Proficiency test for allergens in food 2014

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RIKILT
WAGENINGEN UR

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Distribution list:
22 participating laboratories of which 9 European participants, 10 North-American participants, 2 Asian participants and 1 participant from Oceania.

Contents

Summary

In the autumn of 2014 a proficiency test for allergens in baby cereal was organized by RIKILT, Wageningen UR. This PT-test enabled laboratories to evaluate their competence for the analysis of allergens in baby cereal. Both quantitative and qualitative methods were accepted. The proficiency test was carried out according to ISO/IEC 17043, however this specific test is not part of the accreditation.

For this proficiency test, three test materials were prepared:
A. Baby cereal containing almond (AL) aimed at 20 mg/kg, peanut (PN) aimed at 5 mg/kg and skim milk powder (SMP) aimed at 20 mg/kg;
B. Baby cereal containing almond (AL) aimed at 10 mg/kg, soy flour (SOY) aimed at 25 mg/kg and wheat flour (GL) aimed at 10 mg/kg gluten;
C. Baby cereal containing Peanut (PN) aimed at 15 mg/kg, skim milk powder (SMP) aimed at 5 mg/kg and wheat flour (GL) aimed at 30 mg/kg gluten

The fortified materials were all prepared by spiking baby cereal followed by cryogenic homogenization. During homogeneity testing, all materials proved to be sufficiently homogenous for proficiency testing. The stability test demonstrated that no statistically significant loss of PN and SOY occurred during the timescale of the proficiency test. For AL, GL, and SMP a consequential loss occurred during the storage at room temperature, which was accounted for in the calculations of the z-scores.

Twenty-two laboratories subscribed for participation in this test. All labs reported results. Not all laboratories analysed for all allergens. Only immunochemical assays were applied. Sixteen laboratories applied a method for the analysis of AL. Eighteen laboratories applied a method for the analysis of GL, one lab applied three different methods. Seventeen laboratories applied a method for the analysis of PN. Sixteen laboratories applied a method for the analysis of SMP, one lab applied two different methods, and one lab applied 4 different methods. Fifteen laboratories applied a method for the analysis of SOY. 75% of quantitative results for almond were satisfactory. For gluten 80% of all quantitative results were satisfactory. The uncertainty in the assigned values for PN, SMP and SOY was too high and therefore these values were not used for evaluation of the laboratories performance. Within the participant's scope 5 labs showed optimal performance. Detection of the presence of allergens in spiked samples, i.e. qualitative interpretation of reported results, was obtained in 100% of almond analyses, 95% of gluten analyses, 94% of peanut analyses, 78% of skim milk powder analyses, and 53% of soy analyses.

This proficiency test shows that detection of the presence (qualitative interpretation of results) of AL, GL and PN was successful, even at low concentrations. On the other hand, detection of the presence of SMP and SOY, was not satisfactory. A large variation in the quantitative results was observed for all allergens included. Hence, for all allergens detection methods need further development and harmonisation for accurate estimation of the mass concentration.

1 Introduction

Proficiency testing is conducted to provide laboratories with a powerful tool to evaluate and demonstrate the reliability of the data that are produced. Next to validation and accreditation, proficiency testing is an important requirement of the EU Additional Measures Directive 93/99/EEC [1] and is required by ISO 17025:2005 [2].

The aim of this proficiency test was to give laboratories the possibility to evaluate or demonstrate their competence for the analysis of allergens in baby cereal. Both quantitative and qualitative methods were accepted. The preparation of the materials, including the suitability testing of the materials and the evaluation of the quantitative results were carried out according to ISO 17043 [3] by RIKILT, however this specific test is not part of the accreditation.

2 Material en methods

This proficiency test focused on almond (AL), peanut (PN), soy (SOY), skim milk powder (SMP) and gluten (GL). The labelling rules in European Directives 2003/89/EC, 2006/142/EC and 2011/1169/EC state that 14 (groups) of food allergens must be declared when they are used at any level in food. Cross-contamination is, however, exempt from this regulation. In Australia and New-Zealand the VITAL system is used which provides action levels for labelling cross-contamination based on scientific threshold levels. The action levels for AL, PN, SOY, and SMP for a serving size of 25 grams, the prescribed quantity of baby cereal for 8 month old babies, are presented in Table 1. In regulation (EU) No 828/2014 a threshold of 20 ppm gluten is laid down for using the 'gluten free' statement.

Table 1

Action levels in mg/kg whole compound for AL, PN, SOY, and SMP for a serving size of 25 grams as provided by VITAL [4].

Compound	Action level (mg/kg)
AL	20
PN	32
SOY flour	111
SMP	11

2.1 Sample preparation

Three materials (A, B and C) containing different combinations of AL, PN, SOY flour, SMP and GL (added as wheat flour) at different levels were prepared. All materials were prepared by serial dilution of the selected compound (added as a powder obtained by cryogenic grinding of almonds, peanuts, soy flour, skim milk powder and wheat flour) in finely ground baby cereal aiming at the levels as presented in Table 2. Although the exact gluten content of the wheat flour was unknown, an estimation was made based on the average prolamin content as determined by Mickowska and colleagues [5]. Each of the materials was homogenized under cryogenic conditions according to in-house standard operating procedures [6].

Table 2

Target amount of allergens in the proficiency test materials.

Material	Target amount (mg/kg)				
	AL	GL	PN	SMP	SOY
A	20	-	5	20	-
B	10	10	-	-	25
C	-	30	15	5	-

2.2 Sample identification

After homogenization, the sample materials were divided into sub-portions and stored in polypropylene containers. The samples for the participants (at least 20 grams) were randomly selected and coded through a website application. This website application was developed for

management of proficiency tests organized by RIKILT. For each laboratory a sample set was prepared consisting of one at random selected sample of each material A, B and C.
The codes of the samples belonging to each sample set are presented in Annex 1. The remaining samples were used for homogeneity and stability testing.

2.3 Participants

Twenty-two laboratories subscribed for participation in the proficiency test of which 9 are situated within Europe, 10 in North-America, 2 in Asia and 1 in Oceania. Each participant was asked to indicate *a priori* which compounds were included in their scope. And each participant was asked to report the results through a web application designed for proficiency tests.

2.4 Homogeneity study

The homogeneity of the materials was tested according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [7] and ISO 13528 [8]. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) are compared with the standard deviation for proficiency assessment (σ_P), which is 25% of the grand mean of the homogeneity data [9]. The method applied for homogeneity testing is considered suitable if $s_w < 0.5*\sigma_P$ and a material is considered adequately homogeneous if $s_s < 0.3*\sigma_P$.

Ten containers of material A were analysed in duplicate for PN, ten containers of material B were analysed in duplicate for AL and ten containers of material C were analysed in duplicate for PN to determine the homogeneity of the materials. The homogeneity of other compounds in the materials was not tested, because the homogeneity test of AL/PN was considered adequate to prove the sufficient homogeneity of the material. The results of the homogeneity study and their statistical evaluation are presented in Annex 2-4. All materials demonstrated to be sufficiently homogeneous for use in the proficiency test.

2.5 Sample distribution and instructions

Each of the participating laboratories received a randomly assigned laboratory code, generated by the website application. The sample sets with the corresponding number, consisting of three coded samples (Annex 1) were sent to 21 participating laboratories on November 11th, 2014. One laboratory subscribed in a later stage and to this laboratory a parcel was sent on November 13th, 2014. The sample sets were packed in a paper box and were dispatched to the participants by courier.

