

Accreditation (ISO 17025) of NIR spectroscopic methods: the example of the Community Reference Laboratory for Animal Proteins in Feedingstuffs

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A CRA-W success story ...

1997

7th ICNIRS Conference (Essen, Germany)

1998

NIR microscope at CRA-W

1999

Piroux and Dardenne, proceeding of 9th ICNIRS Conference (Verona, Italy)

2000

NIR imaging system at CRA-W
EC STRATFEED project (*Dardenne and Baeten, NIRNews*)

2003

Interlaboratory study DG-Sanco 2003

2004

Feed Safety Conference in Namur
Interlaboratory studies² DG-Sanco 2004 and STRATFEED
Fernandez Pierna et al., J. of Chemometrics, 18, 341-349.

2005

Baeten et al., ABC, 382, 149-177.
NIR microscope at JRC-IRMM

2006

EC SAFEED-PAP project
Community Reference Laboratory for animal proteins
ISO 17025



COMMISSION DIRECTIVE 2003/126/EC

of 23 December 2003

on the analytical method for the determination of constituents of animal origin for the official control of feedingstuffs

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

HAS ADOPTED THIS DIRECTIVE:

Having regard to the Treaty establishing the European Community,

Article 1

Having regard to Council Directive 70/373/EEC of 20 July 1970 on the introduction of Community methods of sampling and analysis for the official control of feedingstuffs ⁽¹⁾, and in particular Article 2 thereof,

Member States shall provide that where official analysis of feedingstuffs is carried out with a view to officially controlling the presence, identification and/or estimation of the amount of constituents of animal origin in feedingstuffs, in the framework of the coordinated inspection programme in the field of animal nutrition in accordance with Council Directive 95/53/EC ⁽²⁾, it shall be carried out in accordance with the provisions of the Annex to this Directive.

Whereas:

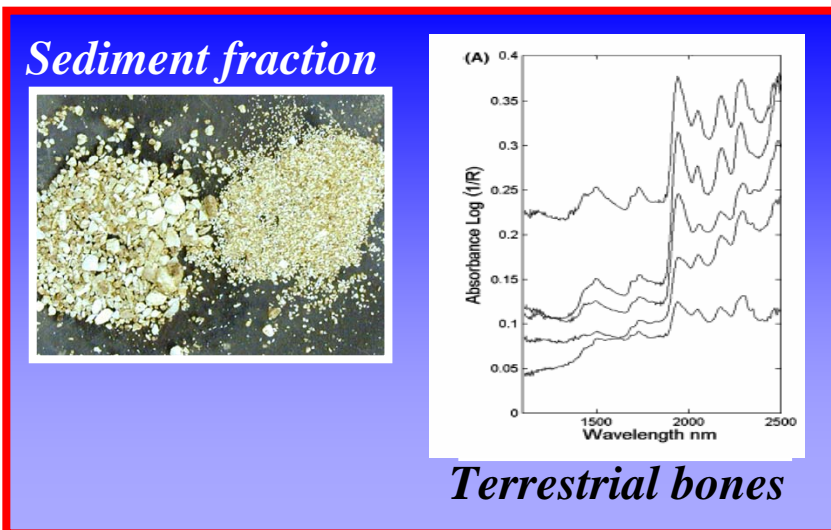
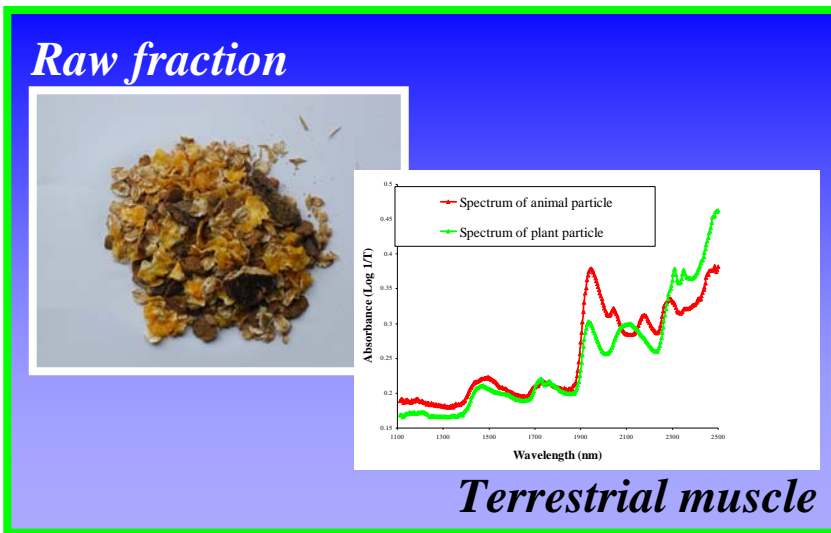
- (1) Pursuant to Directive 70/373/EEC, official controls of feedingstuffs, for the purpose of checking compliance with the requirements of the laws, regulations and administrative provisions governing their quality and composition, are to be carried out using Community sampling and analysis methods.

