

REFERENCE MATERIALS FOR FOOD ALLERGEN ANALYSIS



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NO standard of reference available

DIFFERENT antibodies



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REFERENCE MATERIALS FOR FOOD ALLERGEN ANALYSIS

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1. First objectives

- harmonized validation protocol
- recognized reference materials - RMI
- contaminated with egg and milk proteins at various concentrations
- two food matrices – first cookies and after soy based infant formula
- standards
 - non-fat milk powder NIST, RM 1549
 - spray-dried whole egg for allergen detection NIST, RM 8445

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SPECIAL SECTION ON FOOD ALLERGEN TESTING

Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices

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2. Recipe

based on Scaravelli*'s recipe for cookies incurred with peanut

selected recipe

Qty (g)	Ingredients
2 000	butter (19.6 %)
5 000	wheat flour (49.0 %)
1 875	dust sugar (18.4 %)
600	skimmed milk powder (5.0 %)
675	water (6.6 %)
30	sodium chloride (0.3 %)
12,5	sodium hydrogen carbonate (0.1 %)
15	amonium bisulphate (0.1 %)

Qty (g)	Ingredients
1 500	olive oil (16 %)
5 900	wheat flour (59.0 %)
1 900	dust sugar (19 %)
-	---
650	water (6.5 %)
30	sodium chloride (0.3 %)
10	sodium hydrogen carbonate (0.1 %)
10	amonium bisulphate (0.1 %)

- baking : 16 min at 180 °C
- spiking : serial dilution in butter

* E. Scaravelli *et al.*, Eur Food Res Technol (2008) 227:857-869

3. Baking method

Qty (g)	Ingredients
1 500	olive oil (16 %)
5 900	wheat flour (59.0 %)
1 900	dust sugar (19 %)
650	water (6.5 %)
30	sodium chloride (0.3 %)
10	sodium hydrogen carbonate (0.1 %)
10	amonium bisulphate (0.1 %)

- flatting mill : thickness 8 mm
- diameter of the punch : 60 mm
- weight before baking : 22 g per cookie
- baking : 17 min at 200 °C



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4. Spiking procedure

direct spiking

1000 ppm : 100 mg of each powder in
59 g wheat flour

100 ppm : 10 mg of each powder in
59 g wheat flour

dilution 10x : 1 mg/g

100 ppm: 49 g wheat flour +
10 g of 1 mg/g

vs

serial dilution spiking

solution 50 mg/g : 10 g wheat flour +
500 mg of each powder

dilution 5x : 10 mg/g

1000 ppm: 49 g wheat flour
+ 10 g of 10 mg/g

All solutions were prepared by mixing during 2 h.

4. Spiking procedure

casein		direct	serial	CV (%)
estimated concentration (ppm)	not baked	290	320	7.0
	baked	140	120	10.9

beta-lactoglobulin		direct	serial	CV (%)
estimated concentration (ppm)	not baked	3.4	3.4	0.9
	baked	0.6	0.7	10.9

egg		direct	serial	CV (%)
estimated concentration (ppm)	not baked	1190	1220	1.8
	baked	24	27	8.3

mean of CV = 6.6 %

→ direct spiking

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5. First analyses - CAS

provider	format	standard	LOQ (ppm)	range of quantification (ppm)
CER Groupe	sandwich	casein	0.5	0.5 -20
Elisa Systems	sandwich	skim milk powder	1.0	1.0 – 10.0
R-Biopharm	sandwich	casein	0.5	0.5 – 13.5
Tepnel	competitive	whole milk powder RM8435 from NIST (expressed in casein)	1.6	1.6 – 25
Morinaga	sandwich	milk protein	0.312	0.312 – 20

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5. First analyses - **BLG**

provider	format	standard	LOQ (ppm)	range of quantification (ppm)
CER Groupe	sandwich	beta-lactoglobulin	0.25	0.25 – 5
Elisa Systems	sandwich	beta-lactoglobulin	0.1	0.1 – 1
R-Biopharm^(*)	sandwich	beta-lactoglobulin	0.2	0.2 – 16.2
Tepnel	competitive	beta-lactoglobulin	2.5	2.5 – 40
Morinaga	sandwich	milk protein	0.312	0.312 – 20

(*)

Although RIDASCREEN beta-lactoglobulin is a **qualitative** assay for food samples, it has been used as a **quantitative** one.