The samples were accompanied by a letter (Annex 5) describing the requested analyses (almond, peanut, soy, milk and gluten) and an acknowledgement of receipt form. By e-mail the laboratories received instructions on how to use the web application to report results.

The laboratories were asked to store the samples until analysis at room temperature. A single analysis of each sample was requested. The deadline for submitting results was November 28th 2014, allowing two weeks for the analysis.

2.6 Stability

On November 11th, the day materials were distributed to the participants, 6 randomly selected samples of each material were stored at <-70 °C. It is assumed that the compounds included in this proficiency test are stable at these storage conditions. The remaining samples were stored at room temperature.

Twenty days after distribution of the samples, six samples from material A that had been stored at <-70 °C and six samples from material A that had been stored at room temperature were analysed for PN. 23, 27, 31 and 34 days after distribution of the samples, a similar procedure was applied for AL (material B), SOY (material B), GL (material C), and SMP (material A), respectively. For each set of samples, the average of the results and the standard deviation were calculated.

First it was determined if a 'consequential instability' occurred [7,8]. A consequential instability occurs when the average value of the samples stored at room temperature is more than $0.3_{\sigma H}$ below the average value of the samples stored at <-70 °C. If so, the instability has a significant influence on the calculated z-scores. Second, it was determined whether a statistically significant instability occurred using a Students t-test [8]. The results and statistical evaluation of the stability test are presented in Annex 6.

For PN, and SOY no consequential nor a statistical significant difference was observed among the samples stored at <-70 °C and the samples stored at room temperature. These samples are considered sufficiently stable.

For AL, GL and SMP a consequential difference was observed between the samples stored at <-70 °C and the samples stored at room temperature. The average result was lower than the average of the samples that were stored at <-70 °C. The concentration of AL showed a decrease of 10% during storage at room temperature for 23 days. The concentration of GL showed a decrease of 16% during storage at room temperature for 31 days. The concentration of SMP showed a decrease of 8% during storage at room temperature for 34 days. Therefore, for AL, GL and SMP the instability is incorporated in the calculation of the z'_{ai}-scores.

3 Applied method of analysis

Twenty-two laboratories carried out one or more analyses. An overview of the methods applied in this proficiency test is presented in Annex 7.

3.1 Almond

Sixteen laboratories applied a method for the analysis of AL. One laboratories reported qualitative results using ELISA. Fifteen laboratories reported quantitative results, all using ELISA methods (Annex 7).

3.2 Gluten

Eighteen laboratories applied a method for the analysis of GL, one lab applied three different methods. One laboratory reported qualitative results using a lateral flow device. Seventeen laboratories reported quantitative results, all using ELISA methods (Annex 7).

3.3 Peanut

Seventeen laboratories applied a method for the analysis of PN. One laboratory reported qualitative results using a ELISA. Sixteen laboratories reported quantitative results using ELISA methods (Annex 7).

3.4 Skim milk powder

Sixteen laboratories applied a method for the analysis of SMP, one lab applied two different methods, and one lab applied 4 different methods. One laboratory reported qualitative results using two different lateral flow devices. Fifteen laboratories reported quantitative results using ELISA methods (Annex 7).

3.5 Soy

Fifteen laboratories applied a method for the analysis of SOY. All laboratories reported quantitative results using ELISA (Annex 7).

4 Statistical evaluation

The statistical evaluation of the quantitative part of the study was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [7], elaborated by ISO, IUPAC and AOAC and ISO 13528 [8] in combination with the insights published by the Analytical Methods Committee [10,11] regarding robust statistics.

For the evaluation of the quantitative results the assigned value, the uncertainty of the assigned value, a standard deviation for proficiency assessment and z-scores were calculated.

4.1 Calculation of the assigned value (X)

The assigned value (X) was determined using robust statistics [8,10,11]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in a proficiency test. When using robust statistics, the data does not have to be normally distributed in contrast to conventional outlier elimination methods.

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results using a cut-off value depending on the number of results, was used as the assigned value [8,10]. The assigned value is therefore a consensus value.

4.2 Calculation of the uncertainty of the assigned value (u)

The uncertainty of the assigned value is calculated to determine the influence of this uncertainty on the evaluation of the laboratories. A high uncertainty of the assigned value will lead to a high uncertainty of the calculated participants z_a-scores. If the uncertainty of the assigned value and thus the uncertainty of the z_a-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory. In other words, illegitimate conclusions could be drawn regarding the performance of the participating laboratories from the calculated z_a-scores if the uncertainty of the assigned value is not taken into account.

The uncertainty of the assigned value (the robust mean) is calculated from the estimation of the standard deviation of the assigned value and the number of values used for the calculation of the assigned value [7]:

$$u = 1.25 * \frac{\hat{\sigma}}{\sqrt{n}}$$

where:

u = Uncertainty of the assigned value;
n = Number of values used to calculate the assigned value;
$\hat{\sigma}$ = The estimate of the standard deviation of the assigned value resulting from robust statistics.

According to ISO 13528 [8] the uncertainty of the assigned value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$u \leq 0.3\sigma_P$

where:
u = The uncertainty of the assigned value;
σ_P = Standard deviation for proficiency assessment (§4.3).

In case the uncertainty of the assigned value does not comply with this criterion, the uncertainty of the assigned value should be taken into account when evaluating the performance of the participants regarding the accuracy (§4.4). In case the uncertainty is > $0.7\sigma_P$ the calculated z-scores should not be used for evaluation of laboratories' performance and are presented for information only.

4.3 Calculation of the standard deviation for proficiency assessment (σ_P)

The use of the Horwitz equation is not appropriate for evaluation of results in this proficiency test [8]. Therefore a target standard deviation for proficiency assessment (σ_P) of 25% was taken as an acceptable standard deviation in this test.

$\sigma_P = 0.25c$

where:
σ_P = expected standard deviation in proficiency tests;
c = concentration of the analyte (mg/kg).

4.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating laboratories with regard to the accuracy a z_a-score is calculated. For the evaluation of the performance of the laboratories, ISO 13528 [8] is applied. According to these guidelines z_a-scores are classified as presented in Table 3.

Table 3
Classification of z_a-scores.

$\lvert z_a \rvert \leq 2$	Satisfactory
$2 < \lvert z_a \rvert < 3$	Questionable
$\lvert z_a \rvert \geq 3$	Unsatisfactory

If the calculated uncertainty of the assigned value complies with the criterion mentioned in §4.2, the uncertainty is negligible. In this case the accuracy z-score is calculated from:

$$z_a = \frac{\overline{x} - X}{\sigma_P} \qquad \text{Equation I}$$

where:
z_a = accuracy z-score;
$\overline{x}$ = the average result of the laboratory;
X = assigned value;
σ_P = standard deviation for proficiency assessment.

However, if the uncertainty of the assigned value does not comply with the criterion mentioned in §4.2, it could influence the evaluation of the laboratories. Although, according to ISO 13528 in this case no z-scores can be calculated if a consensus value is used as the assigned value, we feel that

evaluation of the participating laboratories is of main importance justifying the participating laboratories' effort. Therefore in this case, the uncertainty is taken into account by calculating the accuracy z-score [8]:

$$z'_a = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + u^2}}$$ Equation II

where:
z'_a = accuracy z-score taking into account the uncertainty of the assigned value;
$\bar{x}$ = the average result of the laboratory;
X = assigned value;
σ_P = standard deviation for proficiency assessment;
u = uncertainty of the assigned value.

