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# Methods available for re-entry of Processed Animal Proteins in feed

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## **Acknowledgements**

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# Overview of presentation

- Introduction
- Production of reference materials (pilot steriliser)
- Methods for species identification (overview)
- PCR technique for species DNA detection
- Reveal® dipstick method for *screening* ruminant protein
- MELISA-TEK™ for detection of ruminant protein
- Dilemma of feed analysis (allowed products)



# Production of reference materials

**CCL (NL)** has prepared pure porcine and chicken reference materials (PAPs)

∅ in a pilot sterilizer (140 L) with a stirring device

∅ 20 min. 133°C or 159°C

∅ pre-, post- or defatted post-pressure cooking

**PDM (UK)** has prepared pure bovine, ovine, porcine and avian (= chicken) reference materials (PAPs)

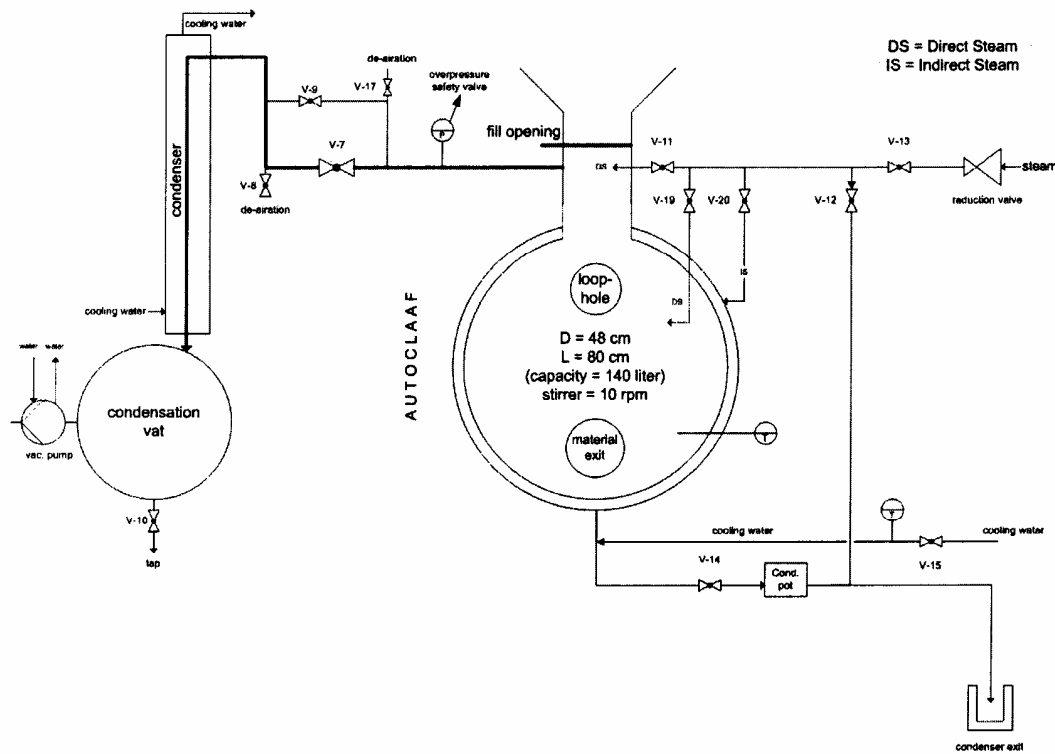
∅ dedicated pilot plant

∅ 20 min. 133°C, 137°C, 141°C and 145°C

∅ post-pressure cooking



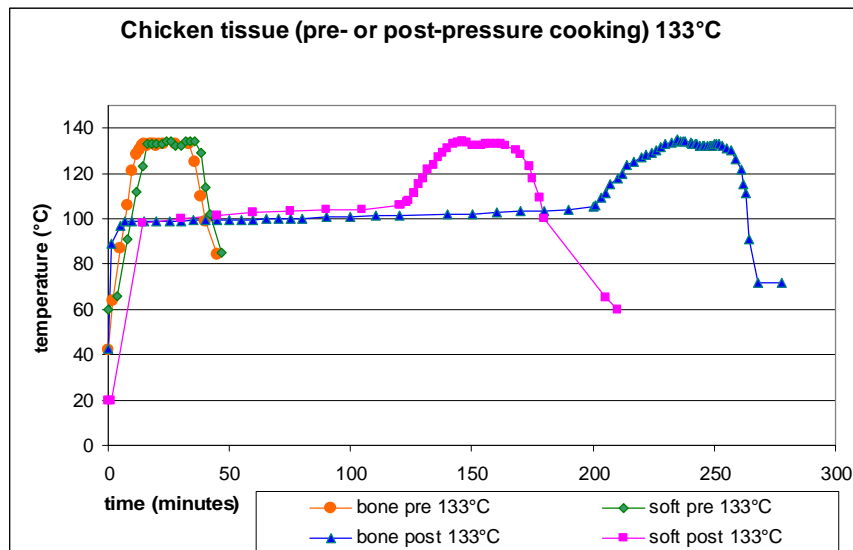
# Sterilisation equipment (used by CCL)



