605	30	+
615	33	+
600	19	+
600	**8**	**+**

MAT 7 Blank - sediment		
Spectra	**Positive spectra**	**Conclusion**
610	0	-
624	0	-
750	**3**	**-**
600	0	-
604	0	-
607	**1**	**+**
600	0	-

Data from one laboratory not considered for the final assessment:

major deviation from the protocol

Data from 7 laboratories

Statistical assessment

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MAT 8 1% Terrestrial PAP + 1% Fishmeal		
Spectra	**Positive spectra**	**Conclusion**
610	4	+
609	11	+
901	2	-
600	2	+
627	8	+
606	4	+
600	2	+

MAT 9 1% Terrestrial PAP		
Spectra	**Positive spectra**	**Conclusion**
610	**2**	**-**
610	**1**	**-**
635	4	+
685	3	+
717	**0**	**-**
725	**2**	**-**
600	0	-
600	2	+
688	3	+
649	6	+
600	3	+
605	3	+
600	3	+
600	4	+

MAT 10 Blank		
Spectra	**Positive spectra**	**Conclusion**
610	0	-
588	0	-
810	0	-
600	0	-
643	0	-
608	0	-
600	0	-

Validation phase: results

Sensitivity (SE) : ability to identify pos. = PA/(PA+ND) *100

Specificity (SP): ability to identify neg.= NA/(PD+NA) *100

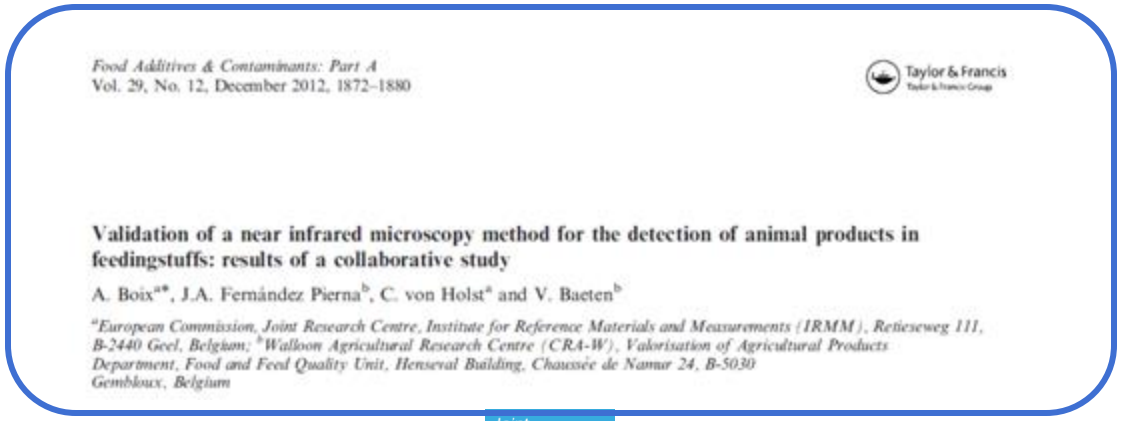
	MAT 5		MAT 6		MAT 7		MAT 8		MAT 9		MAT 10	
	0.5 % Terrest (sed)		0.1 % Terrest (sed)		Blk (sed)		1 % Terrest + 1 % Fish (not sed.)		1 % Terrest (not sed.)		Blk (not sed.)	
n	7		14		7		7		14		7	
	CP	FN	CP	FN	CN	FP	CP	FN	CP	FN	CN	FP
	7	0	14	0	6	1	6	1	9	5	7	0
SE (%)	100		100				86		64			
SP (%)					86						100	

The target of 0.1% MBM in feed is achieved in sediments

The sensitivity of the method for entire samples is between 1% and 2%

Conclusion

- A NIRM method for the detection of animal products in feedingstuffs was successfully validated
- The required sensitivity for official control (0.1 %) is achieved in sediment samples
- Criteria for classification of samples when fewer than 4 spectra are classified as positive need to be set



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Taylor & Francis

Validation of a near infrared microscopy method for the detection of animal products in feedingstuffs: results of a collaborative study

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Thank you

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