If a consequential instability of the proficiency test materials is observed, this can influence the evaluation of the laboratory performance. Therefore, in that case the consequential instability is taken into account when calculating z-scores. Because instability only regards one side of the confidence interval (a decrease of the concentration) this correction only applies to the lower 2s limit and results in an asymmetrical confidence interval.

In the case of a consequential instability the accuracy z-score for the laboratories that reported an amount below the assigned value is corrected for this instability by:

$$z_{ai} = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + \Delta^2}}$$ Equation III

where:
z_{ai} = accuracy z-score taking into account the instability of the assigned value;
$\bar{x}$ = the average result of the laboratory;
X = assigned value;
σ_P = standard deviation for proficiency assessment;
Δ = difference between average concentration of compound stored at -70°C or at room temperature.

In some cases the uncertainty of the assigned value does not comply with the criterion in §4.2 and a consequential instability is observed. In this case the z'_a score for the laboratories that reported an amount below the assigned value is corrected for this instability by:

$$z'_{ai} = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + \Delta^2 + u^2}}$$ Equation IV

where:
z'_{ai} = accuracy z-score taking into account the uncertainty and instability of the assigned value;
$\bar{x}$ = the average result of the laboratory;
X = assigned value;
σ_P = standard deviation for proficiency assessment;
Δ = difference between average concentration of compound stored at -70°C or at room temperature;
u = uncertainty of the assigned value.

5 Results

Twenty-two laboratories registered for the participation and all laboratories reported results. The reported results and the performance of the individual laboratories are presented in Annex 8-10. The false negative results are presented in Annex 11.

5.1 Almond

For each material sixteen laboratories carried out analysis for AL. No false negative results were reported. Eight z-scores > 2 were reported by labs PT194 (once), PT211 (once), PT219 (twice), PT222 (twice), and PT226 (twice).

5.1.1 Material A

Fifteen laboratories carried out quantitative analyses. The lowest value reported is 1.6 mg/kg and the highest value is 23 mg/kg. The assigned value is 14 mg/kg with a robust standard deviation of 3 mg/kg. This is lower than the target standard deviation (RSD 25%): 3.6 mg/kg. The uncertainty of the assigned value is 1.1 mg/kg which does exceed $0.3\sigma_P$ (§4.2), so the uncertainty is taken into account in the evaluation. A consequential instability during storage was observed, so z'_{ai}-scores were calculated. With regard to the accuracy two results were questionable (PT219, PT226) and one was unsatisfactory (PT222).

5.1.2 Material B

Fifteen laboratories carried out quantitative analyses. The lowest value reported is 0.6 mg/kg and the highest value is 20.8 mg/kg. The assigned value is 6 mg/kg with a robust standard deviation of 3 mg/kg. This is more than 2 times higher than the target standard deviation (RSD 25%): 1.5 mg/kg. For this material the uncertainty of the assigned value (1.0 mg/kg) which does exceed $0.3\sigma_P$ (§4.2), so the uncertainty is taken into account in the evaluation. A consequential instability during storage was observed, so z'_{ai}-scores were calculated. With regard to the accuracy three results were questionable (PT194, PT211, PT222) and two were unsatisfactory (PT219 and PT226).

5.1.3 Material C

Material C was not spiked with AL. Two labs (PT194 and PT226) detected very low traces of AL.

5.2 Gluten

For each material eighteen laboratories carried out analysis for GL, one laboratory applied three different methods. Two false negative results were reported by lab PT218. Six z-scores > 2 were reported by labs PT215 (twice), PT220 (twice), PT226 (once) and PT227 (once).

5.2.1 Material A

Material A was not spiked with GL. Seven labs (PT212, PT213, T214, PT215, PT219, PT220, PT226) detected traces of GL.

5.2.2 Material B

Seventeen laboratories carried out quantitative analyses. The lowest value reported is 6.2 mg/kg and the highest value is 466 mg/kg. The assigned value is 13 mg/kg with a robust standard deviation of 7 mg/kg. This is almost 2 times higher than the target standard deviation (RSD 25%): 3.3 mg/kg. The uncertainty of the assigned value is 1.9 mg/kg which does exceed $0.3\sigma_P$ (§4.2) and hence, so the uncertainty is taken into account in the evaluation. A consequential instability during storage was observed, so z'_{ai}-scores were calculated. With regard to the accuracy three results were unsatisfactory (PT215, PT220 and PT227).

5.2.3 Material C

Seventeen laboratories carried out quantitative analyses. The lowest value reported is 18.9 mg/kg and the highest value is 1678 mg/kg. The assigned value is 33 mg/kg with a robust standard deviation of 13 mg/kg. This is higher than the target standard deviation (RSD 25%): 8.3 mg/kg. The uncertainty of the assigned value is 3.8 mg/kg which does exceed $0.3\sigma_P$ (§4.2) and hence, the uncertainty is taken into account in the evaluation. A consequential instability during storage was observed, so z'_{ai}-scores were calculated. With regard to the accuracy three results were unsatisfactory (PT215, PT220 and PT227).

5.3 Peanut

For each material seventeen laboratories carried out analysis for PN. Two false negative results were reported by labs PT216 and PT221. As the uncertainty of the assigned values was very high, i.e. more than $0.7*\sigma_P$ z-scores could not be used for evaluation of laboratories' performance.

5.3.1 Material A

Sixteen laboratories carried out quantitative analyses. Two false negative results were reported by labs PT216 and PT221. The lowest value reported is 1.96 mg/kg and the highest value is 12 mg/kg. The assigned value is 6 mg/kg with a robust standard deviation of 4 mg/kg. This is almost 3 times higher than the target standard deviation (RSD 25%): 1.5 mg/kg. For this material the uncertainty of the assigned value (1.3 mg/kg) is very high, i.e. more than $0.7*\sigma_P$ (1.1 mg/kg) and hence, z-scores could not be used for evaluation of laboratories' performance. If the uncertainty of the assigned value is too large in comparison with the standard deviation for proficiency assessment, there is a risk that laboratories will receive questionable or unsatisfactory z-scores due to inaccuracy of the determination of the assigned value and not as a result of a cause within the laboratory analysis.

5.3.2 Material B

Material B was not spiked with PN and no traces of PN were observed.

5.3.3 Material C

Sixteen laboratories carried out quantitative analyses. The lowest value reported is 2.84 mg/kg and the highest value is 38.5 mg/kg. The assigned value is 19 mg/kg with a robust standard deviation of 12 mg/kg. This is more than two times higher than the target standard deviation (RSD 25%): 4.9 mg/kg. For this material the uncertainty of the assigned value (3.7 mg/kg) is very high, i.e. more than $0.7*\sigma_P$ (1.1 mg/kg) and hence, z-scores could not be used for evaluation of laboratories' performance. If the uncertainty of the assigned value is too large in comparison with the standard deviation for proficiency assessment, there is a risk that laboratories will receive questionable or unsatisfactory z-scores due to inaccuracy of the determination of the assigned value and not as a result of a cause within the laboratory analysis.

5.4 Skim milk powder

For each material sixteen laboratories carried out analysis for SMP, one laboratory applied two different methods and one laboratory applied four different methods. Eleven false negative results were reported by lab PT206A (twice), PT206B (twice), PT211 (once), PT220 (once), PT223 (twice) PT224 (once), PT227 (once), PT228 (once). As the uncertainty of the assigned values was very high, z-scores could not be used for evaluation of laboratories' performance.