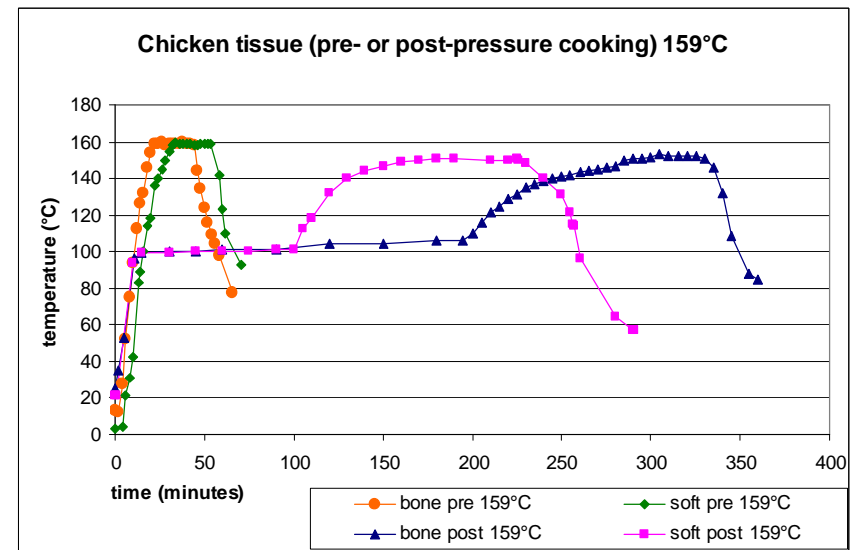


# Temperature profiles of reference materials

20 min. 133°C



20 min. 159°C





## Methods for species identification, used by CCL

**Microscopy (current official method):** detection of animal **tissues**

- very hard to distinct between species
- much education and experience needed

**Immunochemistry (i.e. ELISA):** specific **protein** detection

- proteins not present in every tissue cell
- easy to perform

**PCR:** specific **DNA** detection

- present in every tissue cell
- experience needed



## PCR technique for detection cattle/ ruminant tissue (DNA)

**Community Reference Laboratory (CRA-W, Gembloux, B):**

PCR test for **cattle**

∅ Testkit operational at CCL

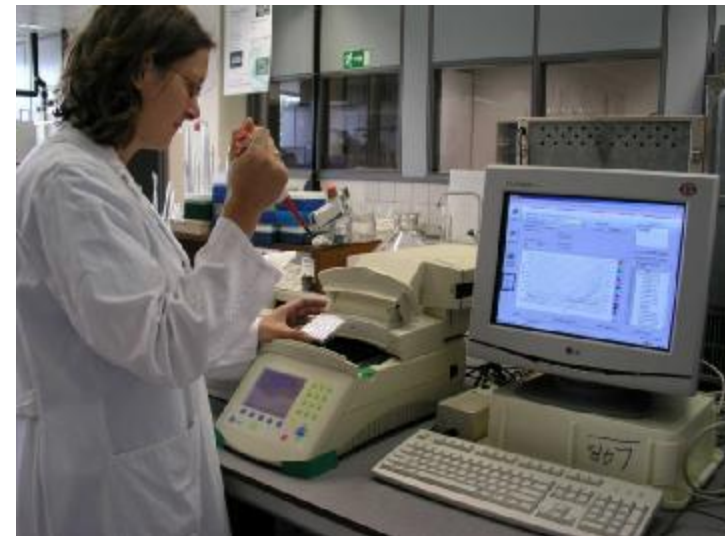
∅ In future commercial available

**TNO (Zeist, NL):**

PCR test for **ruminants**

∅ Analysis by TNO

∅ Test kit not (yet) commercial available





# PCR Cattle/ruminant detection in pure reference materials (PAPs)

		PCR CRA-W	PCR TNO			PCR CRA-W	PCR TNO
Pork soft	133°C pre-pressure cooking			Bovine carcass	133°C		
	159°C pre-pressure cooking				137°C		
	133°C post-pressure cooking				141°C		
	159°C post-pressure cooking				145°C		
Pork bones	133°C defatted post-pressure cooking			Ovine carcass	133°C		
	159°C defatted post-pressure cooking				137°C		
	133°C pre-pressure cooking				141°C		
	159°C pre-pressure cooking				145°C		