Article 2

Microscopic method



Classical microscopy is based on the visual observation of morphologic features of ingredient particles



NIR microscopy methods advantage

ISO 17025



- 1 Instrumentation
- 2 Analysis of the internal standard
- 3 Procedure of analysis
- 4 Verification of the procedure of data conversion
- 6 Export the data and conversion of analyzed samples
- 7 Preparation of files and equations for prediction
- 8 Verification of the procedure of prediction
- 9 Prediction
- 10 Visual inspection of the spectra

Analysis of the sediment – samples spiked at 0-1%

Table 1 Results from NIRM analysis of the samples of the sample set B

Sample number	True MBM (%)	Sediment ^a (%)	No. of particles analysed	No. of animal particles ^b	Bones in the sediment ^c (%)	Weight of bones in the sample ^d (g)	Animal ingredients in the sample ^e (%)	Conclusion mean
One-solvent method								
254	0	2.977	157	0 (11)	0.000	0.000	0.000	0.000
264	0	2.665	135	0 (5)	0.000	0.000	0.000	0.000
363	0	2.673	161	0 (5)	0.000	0.000	0.000	0.000
256	0.05	2.862	155	3 (20)	1.935	0.006	0.198	0.186
285	0.05	2.892	108	2 (7)	1.852	0.005	0.191	0.186
365	0.05	2.753	116	2 (9)	1.724	0.005	0.170	0.186
255	0.1	2.788	182	1 (19)	0.549	0.002	0.055	0.266
265	0.1	2.829	140	8 (13)	5.714	0.016	0.577	0.266
364	0.1	2.857	122	2 (5)	1.639	0.005	0.167	0.266
257	0.5	2.857	168	13 (20)	7.738	0.022	0.789	0.932
267	0.5	2.815	124	10 (12)	8.065	0.023	0.811	0.932
366	0.5	2.876	103	12 (15)	11.650	0.034	1.197	0.932
258	1	2.969	142	20 (31)	14.085	0.042	1.493	1.508
268	1	2.845	144	20 (29)	13.889	0.040	1.411	1.508
367	1	2.835	100	16 (18)	16.000	0.045	1.620	1.508
Two-solvent method								
249	0	0.554	144	0 (10)	0.000	0.000	0.000	0.000
259	0	0.511	159	0 (17)	0.000	0.000	0.000	0.000
369	0	0.488	129	0 (21)	0.000	0.000	0.000	0.000
251	0.05	0.588	158	3 (15)	1.899	0.001	0.047	0.089
261	0.05	0.571	105	5 (10)	4.762	0.003	0.113	0.089
371	0.05	0.604	117	5 (14)	4.274	0.003	0.108	0.089
250	0.1	0.623	156	8 (23)	5.128	0.003	0.133	0.130
260	0.1	0.609	150	7 (17)	4.667	0.003	0.118	0.130
370	0.1	0.543	132	7 (19)	6.061	0.003	0.137	0.130
252	0.5	0.766	140	19 (31)	13.571	0.010	0.433	0.544
262	0.5	0.717	132	26 (35)	19.697	0.014	0.588	0.544
372	0.5	0.722	118	24 (32)	20.339	0.015	0.612	0.544
253	1	0.851	137	39 (46)	28.467	0.024	1.009	1.018
263	1	0.936	136	36 (46)	26.471	0.025	1.032	1.018
373	1	0.932	142	37 (45)	26.056	0.024	1.012	1.018