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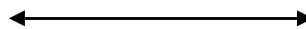
5. First analyses - egg

provider	format	standard	LOQ (ppm)	range of quantification (ppm)
CER Groupe	sandwich	whole egg powder protein RM8415 (NIST)	2	2 – 50
Elisa Systems	sandwich	egg white protein	1	1 – 5
R-Biopharm	sandwich	egg white protein	1	1 – 27
Tepnel	competitive	ovomucoid (expressed in egg white protein)	0.5	0.5 – 10
Morinaga	sandwich	egg protein	0.312	0.312 – 20

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5. First analyses

CAS
BLG
egg



CER Groupe
ELISA Systems
R-Biopharm
Tepnel
Morinaga

Samples

- 0 ppm
- 10 ppm (100 ppm 10 times diluted)
- 100 ppm
- 1000 ppm

} egg and milk NIST RM powder

baked or **not** baked

Interpretation

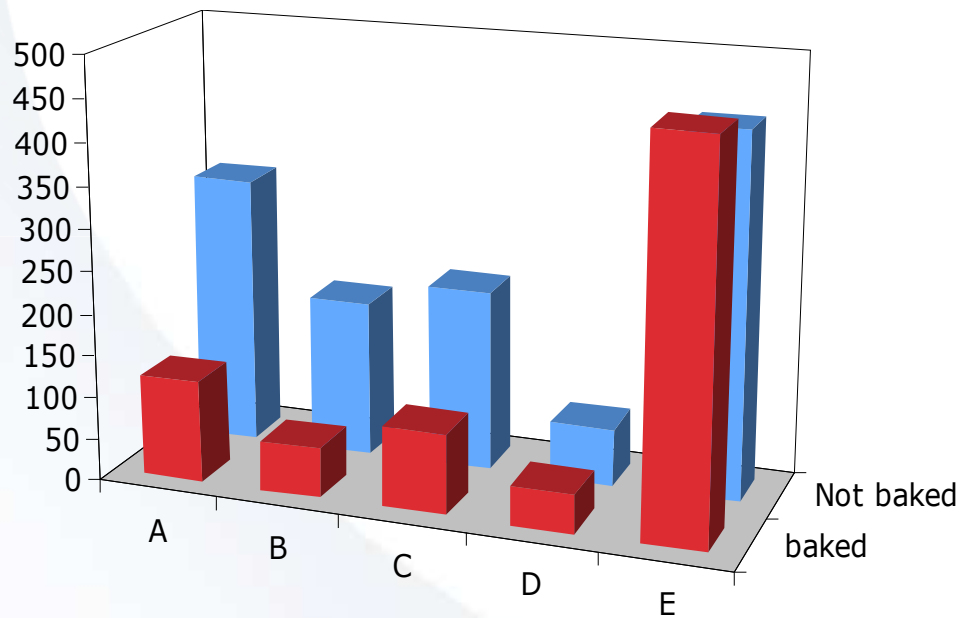
- estimated concentration -> calculated for 1000 ppm samples
- lost in water (+/- 15 %) -> not taken into account
- same graph for the different kits providers but not the same units
- 'detection at 10 ppm' = ability of test to detect positive baked cookies at 10 ppm