Chicken soft	133°C post-pressure cooking			Porcine carcass	133°C		
	159°C post-pressure cooking				137°C		
	133°C defatted post-pressure cooking				141°C		
	159°C defatted post-pressure cooking				145°C		+
Chicken bones	133°C pre-pressure cooking			Avian carcass	133°C		
	159°C pre-pressure cooking				137°C		
	133°C post-pressure cooking				141°C		
	159°C post-pressure cooking				145°C		
Chicken bones	133°C defatted post-pressure cooking			Bovine muscle	133°C		
	159°C defatted post-pressure cooking				137°C		
	133°C pre-pressure cooking				141°C		
	159°C pre-pressure cooking				145°C		
Chicken bones	133°C post-pressure cooking			Ovine muscle	133°C		
	159°C post-pressure cooking				137°C		
	133°C defatted post-pressure cooking				141°C		
	159°C defatted post-pressure cooking				145°C		
right				Porcine muscle	133°C		
wrong					137°C		
					141°C		
					145°C		
				Avian muscle	133°C		
					137°C		
					141°C		
					145°C		





## Results PCR cattle (CRA-W) with non ruminant PAPs, spiked with ruminant material

Non ruminant PAP	Inclusion level; % Ruminant PAP "spike"					
	Zero	0,1	0,5	1	2	5
Porcine PAP a		-				
Porcine PAP b		-				
Porcine PAP c		-	-	-	-	
Avian PAP a		-				
Avian PAP b						
Avian PAP c						
Feather PAP a						
Feather PAP b						
Fishmeal PAP		-				



# Reveal equipment & interpretation

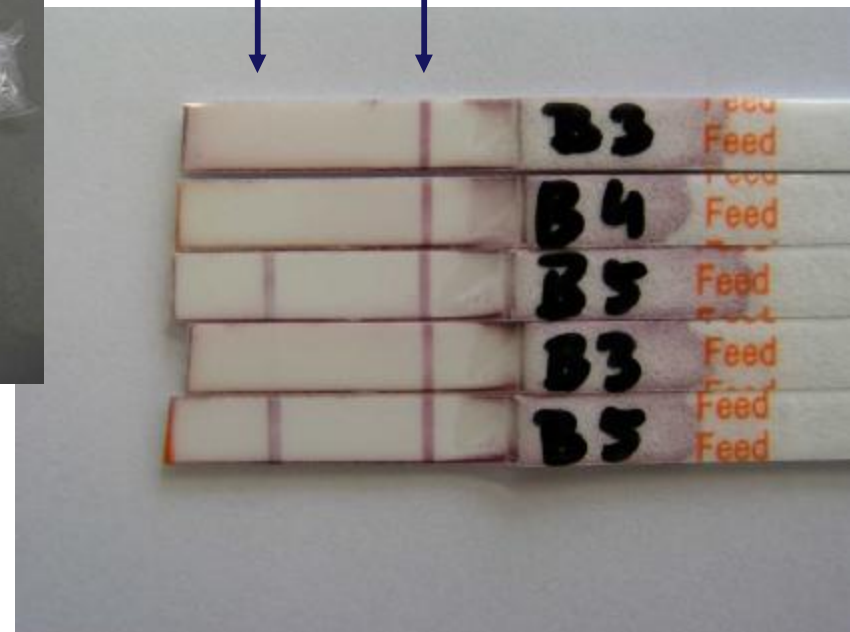


Conical flasks (e.g. 300 ml)