^aSediment (%) = (weight sediment/weight sample) × 100

^bNo. of animal particles = number of particles classified as being of animal origin. The results from the decision rule model are given in parentheses

^cBones in the sediment (%) = (No. of animal particles/No. of analysed particles) × 100

^dWeight of bones in the sample = [Bones in the sediment (%) / 100 × weight sediment (g)]

^eAnimal ingredients in the sample (%) = [weight bones in the sample (g) / % bones in the animal feed ingredient used to spike the sample (=f factor)]

Baeten V., von Holst C., Garrido A., Vancutsem J., Michotte Renier A. and Dardenne P. (2005). **Detection of banned meat and bone meal in feedstuffs by near-infrared microscopic analysis of the dense sediment fraction**, *Anal. Bioanal. Chem.*, **382**, 149-157.

Sensibility (feed sample spiked at 0.1%)

Results of the NIRM homogeneity study

DQ/04/0267/10 - MAT04-VII - 0.1% MBM

	Code	Weight sample [mg]	Weight sediment [mg]	Sediment (a) [%]	Nb analysed particles	Nb animal particles (b)	Bones in the sediment (c) [%]
a	807	10048.1	121.9	1.21	149	17	11.41
b	808	10087.2	132	1.31	181	19	10.50
c	809	10087.2	130.6	1.29	171	19	11.11
d	810	10091.1	142.6	1.41	136	21	15.44
e	811	10046.4	123.2	1.23	155	15	9.68
f	812	10057	125.5	1.25	148	12	8.11
g	813	10058.8	129.5	1.29	120	17	14.17
h	814	10128.7	121.7	1.20	143	16	11.19
i	815	10073.1	120.7	1.20	158	19	12.03
j	816	10012.4	135.2	1.35	154	14	9.09
	<i>Xm (d)</i>			1.27			11.27
	<i>SD (e)</i>			0.07			2.22

- Legend:
- (a) % sediment = (Weight sediment/Weight sample) * 100
 - (b) Nb animal particles = Number of particles clearly identified (by means of their infrared spectrum) a
 - (c) % bones in the sediment = ((Nb animal particles/Nb analysed particles) * 100) / % of particles of th

Sensibility (various feed samples spiked at 0.1%)