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6. Results : NIST RM 1549 non-fat milk powder

casein

1000 ppm



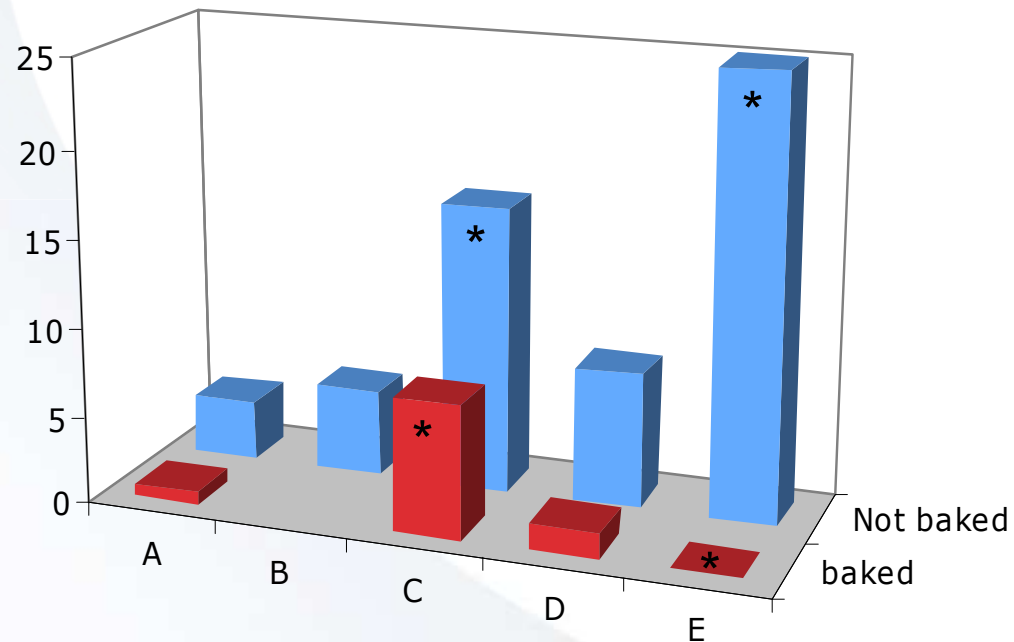
	RECOVERY (%)	DETECTION at 10 ppm
A	49	OK
B	32	n.d.
C	45	OK
D	54	n.d.
E	105	OK

$$\text{RECOVERY} = \frac{\text{Cc baked} \times 100}{\text{Cc not baked}}$$

6. Results : NIST RM 1549 non-fat milk powder

beta-lactoglobulin

1000 ppm



	RECOVERY (%)	DETECTION at 10 ppm
A	20	n.d.
B	/	n.d.
C	46	OK
D	19	n.d.
E	3	n.d.

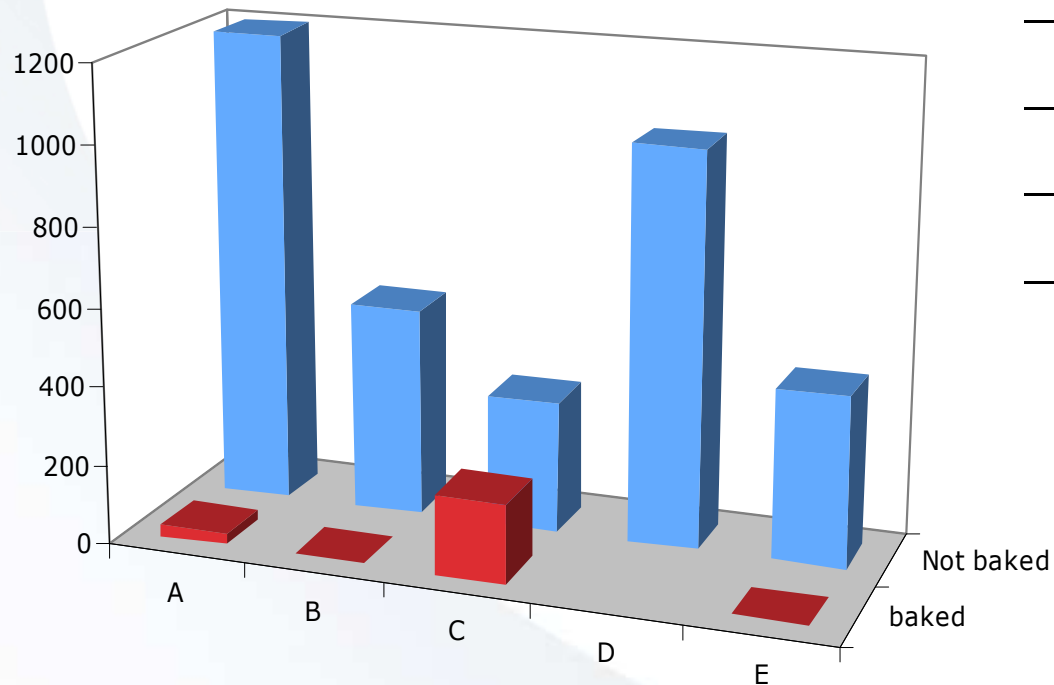
$$\text{RECOVERY} = \frac{C_c \text{ baked} \times 100}{C_c \text{ not baked}}$$