Water bath 100°C

Timer

sample zone:  
line = positive      control zone:  
line = correct





# Results <Reveal for Ruminant in Feed> with pure PAPs

		Reveal			Reveal
Pork soft	133°C	pre-pressure cooking		Bovine carcass	133°C
	159°C				137°C
	133°C				141°C
	159°C				145°C
	133°C				133°C
Pork bones	159°C	post-pressure cooking		Ovine carcass	137°C
	133°C				141°C
	159°C				145°C
	133°C				133°C
	159°C				137°C
Chicken soft	133°C	defatted post-pressure cooking		Porcine carcass	141°C
	159°C				145°C
	133°C				133°C
	159°C				137°C
	133°C				141°C
Chicken bones	159°C	pre-pressure cooking		Avian carcass	145°C
	133°C				133°C
	159°C				137°C
	133°C				141°C
	159°C				145°C
Chicken bones	133°C	post-pressure cooking		Bovine muscle	133°C
	159°C				137°C
	133°C				141°C
	159°C				145°C
	133°C				133°C
Chicken bones	159°C	defatted post-pressure cooking		Ovine muscle	137°C
	133°C				141°C
	159°C				145°C
	133°C				133°C
	159°C				137°C
Chicken bones	133°C	pre-pressure cooking		Porcine muscle	141°C
	159°C				145°C
	133°C				133°C
	159°C				137°C
	133°C				141°C
Chicken bones	159°C	post-pressure cooking		Avian muscle	145°C
	133°C				133°C
	159°C				137°C
	133°C				141°C
	159°C				145°C

right  
wrong



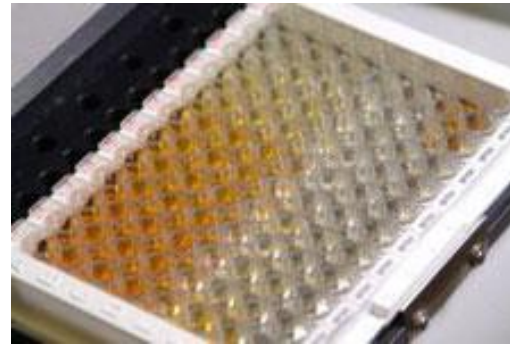
## Results <Reveal for ruminant in Feed> for control of PAPs for aqua feed

Non ruminant PAP for aquafeed	Inclusion level; % Ruminant PAP “spike”					
	Zero	0,1	0,5	1	2	5
Porcine PAP a		-				
Porcine PAP b		-	-			
Porcine PAP c		-	-	-	-	-
Avian PAP a						
Avian PAP b		-	-			
Avian PAP c		-				
Feather PAP a		-				
Feather PAP b		-				
Fishmeal PAP		-				



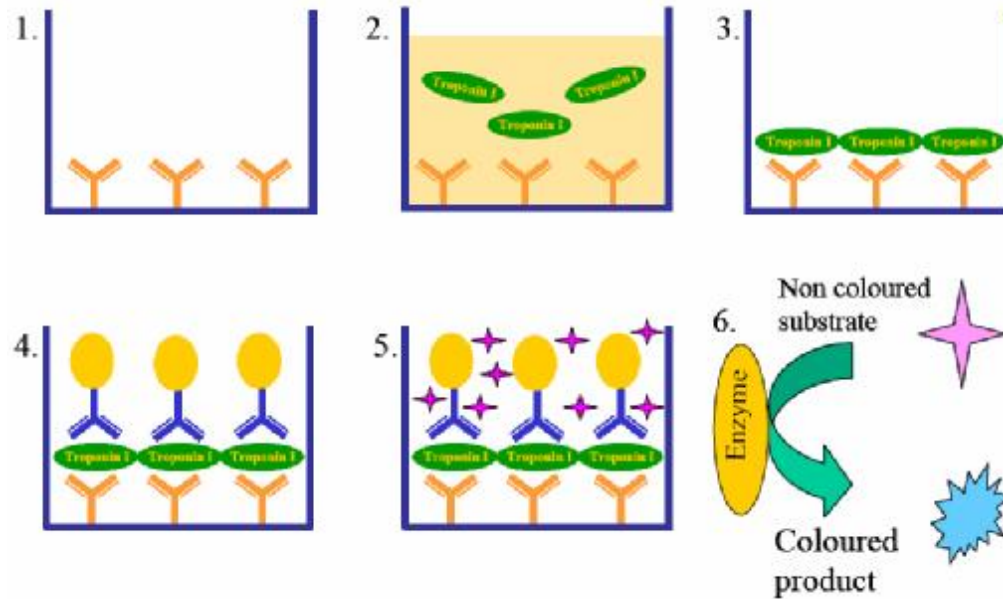
# ELISA-TEK Ruminant (bovine/ Ovine) KIT, ELISA Technologies, Inc. Including the high sensitivity extraction kit.