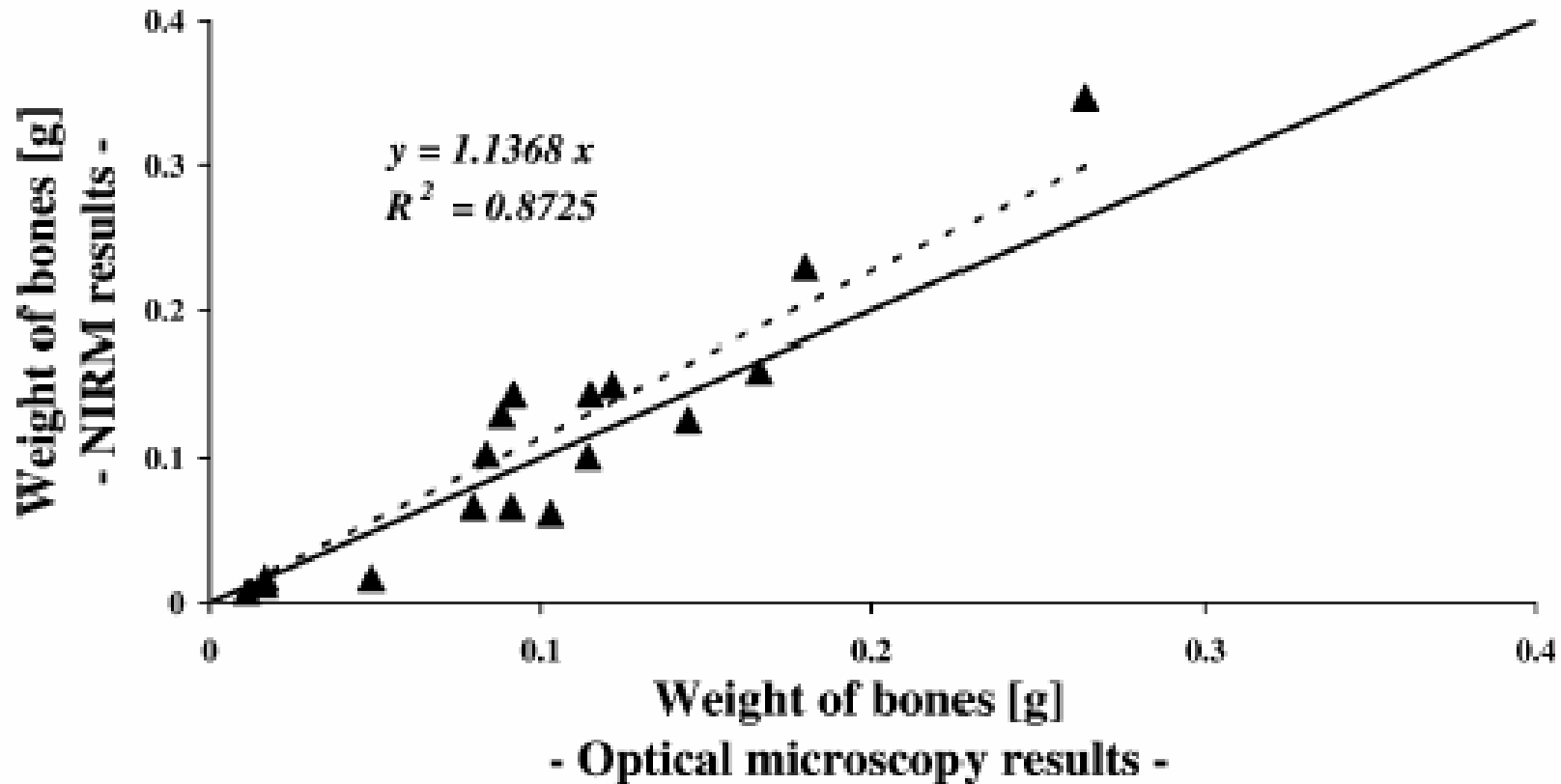
Table : Results of detection of MBM particles by nirm in feedingstuffs contaminated at 0.1%.

Samples containing 0.1% of MBM				
	DQ-03-0027	DQ-04-267-07	DQ-04-267-10	DQ-03-0781-08
Detected particles in 10 g	4	10	17	10
	6	7	19	7
	4	3	19	6
	1	7	21	6
	1	7	15	5
	3	3	12	8
	1	7	17	5
	1	6	16	11
	1	3	19	4
	1	2	14	6
		2		



(Samples from several European interlaboratory studies conducted for CM, PCR and Immuno methods)

NIR Microscopy *versus* Classical Microscopy



Baeten V., von Holst C., Garrido A., Vancutsem J., Michotte Renier A. and Dardenne P. (2005). **Detection of banned meat and bone meal in feedstuffs by near-infrared microscopic analysis of the dense sediment fraction**, *Anal. Bioanal. Chem.*, **382**, 149-157.

Preparation of a standard for the NIRM method

DETAILED REPORT

Groupes	Nber of mesures	Sum	Mean	Variance
1	2	12	6.0	0.0
2	2	21	10.5	0.5
3	2	14	7.0	18.0
4	2	15	7.5	0.5
5	2	16	8.0	2.0
6	2	16	8.0	2.0
7	2	17	8.5	24.5
8	2	23	11.5	4.5
9	2	15	7.5	0.5
10	2	20	10.0	32.0

Table 7c: Variance analysis: one factor (second part)

ANALYSIS OF VARIANCE

Orig. of v°	Σ of squares	D° of freedom	mean squares	F	Prob.	Crit. val. for F
Betw. groupes	52.4500	9	5.8278	0.6897	0.7061	3.0204
Inside of the g.	84.5000	10	8.4500		> 0,05	
Total	136.9500	19			homogenous sub-samples	

The analysis of the variance clearly shows that sub-samples are homogenous as the calculated probability is higher than 0.05.