* : /10 for clarity

6. Results : NIST RM 8445 spray-dried whole egg

egg

1000 ppm



	RECOVERY (%)	DETECTION at 10 ppm
A	2	n.d.
B	0.5	n.d.
C	61	OK
D	/	n.d.
E	0.4	n.d.

$$\text{RECOVERY} = \frac{C_c \text{ baked} \times 100}{C_c \text{ not baked}}$$

7. Observations & conclusions

- **BLG & egg proteins** => very **sensitive** to heating
 - > under-estimation of presence of these allergens in final product
 - > could be interesting to compare ELISA detection and allergic reaction
- each kit -> own calibrators and own quantification procedure
estimated concentrations => **different**
(even with information on calibrators and unit conversion factors given by some providers e.g. Elisa Systems)
- food processing -> affect **extraction** and **detection** of proteins
- food allergen detection methods -> detect **native** and **modified** proteins

=> **NEED of incurred reference material**

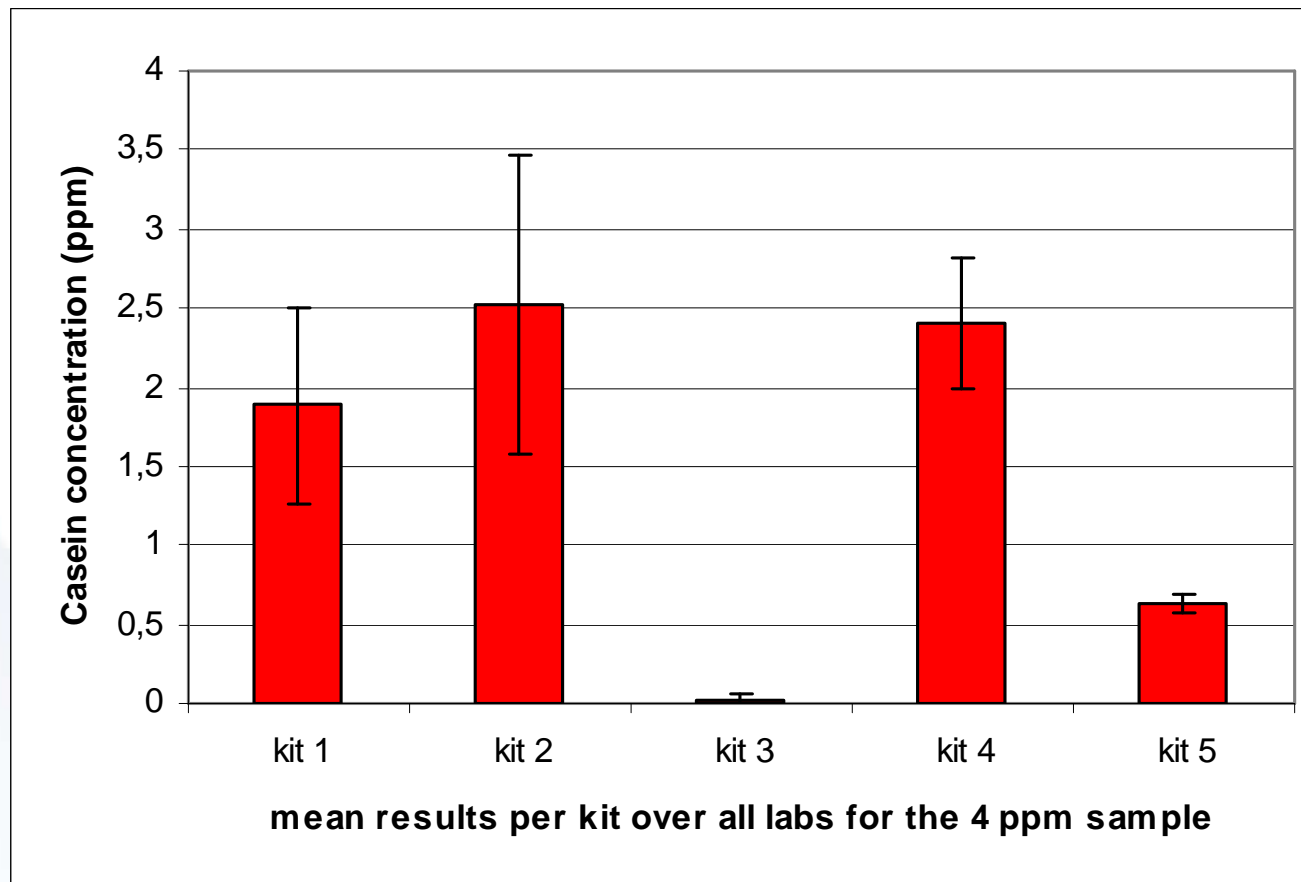
8. Pre ring trial

- ❑ **Protocol**
 - focus on **casein** detection only
 - 3 labs involved : **CER, IFR, Eurofins**
 - ELISA kits providers : R-Biopharm, Tepnel, Elisa Systems, Morinaga, Neogen
 - **1 kit of each provider** supplied to each lab
 - 3 blind coded cookies samples /kit : **0 - 2 - 4 ppm casein** (standard = NIST RM 1549)

- ❑ **For harmonised validation protocol** : 2nd RMI needed
 - > soy based infant formula
 - spiking at 8.3 and 16.7 ppm NIST RM 1549 milk powder (2 and 4 ppm casein)
 - pre-ring trial like cookies