5.4.1 Material A

Sixteen laboratories carried out quantitative analyses. Two false negative results were reported by labs PT206A, and PT206B. The lowest value reported is 0.5 mg/kg and the highest value is 30.6 mg/kg. The assigned value is 9 mg/kg with a robust standard deviation of 8 mg/kg. This is 3 times higher than the target standard deviation (RSD 25%): 2.4 mg/kg. For this material the uncertainty of the assigned value (7.8 mg/kg) is very high, i.e. more than $0.7*\sigma_P$ (1.7 mg/kg) and hence, z-scores could not be used for evaluation of laboratories' performance. If the uncertainty of the assigned value is too large in comparison with the standard deviation for proficiency assessment, there is a risk that laboratories will receive questionable or unsatisfactory z-scores due to inaccuracy of the determination of the assigned value and not as a result of a cause within the laboratory analysis.

5.4.2 Material B

Material A was not spiked with SMP. One lab (PT213) detected traces of SMP.

5.4.3 Material C

Sixteen laboratories carried out quantitative analyses. Seven false negative results were reported by labs PT206A, PT206B, PT211, PT220, PT224, PT227, PT228. The lowest value reported is 0.6 mg/kg and the highest value is 6.6 mg/kg. The assigned value is 3 mg/kg with a robust standard deviation of 3 mg/kg. This is 3 times higher than the target standard deviation (RSD 25%): 0.8 mg/kg. For this material the uncertainty of the assigned value (1.2 mg/kg) is very high, i.e. more than $0.7*\sigma_P$ (0.6 mg/kg) and hence, z-scores could not be used for evaluation of laboratories' performance. If the uncertainty of the assigned value is too large in comparison with the standard deviation for proficiency assessment, there is a risk that laboratories will receive questionable or unsatisfactory z-scores due to inaccuracy of the determination of the assigned value and not as a result of a cause within the laboratory analysis.

5.5 Soy

For each material fifteen laboratories carried out analysis for SOY. Seven false negative results were reported by lab PT206, PT213, PT214, PT216, PT218, PT220, PT223. As the uncertainty of the assigned values was very high, z-scores could not be used for evaluation of laboratories' performance.

5.5.1 Material A

Material A was not spiked with SOY. Two lab (PT211 and PT228) detected traces of SOY.

5.5.2 Material B

Fifteen laboratories carried out quantitative analyses. Seven false negative results were reported by lab PT206, PT213, PT214, PT216, PT218, PT220, PT223. The lowest value reported is 2 mg/kg and the highest value is 41 mg/kg. The assigned value is 4 mg/kg with a robust standard deviation of 5 mg/kg. This is 4 times higher than the target standard deviation (RSD 25%): 1.0 mg/kg. For this material the uncertainty of the assigned value (1.8 mg/kg) is very high, i.e. more than $0.7*\sigma_P$

(0.7 mg/kg) and hence, z-scores could not be used for evaluation of laboratories' performance. If the uncertainty of the assigned value is too large in comparison with the standard deviation for proficiency assessment, there is a risk that laboratories will receive questionable or unsatisfactory z-scores due to inaccuracy of the determination of the assigned value and not as a result of a cause within the laboratory analysis.

5.5.3 Material C

Material C was not spiked with SOY. Two lab (PT211 and PT228) detected traces of SOY.

6 Conclusions

Twenty-two laboratories reported results for the proficiency test for allergens in baby cereal. Each participant was *a priori* asked to list the compounds included in their methods. This allowed us to evaluate the results with respect to the laboratories' scope. Only compounds that were not detected even though they were within the laboratory's scope, were considered a false negative result.

Within the participant's scope 5 labs showed optimal performance: PT210, PT212, PT217, PT225, and PT229. An overview of each participant's performance is shown in Annex 12. Quantitative results for PN, SOY and SMP were not taken into account, due to the high uncertainty in the assigned values. However, for all allergens false negative results were accounted for.

Overall, 75% of quantitative results for almond were satisfactory. For gluten 80% of all quantitative results were satisfactory. The uncertainty of the assigned value for analysis of peanut, skim milk powder and soy was too high and hence, there is a risk that laboratories will receive questionable or unsatisfactory results due to inaccuracy of the determination of the assigned value and not as a result of a cause within the laboratory analysis. Overall, correct classification of spiked samples, i.e. detection of the presence of allergens, was obtained in 100% of almond analyses, 95% of gluten analyses, 94% of peanut analyses, 78% of skim milk powder analyses, and 53% of soy analyses. Satisfactory results for each allergen in each material are shown in Table 4.

This proficiency test shows that detection of the presence (qualitative interpretation of reported results) of almond, gluten and peanut was successful, even at low concentrations. On the other hand, detection of the presence of skim milk powder and soy, was not satisfactory. A large variation in the quantitative results was observed for all allergens included. Hence, for all allergens detection methods need further development and harmonisation for accurate estimation of the mass concentration.

Table 4

Overview of the results of the proficiency test.

		Target amount (mg/kg)	Assigned value (mg/kg)	Satisfactory results (%)	Correct classification spike (%)
Material A	Almond	20	14	80	100
	Gluten	-	-	-	-
	Peanut*	5	6		88
	Skim milk powder*	20	9		90
	Soy flour	-	-		-
Material B	Almond	10	6	67	100
	Gluten	10	13	84	95
	Peanut	-	-		-
	Skim milk powder	-	-		-
	Soy flour*	25	4		53
Material C	Almond	-	-		-
	Gluten	30	33	84	95
	Peanut*	15	19		100
	Skim milk powder*	5	3		65
	Soy flour	-	-		-

* The uncertainty of assigned value is too high to allow proper evaluation of laboratory performance.

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8 ISO 13528:2005(E). 2005. Statistical methods for use in proficiency testing by inter-laboratory comparison, 1st edition.

9 Owen, L, Gilbert, J. 2009. Proficiency testing for quality assurance of allergens methods. Anal. Bioanal. Chem. (395):147-153.

10 Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 1. Basic concepts. Analyst 114:1693-1697.

11 Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 2. Inter-laboratory trials. Analyst. 114:1699-1702.

Annex 1 Codification of the samples

Lab code	Material A*	Material B*	Material C*
PT194	735	184	623
PT206	325	718	879
PT210	371	918	912
PT211	408	114	446
PT212	613	818	633
PT213	673	825	199
PT214	420	938	696
PT215	742	487	874
PT216	367	100	527
PT217	794	392	413
PT218	559	300	585
PT219	900	550	506
PT220	296	161	627
PT221	270	791	442
PT222	779	749	766
PT223	215	829	988
PT224	676	522	883
PT225	747	375	619
PT226	262	122	572
PT227	401	360	974
PT228	976	639	508
PT229	953	298	402

* All sample codes start with Food Allergens/2014/.

Annex 2 Statistical evaluation of homogeneity data of material A for PN

	PN (mg/kg)	
Sample number	Replicate 1	Replicate 2
Hom/A001	10	10
Hom/A002	9	9
Hom/A003	9	10
Hom/A004	10	9
Hom/A005	10	10
Hom/A006	9	9
Hom/A007	9	8
Hom/A008	9	9
Hom/A009	9	9
Hom/A010	8	9
Grand mean	9	
Cochran's test		
C	0.250	
C_{crit}	0.602	
$C<C_{crit}$?	NO OUTLIERS	
Target s = 0.25 grand mean	2.3	
s_x	0.54	
s_w	0.45	
s_s	0.43	
Critial = 0.3 target s	4.64	
s_s<critical?	ACCEPTED	

s_x = standard deviation of the sample averages.

s_w = within-sample standard deviation.

s_s = between-sample standard deviation.