(Source : Validation dossier of the NIRM method)

Control card

DQ/04/0752

CRAgx code	Analysis	Number of analysed particles	Number of animal particles	Percentage of animal particles
30/08/04	Sed	60	8	13.33%
31/08/04	Sed	60	7	11.67%
01/09/04	Sed	60	4	6.67%
02/09/04	Sed	60	7	11.67%
08/09/04	Sed	60	11	18.33%
09/09/04	Sed	60	12	20.00%
07/10/04	Sed	60	10	16.67%
08/10/04	Sed	60	8	13.33%
11/10/04	Sed	60	13	21.67%
13/10/04	Sed	60	14	23.33%
15/10/04	Sed	60	7	11.67%
18/10/04	Sed	60	4	6.67%
19/10/04	Sed	60	9	15.00%
22/10/04	Sed	60	7	11.67%
25/10/04	Sed	60	9	15.00%
22/11/04	Sed	60	4	6.67%
23/11/04	Sed	60	13	21.67%
25/11/04	Sed	60	4	6.67%
04/01/05	Sed	60	9	15.00%
06/01/05	Sed	60	2	3.33%
12/01/05	Sed	60	11	18.33%
19/01/05	Sed	60	7	11.67%
20/01/05	Sed	60	4	6.67%
Mean (m)		8.00		
Standard deviation (SD)		3.36		
lim sup. = m + 2 x SD =		14.71		
lim inf. = m - 2 x SD =		1.29		

Table : Result of 23 days of NIRM analysis of 60 particles collected in the same sediment sample