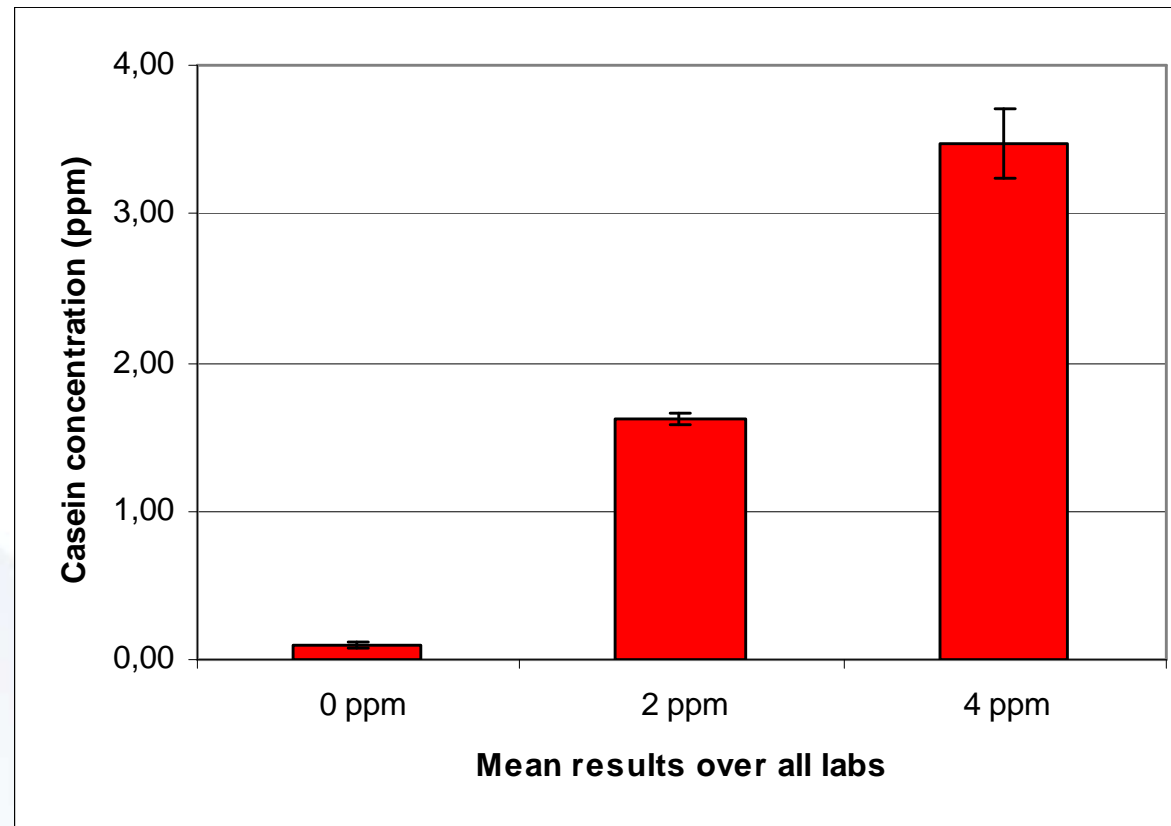
8. Pre ring trial

- Results – cookies



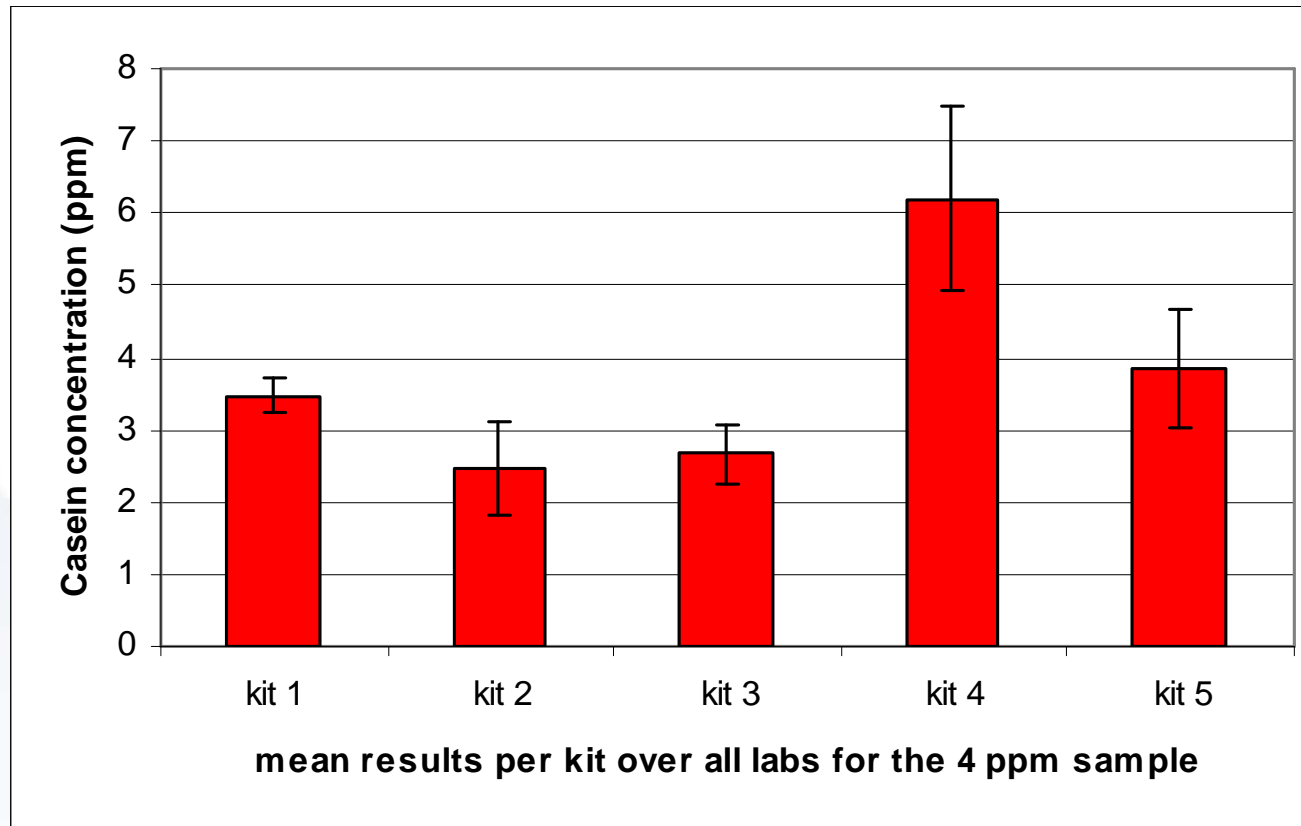
8. Pre ring trial

- Results – soy based infant formula



8. Pre ring trial

- Results – soy based infant formula



8. Pre ring trial

□ **Conclusions**

- materials seem to be suitable as **reference materials**
- useful for testing **performance** of test kits
- calculated spiking level: 0 – 1 – 2 – 5 – 10 – 50 ppm casein



□ **Scaling-up**

- production of 5 kg of cookies per level
- production of 2 kg of soy based infant formula per level

9. Production

- **Cookies** – 40 kg negative dough and 15 kg of 1000 ppm milk powder RM1549 containing dough

Previous (%)	Ingredients	Actual (%)
16.0	olive oil	15.1
59.0	wheat flour	53.7
19.0	dust sugar	16.1
6.5	water	14.6
0.3	sodium chloride	0.3
0.1	sodium hydrogen carbonate	0.1
0.1	amonium bisulphate	0.1

 **Increasing water content**

flattening mill : thickness = 7.5 mm
diameter of the punch : 60 mm
baking : vault 70/30, single plate, 200°C, 21 min
mean weight before baking : 34 g (n=35)
mean weight after baking : 28 g (n=35) => lost in water = 17.6%

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9. Production

- ❑ **Cookies – grinding**
 - IRMM
 - milled at 2 mm => mean particule size between 120 µm and 250 µm

- ❑ **Soy based infant formula – provided by Danone**

- ❑ **Both RMI – mix ~ sampling ~ homogeneity ~ stability**
 - 1000 ppm milk powder ⇔ 240 ppm casein – diluted with ground negative cookies to reach 1 – 2 – 5 – 10 – 50 ppm casein
 - 90 tubes x 5 kits for each level for each matrix
 - homogeneity assay -> **passed** for each level and each matrix
 - stability: at least 65 days at 37°C = OK

10. Perspectives

□ **MoniQA Casein Validation Study**

- according to the harmonised validation protocol
- 20 international labs involved
- 5 ELISA kits providers as for the pre ring trial
- started 26/04/2010 -> sending of 180 coded samples and 5 kits/ lab
- achieved -> **Results are being analysed**