Annex 3 Statistical evaluation of homogeneity data of material B for AL

	AL (mg/kg)	
Sample number	Replicate 1	Replicate 2
Hom/B001	5	5
Hom/B002	4	5
Hom/B003	4	6
Hom/B004	4	5
Hom/B005	4	6
Hom/B006	4	5
Hom/B007	5	4
Hom/B008	5	4
Hom/B009	5	4
Hom/B010	5	5
Grand mean	5	
Cochran's test		
C	0.286	
C_{crit}	0.602	
$C<C_{crit}$?	NO OUTLIERS	
Target s = 0.25 grand mean	1.18	
s_x	0.26	
s_w	0.84	
s_s	0.00	
Critial = 0.3 target s	0.35	
s_s<critical?	ACCEPTED	

s_x = standard deviation of the sample averages.

s_w = within-sample standard deviation.

s_s = between-sample standard deviation.

Annex 4 Statistical evaluation of homogeneity data of material C for PN

	PN (mg/kg)	
Sample number	Replicate 1	Replicate 2
Hom/C001	33	33
Hom/C002	35	35
Hom/C003	35	30
Hom/C004	34	33
Hom/C005	31	31
Hom/C006	31	32
Hom/C007	31	30
Hom/C008	28	29
Hom/C009	29	31
Hom/C010	32	34
Grand mean	32	
Cochran's test		
C	0.676	
C_{crit}	0.602	
$C<C_{crit}$?	NO OUTLIERS	
Target s = 0.25 grand mean	7.9	
s_x	1.92	
s_w	1.36	
s_s	1.66	
Critial = 0.3 target s	2.39	
s_s<critical?	ACCEPTED	

s_X = standard deviation of the sample averages.

s_W = within-sample standard deviation.

s_S = between-sample standard deviation.

Annex 5 Instruction letter

RIKILT
WAGENINGEN UR

For quality of life

P.O. Box 230 | 6700 AE WAGENINGEN | The Netherlands

Veterinary Drugs

DATE
September 12, 2014

SUBJECT
inter-laboratory study allergens in food

OUR REFERENCE
14/RIK0587

POSTAL ADDRESS
P.O. Box 230
6700 AE WAGENINGEN
The Netherlands

VISITORS' ADDRESS
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Building 123
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CoC NUMBER
09098104

HANDLED BY
dr. ir. M.G.E.G. Bremer

TELEPHONE
+31 (0)317 482263

EMAIL
monique.bremer@wur.nl

ANNEX: 1

Dear colleague,

RIKILT Wageningen UR is planning to organize an inter-laboratory study regarding allergens in food. This study will mainly focus on the quantification of the detected compounds. However, qualitative methods also will be evaluated. The primary goal of this inter-laboratory study is to give laboratories the opportunity to evaluate or demonstrate their performance regarding the analysis of allergens in food.
Laboratories are encouraged to use their own methods and procedures to reflect the handling of real samples as closely as possible. RIKILT Wageningen UR organizes proficiency studies according to ISO 17043. If the number of participants is insufficient, the inter-laboratory study cannot proceed.
I would like to invite you to participate in this study.

Please forward this invitation to other laboratories in your country or other contacts that could be interested in participating. The following issues are important for participation in the proficiency test:

1. Samples

- Three baby cereal samples (approximately 25 grams) will be supplied for the quantitative or qualitative analysis of allergens.
- The samples may contain one or more allergenic compounds. Please note not all allergens that must be labeled according to EU legislation will be present.
- Samples will be sent end of October 2014. The distribution of the samples will be announced by e-mail.
- The participant should arrange the necessary import permits for the sample materials.

2. Analysis

- Please indicate in Annex 1 which compounds are included in your analytical method(s).
- The results have to be reported within 3 weeks after shipment of the samples

3. Report

- A report of the inter-laboratory study will be dispatched in February 2015.
- Results of the inter-laboratory study will be presented anonymously.

Wageningen UR (Wageningen University and various research institutes) is specialised in the domain of healthy food and living environment.

RIKILT, part of Wageningen UR, carries out research into the safety, quality and health of food and feed and provides consultancy services to (inter)national governmental authorities. RIKILT is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014 and R013).

September 12, 2014

14/RIK0587

2 of 2

4. Additional information

- RIKILT Wageningen UR is allowed to use the anonymous results of the inter-laboratory study in presentations, seminars and publications.
- RIKILT Wageningen UR will never inform third parties (e.g. accreditation bodies) on specific laboratory results without informing the laboratory first.

5. Costs

- For the participation, we request a fee of € 500,- (ex. VAT) as a compensation for the preparation and transportation of the samples.
- If an extra batch of samples is needed after the first shipping, the courier costs will be charged.

If you would like to participate, please fill out the enclosed participation form (Annex 1) (preferably digitally) and send it to me before the 10th of October 2014 by fax (+31 317 417 717) or e-mail (monique.bremer@wur.nl). If you choose the latter, please print the document, sign it and e-mail a scan of the signed document.

Hoping to welcome you for this study,

Dr. ir. M.G.E.G. Bremer
RIKILT Wageningen UR

Annex 6 Statistical evaluation of stability data

Statistical evaluation for PN in material A		
Storage temp	-70 °C	RT
Time at RT (days)		20
Calculated amounts (mg/kg)	8	9
	9	9
	10	9
	9	8
	9	9
	9	8
Average amount (mg/kg)	9	9
n	6	6
Standard deviation (mg/kg)	0.632	0.516
Difference		0.33
$0.3\sigma_p$		0.67
Consequential difference? Diff > 0.3 σ_p		**NO**
T		1.00
t_{crit}		2.23
Statistical difference? t > t_{crit}		**NO**

Statistical evaluation for AL in material B		
Storage temp	-70 °C	RT
Time at RT (days)		23
Calculated amounts (mg/kg)	5	5
	5	4
	6	5
	5	6
	5	4
	5	4
Average amount (mg/kg)	5	5
n	6	6
Standard deviation (mg/kg)	0.4	0.8
Difference		0.5
$0.3\sigma_p$		0.3
Consequential difference? Diff > 0.3 σ_p		**YES**
t		1.34
t_{crit}		2.23
Statistical difference? t > t_{crit}		**NO**

Statistical evaluation for SOY in material B		
Storage temp	-70 °C	RT
Time at RT (days)		27
Calculated amounts (mg/kg)	29	23
	23	27
	25	26
	26	22
	22	22
	25	22
Average amount (mg/kg)	25	24
n	6	6
Standard deviation (mg/kg)	2.4	2.3
Difference		1.3
$0.3\sigma_p$		1.9
Consequential difference? Diff > 0.3 σ_H		**NO**
t		1.0
t_{crit}		2.2
Statistical difference? t > t_{crit}		**NO**

Statistical evaluation for GL in material C		
Storage temp	-70 °C	RT
Time at RT (days)		31
Calculated amounts (mg/kg)	35	26
	31	23
	26	22
	27	27
	29	25
	30	26
Average amount (mg/kg)	30	25
n	6	6
Standard deviation (mg/kg)	3.2	1.9
Difference		4.8
$0.3\sigma_p$		2.2
Consequential difference? Diff > 0.3 σ_p		**YES**
t		3.16
t_{crit}		2.23
Statistical difference? t > t_{crit}		**YES**

Statistical evaluation for SMP in material A		
Storage temp	-70 °C	RT
Time at RT (days)		34
Calculated amounts (mg/kg)	10	7
	9	8
	10	10
	14	9
	7	7
	7	11
Average amount (mg/kg)	10	9
n	6	6
Standard deviation (mg/kg)	3.2	1.9
Difference		0.8
$0.3\sigma_p$		0.71
Consequential difference? Diff > 0.3 σ_p		**YES**
t		0.67
t_{crit}		2.23
Statistical difference? t > t_{crit}		**NO**