Control card

NIRM control card - PA-IR2-NIRM

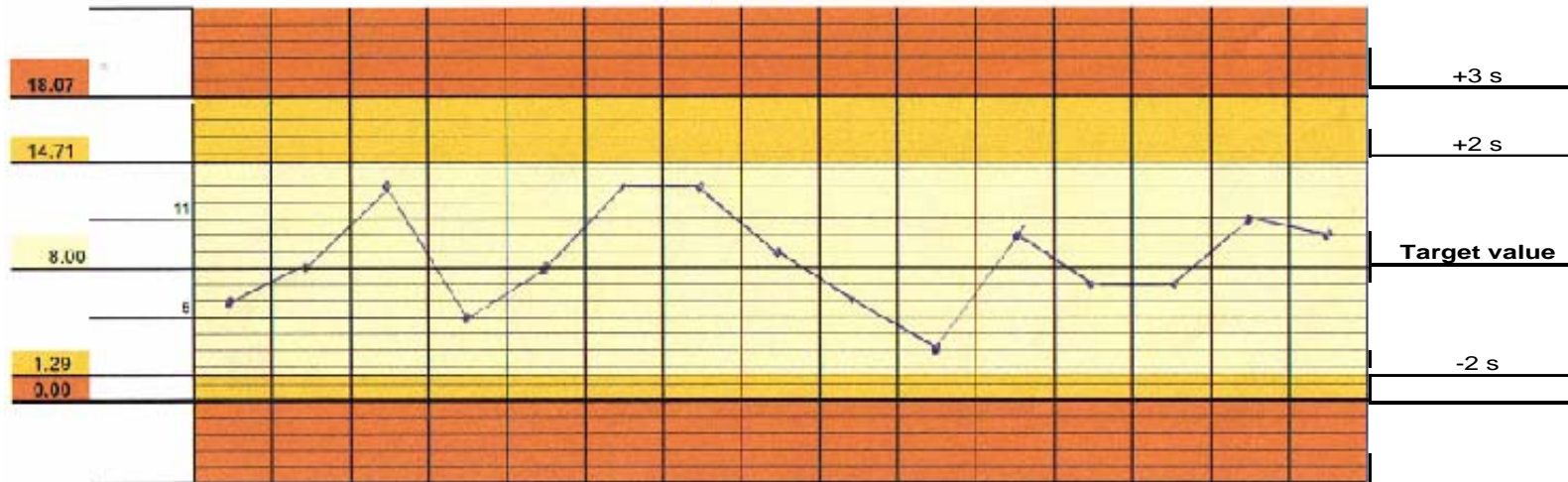


Sample :	DQ-04-0752	Analytical parameter :	Animal protein	Period :	May 2005 -
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Analyst	IF	IG	IF	IF	IF	IF	IF	IF	IG	IF	IF	IF	IF	IF	IF
Date	11.05.05	12.05.05	13.05.05	14.05.05	15.05.05	16.05.05	17.05.05	18.05.05	19.05.05	20.05.05	21.05.05	22.05.05	23.05.05	24.05.05	25.05.05
Nbr Ani. Partic.	6	8	13	5	8	13	13	5	6	3	16	7	7	11	12
Corrective act.															

Visa Res. Labo :
Date :

Handwritten signatures and dates:
 10/05/05
 11/05/05
 12/05/05
 13/05/05
 14/05/05
 15/05/05
 16/05/05
 17/05/05
 18/05/05
 19/05/05
 20/05/05
 21/05/05
 22/05/05
 23/05/05
 24/05/05
 25/05/05



Samples	Level of contamination	XM	SD
DQ-03-0027	0.10%	0.053%	0.043
DQ-03-0028	0.50%	0.208%	0.096
DQ-04-267-07	0.10%	0.155%	0.069
DQ-04-267-10	0.10%	0.174%	0.040
DQ-04-267-17	0.50%	0.750%	0.163
DQ-03-0781-08	0.10%	0.112%	0.051

Table 4: Comparison of the expected contamination level and the percentage detected.

Cross-contamination checking (blind samples)