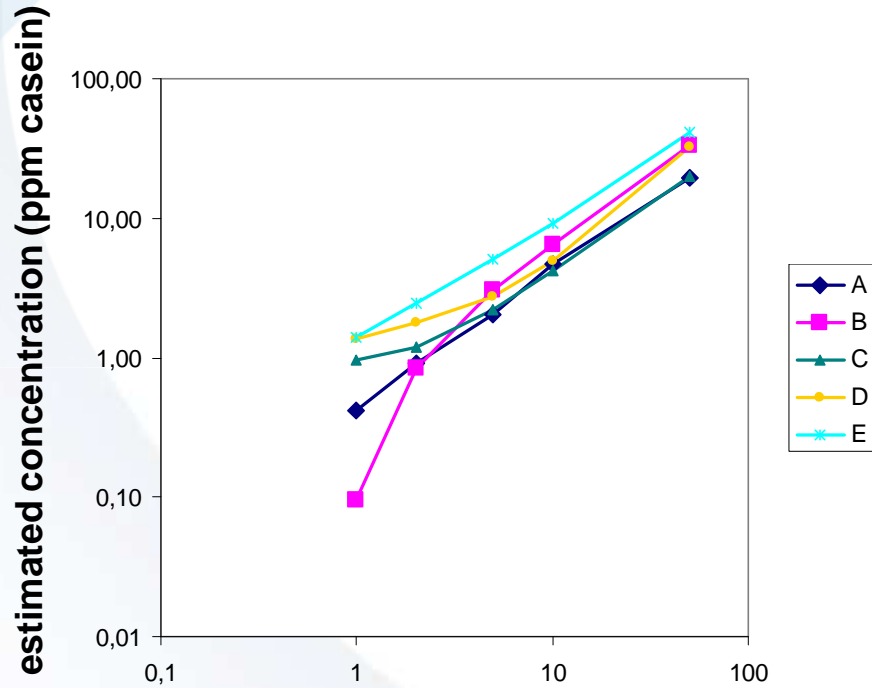
□ **Goals**

- prove/promote the **use of RMI**
- implement/validate the **harmonised validation protocol**
- document **validation data** of food allergen detection methods

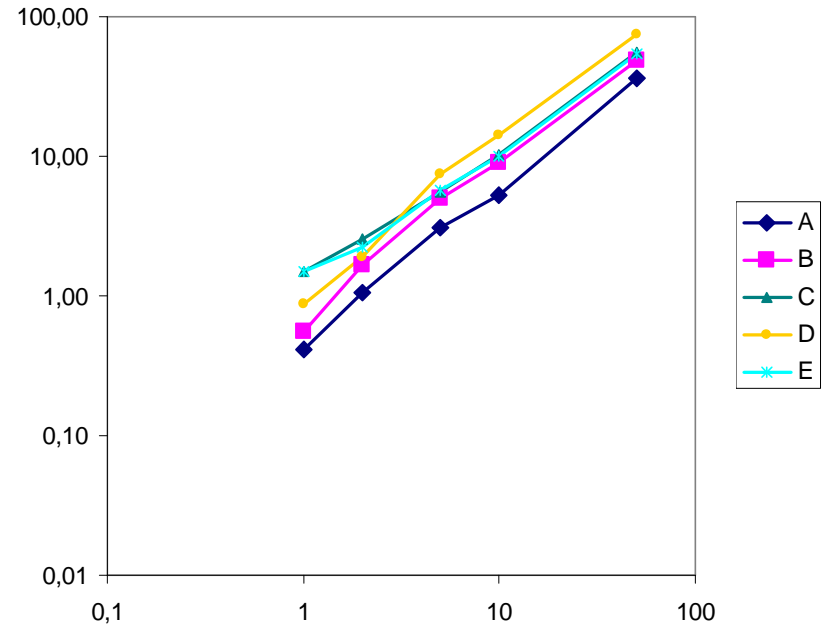


10. Perspectives – first results

cookies



soy based infant formula



expected concentration (ppm casein)

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