Detection of Troponin I,  
heat stable, muscle specific protein.





# Principle of MELISA-TEK™



source: O. Fumière, G. Berben and V. Baeten, October 2008.



## MELISA-TEK results for pure samples

PAP samples	% ruminant	Samples tested (N)	Samples correct (n)	Notes
Bovine PAP	100	8	8	ruminant detected
Ovine PAP	100	8	8	ruminant detected
Poultry PAP	0	22	21	1 false positive
Hydrolysed feathermeal	0	2	2	no rum. detected
Porcine PAP	0	32	32	no rum. detected
Pork/poultry	0	12	12	no rum. detected
Fishmeal	0	2	2	no rum. detected
Milk powder	0	2	2	no rum. detected



## Results MELISA-TEK, with spiked samples

Non ruminant PAP	Inclusion level; % Ruminant PAP "spike"					
	Zero	0,1	0,5	1	2	5
Porcine PAP a	Green	Green	Green	White	White	White
Porcine PAP b	Green	White	White	Red -	Green	White
Porcine PAP c	Green	White	Green	Green	White	White
Avian PAP a	Green	Green	Green	White	White	White
Avian PAP b	Green	White	Green	Green	White	White
Avian PAP c	Green	Green	Green	White	White	White
Feather PAP a	Green	White	Green	Green	Green	White
Feather PAP b	Green	White	Green	Green	White	White
Fishmeal PAP a	Green	Red -	Red -	Green	Green	Green

Blanc cell is not tested





## MELISA-TEK results for spiked samples

samples	% ruminant spike for the sample			
	0	0.5	1.0	2.0
Poultry PAP		7 / 7 *	8 / 8	not tested
feathermeal Hydrolysed		6 / 6	6 / 6	4 / 4
porcine PAP		7 / 7	50 / 55	37 / 37
fishmeal PAP		7 / 8	9 / 9	2 / 2

green cells: all results correct; yellow cells: not all results correct.

\* n / N = number of correct samples / Number of samples tested



## MELISA-TEK results commercial samples

<b>samples</b>	<b>% * ruminant</b>	<b>Samples tested (N)</b>	<b>Samples correct (n)</b>	<b>Notes</b>
ruminant PAP	100	2	2	ruminant detected
ruminant PAP	estimate 50 - 90	7	7	ruminant detected
ruminant PAP	40	1	1	ruminant detected
ruminant PAP	5	1	1	ruminant detected

\* According to supplier of the material



## Method characteristics of MELISA-TEK (ruminant spike level $\geq 1\%$ )

		Sample with ruminant spike	
		+	-
MELISA-TEK result	+	true positive (TP) 169	false positive (FN) 1
	-	false negative (FN) 6	true negative (TN) 71
		Sensitivity = TP/(TP+FN) = <b>97%</b>	Specificity = TN/(FP+TN) = <b>99%</b>



## Method characteristics of MELISA-TEK (ruminant spike level $\geq 2\%$ )

		Sample with ruminant spike	
		+	-
MELISA-TEK result	+	true positive (TP) 82	false positive (FN) 1
	-	false negative (FN) 0	true negative (TN) 71
		Sensitivity = $TP/(TP+FN) = 100\%$	Specificity = $TN/(FP+TN) = 99\%$