Annex 7 Overview of the applied methods for allergens

Lab code	Almond	Gluten	Skim milk powder	Peanut	Soy flour
PT194	BioFront Almond ELISA Kit (BioFront Technologies)	-	-	BioFront MonoTrace™ Peanut ELISA Kit (BioFront Technologies)	-
PT206	RIDASCREEN (R-Biopharm)	R7001 RIDASCREEN (R-Biopharm)	**A:** Casein: R4612 – RIDASCREEN (R-Biopharm) **B:** β-lactoglobulin: R4901 RIDASCREEN (R-Biopharm)	R6402 – Fast ei (R-Biopharm)	Soy protein (ELISA Systems)
PT210	-	RIDASCREEN Gliadin (Bioscience Diagnostics Pte Ltd)	-	Biokit Peanut Assay Kit (Chokim Scientific (S) Pte Ltd)	-
PT211	RIDASCREEN®FAST Mandel / Almond (R-Biopharm)	RIDASCREEN® Gliadin (R-Biopharm)	RIDASCREEN®FAST Milk (R-Biopharm)	RIDASCREEN®FAST Peanut (R-Biopharm)	RIDASCREEN®FAST Soya (R-Biopharm)
PT212	-	In house method analysis : Determination Gluten in Food / RIDASCREEN. Enzyme Immunoassay for the Quantitative Analysis of Gliadins and Corresponding Prolamin (R- Biopharm)	-	-	-
PT213	Veratox Quantitative Almond Allergen test #8440 (Neogen)	RIDASCREEN Gliadin R7001 (R-Biopharm)	Casein Residue ESCASPRD-48 (ELISA Systems)	Veratox Peanut Allergen Quantitative Test Kit #8430 (Neogen)	Veratox Quantitative Soy Allergen test #8410 (Neogen)
PT214	Veratox for Almond Allergen (8440) (Neogen)	RIDASCREEN Gliadin (R7001) (R-Biopharm)	Casein Residue (ESCASPRD-48) (ELISA Systems)	Veratox for Peanut Allergen (8430) (Neogen)	Veratox for Soy (8410) (Neogen)
PT215	Immunolab Almond ELISA (Immunonlab GmbH)	Immunolab Gliadin ELISA (Immunonlab GmbH)	Immunolab Casein ELISA (Immunonlab GmbH)	Immunolab Peanut ELISA (Immunonlab GmbH)	Immunolab Soy ELISA (Immunonlab GmbH)
PT216	ELISA SYSTEMS Almond Residue assay (ELISA Systems)	ELISA SYSTEMS Gliadin assay (ELISA Systems)	ELISA SYSTEMS Casein Residue assay (ELISA Systems)	ELISA SYSTEMS Peanut Residue assay (ELISA Systems)	ELISA SYSTEMS Enhanced Soy residue assay (ElLISA Systems)
PT217	Veratox (Neogen)	RIDASCREEN Fast Gliadin (R-Biopharm)	Veratox (Neogen)	Veratox (Neogen)	Veratox (Neogen)
PT218	-	?	?	?	?
PT219	RIDASCREEN FAST Almond (R6901) (R-Biopharm)	RIDASCREEN Gliadin (R7001) (R-Biopharm)	AgraQuant Casein (COKAL1200) (Romer Labs)	Biokits Peanut Assay Kit (902048Q) (Neogen)	Veratox for Soy Allergen (8410) (Neogen)

Lab code	Almond	Gluten			Skim milk powder				Peanut	Soy flour
PT220	Almond Protein Residue Assay (ELISA Systems)	Aller-Tek™ Gluten ELISA (ELISA Technologies)			Casein Residue Assay (ELISA Systems)				RIDASCREEN Fast Peanut Residue Assay (R-Biopharm)	Enhanced Soy Residue Protein Assay (ELISA Systems)
PT221	ESARD-48 (ELISA Systems)	ESGLISS-48 (ELISA Systems)			ESCASPRD-48 (ELISA Systems)				ESPRDT-48 (ELISA Systems)	ESSOYPRD-48 (ELISA Systems)
PT222	CER Almond Kit (in-house developed test)	-			CER Casein Kit (in-house developed test)				-	-
PT223	Veratox for Almond Allergen (8440) (Neogen)	Veratox for Gliadin R5 (8510) (Neogen)			-				Veratox for Peanut Allergen (8430) (Neogen)	Veratox for Soy Allergen (8410) (Neogen)
PT224	-	RIDASCREEN Gliadin kit (R-Biopharm)			RIDASCREEN FAST Casein kit (R-Biopharm)				RIDASCREEN FAST Peanut kit (R-Biopharm)	-
PT225	Alertox ELISA Allergen-Almond (Biomedal Diagnostics)	**A**:INGEZIM Gluten (Biomedal Diagnostics)	**B**:GlutenTox ELISA Sandwich G12 (Biomedal Diagnostics)	**C**:GlutenTox Stick Plus (Biomedal Diagnostics)	**A**:Alertox ELISA Allergen-Casein (Biomedal Diagnostics)	**B**:Alertox ELISA Allergen- β-lactoglobulin (Biomedal Diagnostics)	**C**:Alertox Stick Casein (Biomedal Diagnostics)	**D**:Alertox Stick β - lactoglobulin (Biomedal Diagnostics)	?	Alertox ELISA Allergen-Soy (STI) (Biomedal Diagnostics)
PT226	MonoTrace Almond ELISA (BioFront Technologies)	Gluten Aller-Tek ELISA (ELISA RIDASCREENTechnologies)			-				MonoTrace Peanut ELISA (BioFront Technologies)	-
PT227	-	Gluten-Check (Bio-Check (UK))			BLG Residue Detection (Elisa Systems)				-	Soy Residue Detection (Elisa Systems)
PT228	-	-			RIDASCREEN FAST β-lactoglobulin (R-Biopharm)				-	RIDASCREEN FAST Soya (R-Biopharm)
PT229	ELISA – Almond (BioFront Technologies)	-			-				ELISA – Peanut (BioFront Technologies)	-

Annex 8 Results for material A

Lab code	AL (mg/kg)	z'_{ai}-score	GL (mg/kg)	PN* (mg/kg)	SMP* (mg/kg)	SOY (mg/kg)
PT194	15.01	0.21		1.96		
PT206A	10.6	-0.94	nd <10	8.6	**nd <2.5**	nd <2.5
PT206B					**nd <5**	
PT210			nd	2.58		
PT211	21.3	1.90	nd	8.5	10.8	12.6
PT212			5.64			
PT213	14.7	0.13	5.3	5.6	5.5	nd
PT214	16	0.48	6	5.5	20	nd
PT215	15	0.21	82	12	22	nd
PT216	6.8	-1.93	0	**0**	2.9	0
PT217	13	-0.32	nd <10	3	4	<1
PT218	nt		nd	11.6	7.9	nd
PT219	23	**2.36**	traces	4.2	16	nd
PT220	10.7	-0.91	10.4	10.4	11.4	nd
PT221	12.8	-0.37	nd< 2	**nd < 3.3**	2.6	nd< 2.9
PT222	1.6	**-3.27**			2.5	
PT223	13	-0.32	nd	6.1	nt	nd
PT224			nd	4.68	5.29	
PT225A	15	0.21	nd <5	10.1	14.4	nd < 1
PT225B			nd <0.6		6.1	
PT225C			nd		detected	
PT225D					detected	
PT226	22.3	2.17	10.1	2.8	nt	nt
PT227	nt		nd	nt	0.5	nd
PT228	nt		nt	nt	30.6	9.1
PT229	detected			detected		