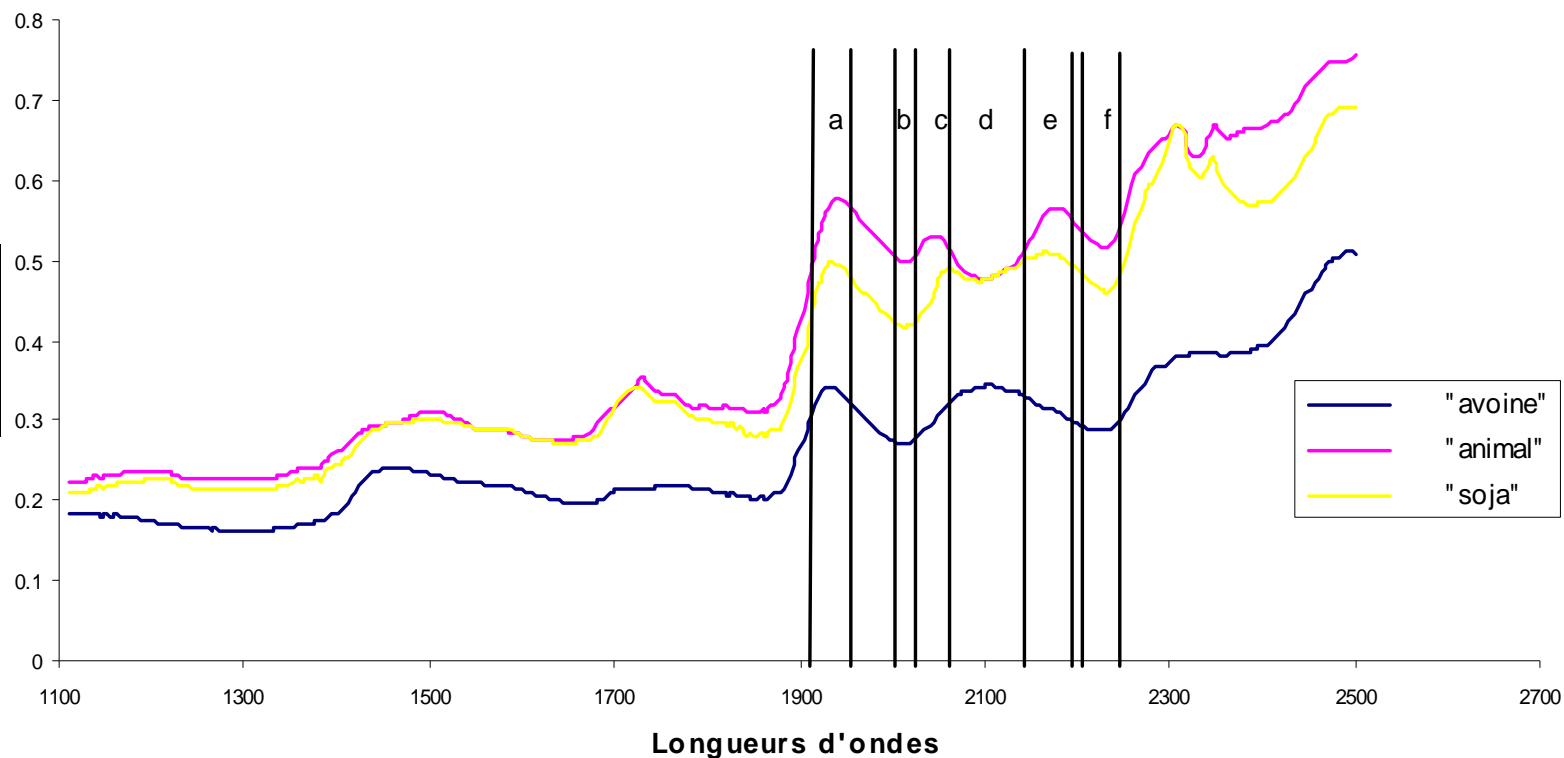
Table : Results of alternated samples analysis to test if there are cross-contaminations

Sub-samples	Sediment code	Theoretical percentage	MBM particles detection
DQ-04-0267-12-1	905	5% MBM	yes
DQ-02-1017-02-a	906	0% MBM	no
DQ-04-0267-12-2	907	5% MBM	yes
DQ-02-1017-02-b	908	0% MBM	no
DQ-04-0267-12-3	909	5% MBM	yes
DQ-02-1017-02-c	910	0% MBM	no