- Techniques **available today** for detection of prohibited species in single species PAPs

	prohibited species tissues	control technique
ruminant MBM	not allowed in feed for farmed animals	control not necessary
<b>porcine or poultry PAPs or fish meal for aquafeed</b>	<b>ruminant tissues</b>	<b>MELISA-TEK (or Reveal) PCR for confirmation</b>
porcine PAPs for poultry feed	ruminant tissues poultry tissues	<b>MELISA-TEK (or Reveal) for ruminant tissues</b> PCR for poultry tissues
poultry PAPs for pig feed	ruminant tissues porcine tissues	<b>MELISA-TEK (or Reveal) for ruminant tissues</b> PCR for porcine tissues



## Techniques for detection of (prohibited) species in compound feed for farmed animals

	prohibited species tissues	Control technique
ruminant feed	all animal tissues	microscopy
aqua feed	ruminant tissues	MELISA-TEK
pig feed	ruminant / porcine tissues (except <i>allowed products</i> )	MELISA-TEK for ruminant tissues PCR for porcine tissues
poultry feed	ruminant / poultry tissues (except <i>allowed products</i> )	MELISA-TEK for ruminant tissues PCR for poultry tissues



**Leads to dilemma**



## Dilemma

Some animal proteins are already allowed in compound feed,  
e.g. hydrolysed proteins, milk, egg, fat, DCP, TCP,  
gelatine and bloodproducts,  
feed materials of plant origin with (i.e. rodent, bird)  
bone spicules.

How to discriminate between prohibited and allowed products  
in feed?



## Solution for this dilemma

Use analytical methods

for control of raw materials (PAPs)

not for control of feed,

but, combine it with administrative monitoring  
of compound feed (tracking and tracing).





# Conclusion: Methods are available today

- Raw materials for aqua feed:

- screening for absence of ruminant material with MELISA-TEK and confirmation with PCR.

- lifting this ban is in discussion with DG-Sanco.

- Raw materials for feed for farmed animals:

- screening for absence of ruminant material with MELISA-TEK and confirmation with PCR.

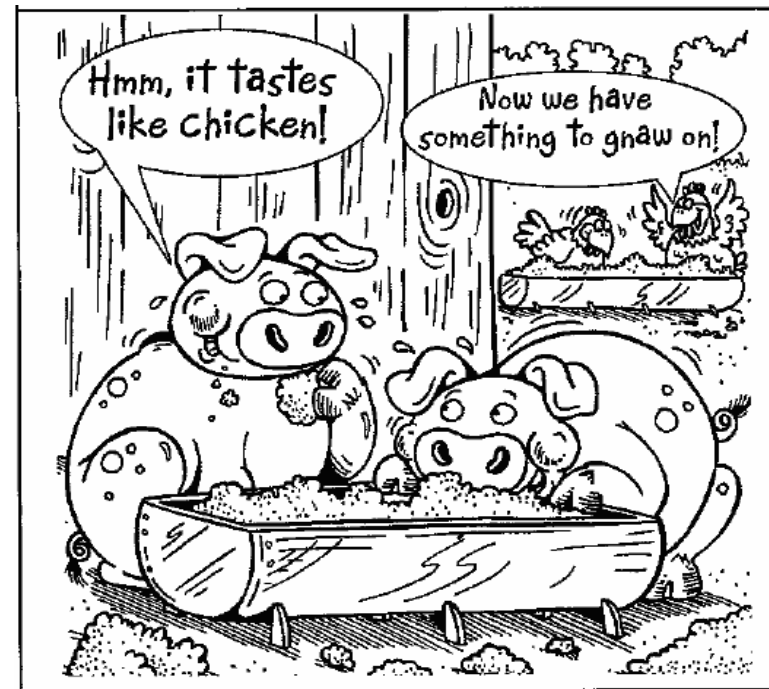
- but for lifting this ban, the following methods have to be further validated:

- 1) Test for poultry material (for acceptance porcine PAP in poultry feed)

- 2) Test for porcine material (for acceptance poultry PAP in pig feed)



**Thank you for your attention**



Source: Feed Tech 11.5 2007  
[www.AllAboutFeed.net](http://www.AllAboutFeed.net) - 6