** As the uncertainty of the assigned value is very high, z -scores could not be calculated*

**nd: not detected

*** nt: not tested

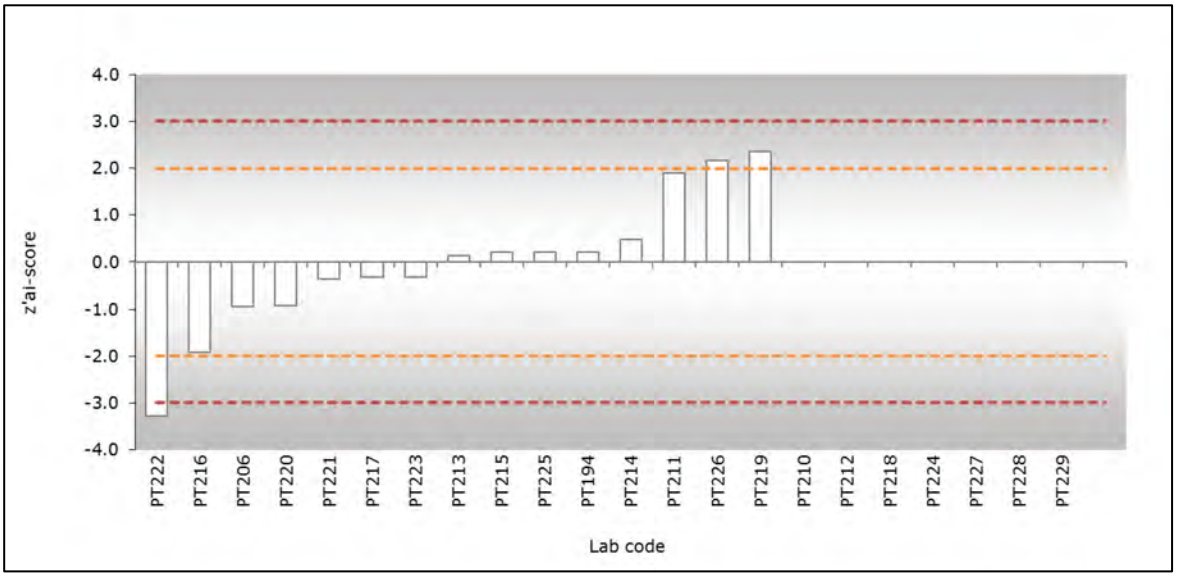

Figure a *Graphical representation of z'_{ai}-scores for AL in material A.*
The $X \pm 2\sigma_p$ and $X \pm 3\sigma_p$ lines (yellow and red) are calculated according to equation II in §4.4.

Annex 9 Results for material B

Lab code	AL (mg/kg)	Z'_{ai}-score	GL (mg/kg)	Z'_{ai}-score	PN (mg/kg)	SMP (mg/kg)	SOY* (mg/kg)
PT194	2.23	**-2.04**			nd		
PT206A	3.8	-1.22	18	1.27	nd <2.5	nd <2.5	**nd <2.5**
PT206B						nd <5	
PT210			12.14	-0.23	nd		
PT211	10.7	**2.47**	14.1	0.24	nd	nd	25.5
PT212			14.94	0.46			
PT213	6.3	0.09	17	1.00	nd	1.7	**nd**
PT214	7.6	0.79	10	-0.70	nd	nd	**nd**
PT215	8	1.01	466	**118.85**	nd	nd	41
PT216	4.1	-1.06	7	-1.36	nd	nd	**nd**
PT217	5	-0.59	16	0.74	nd <1	nd <1	2
PT218	nt		**nd**		nd	nd	**nd**
PT219	13	**3.71**	11	-0.48	nd	nd	2.5
PT220	5.9	-0.12	73.9	**15.94**	nd	nd	**nd**
PT221	5.3	-0.43	7	-1.36	nd < 3.3	nd < 0.5	pos < 5.8
PT222	0.6	**-2.89**				nd	
PT223	7.8	0.90	7	-1.36	nd	nt	**nd**
PT224			7.48	-1.25	nd	nd	
PT225A	7.4	0.68	9	-0.92	< 1.0	< 0.9	12.5
PT225B			12.5	0.15		<0.9	
PT225C			detected			nd	
PT225D						nd	
PT226	20.8	**7.93**	55.3	-0.15	nd	nt	nt
PT227	nt		6.2	**11.06**	nt	nd	3
PT228	nt		nt	-1.53	nt	nd	38.5
PT229	detected				nd		

** As the uncertainty of the assigned value is very high, z -scores could not be calculated.*

** nd: not detected

*** nt: not tested

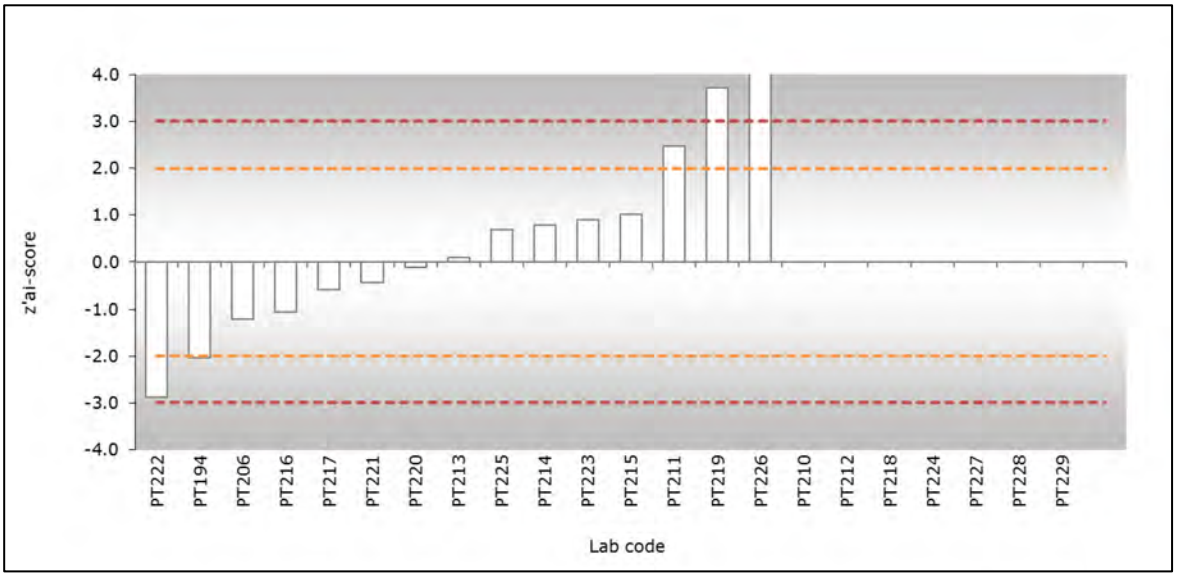

Figure a *Graphical representation of z'_{ai}-scores for AL in material B. The $X \pm 2\sigma_p$ and $X \pm 3\sigma_p$ lines (yellow and red) are calculated according to equation II in §4.4.*