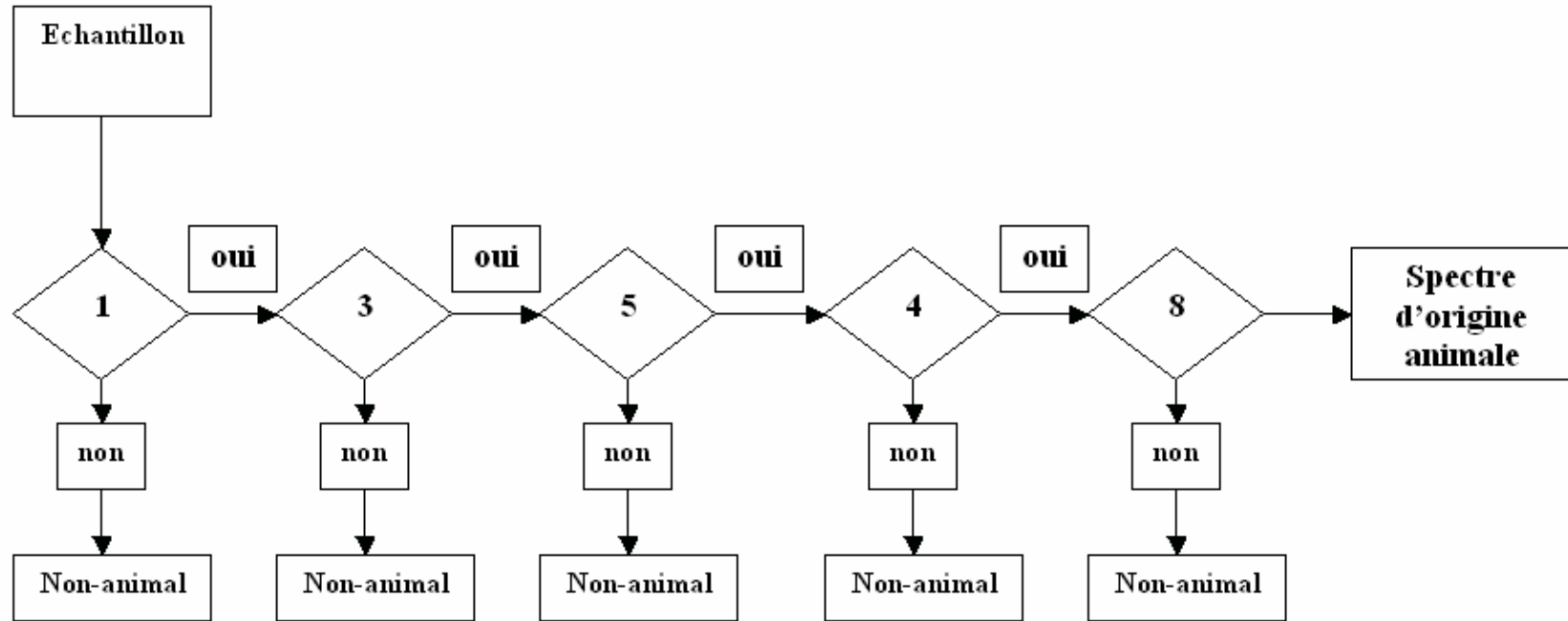
(Source : Validation dossier of the NIRM method)

Visual control of the spectra

Spectres moyens types



Visual control of the spectra



- Condition 1 : 1920-1960 : pic en (a)
- Condition 2 : 2010-2030 : puit en (b)
- Condition 3 : 2030-2070 : pic en (c)
- Condition 4 : 2070-2150 : puit ou pic en (d)
- Condition 5 : 2150-2200 : pic en (e)
- Condition 6 : 2210-2250 : puit en (f)
- Condition 8 : $(b+f)/2 > d$



Organisme belge d'Accréditation
Belgische Accreditatieinstelling
Belgische Akkreditierungsstelle
Belgian Accreditation Body

Signatory to EA, ILAC and IAF
Multilateral Agreements

Accreditation Certificate No. 300-TEST

In compliance with the provisions of the Royal Decree of 31 January 2006 setting up BELAC, the Accreditation Board hereby declares, that the test laboratory

**MINISTÈRE DE LA RÉGION WALLONNE
CENTRE WALLON DE RECHERCHES AGRONOMIQUES
DÉPARTEMENT QUALITÉ DES PRODUCTIONS AGRICOLES
Chaussée de Namur, 24
5030 GEMBLoux - Belgium**

has the competence to perform the tests as described in the annex which is an integral part of the present certificate, in accordance with the requirements of the standard NBN EN ISO/IEC 17025:2000. The present accreditation is the subject of regular surveillance in order to confirm the compliance with the accreditation conditions.

The Chair of the Accreditation Board BELAC,



Nicole MEURÉE-VANLAETHEM

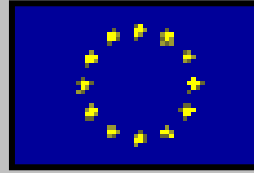
Issue date : **2006-09-05**

Validity date : **2009-06-18**

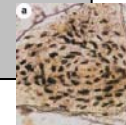
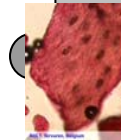
Original version of this certificate is in French.

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Ring trial & training



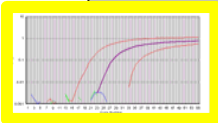
Microscopy



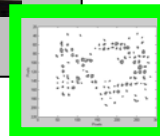
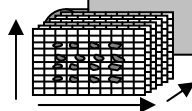
Immuno



PCR



Imaging



NIRM