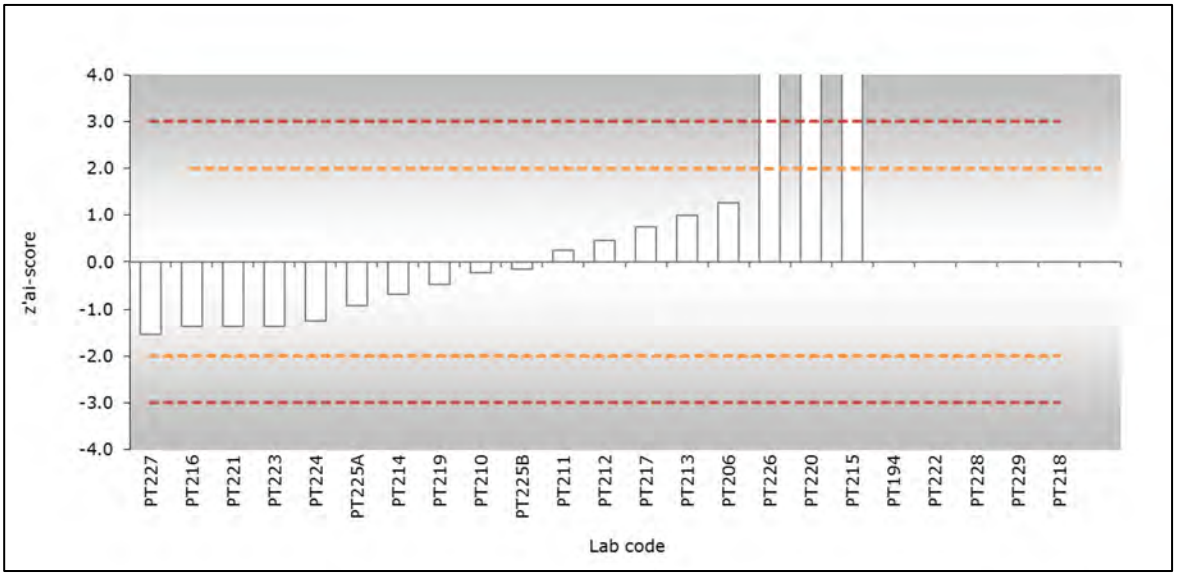

Figure b *Graphical representation of z'_{ai}-scores for GLU in material B. The $X \pm 2\sigma_p$ and $X \pm 3\sigma_p$ lines (yellow and red) are calculated according to equation II in §4.4.*

Annex 10 Results for material C

Lab code	AL (mg/kg)	GL (mg/kg)	Z'_{ai}-score	PN* (mg/kg)	SMP* (mg/kg)	SOY (mg/kg)
PT194	1.33			2.84		
PT206A	nd <2.4	33	-0.02	29.6	**nd <2.5**	nd <2.5
PT206B					**nd <5**	
PT210		34.14	0.11	12.5		
PT211	nd	27.3	-0.57	25.4	**nd**	12.5
PT212		35.72	0.28			
PT213	nd	48.1	1.64	18.1	4.2	nd
PT214	nd	31	-0.21	23	6.6	nd
PT215	nd	1678	**180.60**	32	4.7	nd
PT216	0	22.3	-1.05	9.4	0.8	0
PT217	nd <1	30	-0.31	14	1	nd <1
PT218	nt	**nd**		38.5	1	nd
PT219	nd	32	-0.11	12	4.5	nd
PT220	nd	238	**22.49**	28.3	**nd**	nd
PT221	nd < 1.2	22	-1.08	pos < 6.7	pos< 1.0	nd < 2.9
PT222	nd				0.6	
PT223	nd	21	-1.18	20	nt	nd
PT224		40.65	0.82	13.17	**< LOD**	
PT225A	nd < 1	18.9	-1.38	29.8	6.5	nd < 1
PT225B		21.9	-1.09		1.3	
PT225C		detected			detected	
PT225D					detected	
PT226	1	101.5	**7.50**	3.8	nt	nt
PT227	nt	25	-0.79	nt	**nd**	nd
PT228	nt	nt		nt	**nd**	14.1
PT229	detected			detected		

** As the uncertainty of the assigned value is very high, z -scores could not be calculated.*

** nd: not detected

*** nt: not tested

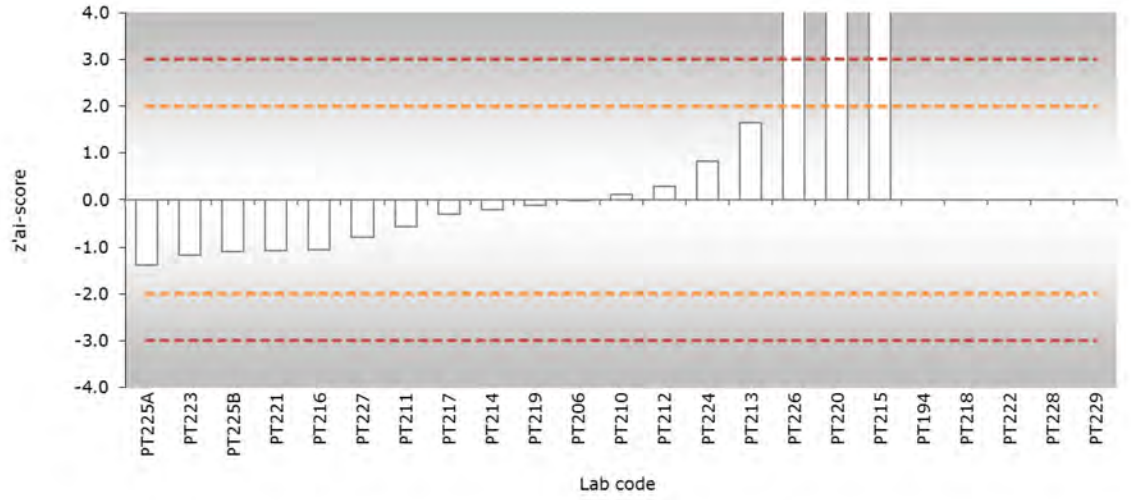

Figure a *Graphical representation of z_{ai}-scores for GL in material C.*
The $X \pm 2\sigma_p$ and $X \pm 3\sigma_p$ lines (yellow and red) are calculated according to equation I in §4.4.

Annex 11 False negative results

Material A					Material B					Material C				
AL	GL	PN	SMP	SOY	AL	GL	PN	SMP	SOY	AL	GL	PN	SMP	SOY
		PT216	PT206A			PT218			PT206		PT218		PT206A	
		PT221	PT206B						PT213				PT206B	
									PT214				PT211	
									PT216				PT220	
									PT218				PT224	
									PT220				PT227	
									PT223				PT228	

Annex 12 Overall score participants

Lab code	Scores*	Remarks
PT194	1 satisfactory out of 2 z-scores	
PT206	4 satisfactory out of 4 z-scores, 5 FN	
PT210	2 satisfactory out of 2 z-scores	Optimal performance within scope
PT211	3 satisfactory out of 4 z-scores, 1 FN	
PT212	2 satisfactory out of 2 z-scores	Optimal performance within scope
PT213	4 satisfactory out of 4 z-scores, 1 FN	
PT214	4 satisfactory out of 4 z-scores, 1 FN	
PT215	2 satisfactory out of 4 z-scores	
PT216	4 satisfactory out of 4 z-scores, 2 FN	
PT217	4 satisfactory out of 4 z-scores	Optimal performance within scope
PT218	2 satisfactory out of 4 z-scores, 3 FN	
PT219	2 satisfactory out of 4 z-scores	
PT220	2 satisfactory out of 4 z-scores, 2 FN	
PT221	4 satisfactory out of 4 z-scores, 1 FN	
PT222	0 satisfactory out of 2 z-scores	
PT223	4 satisfactory out of 4 z-scores, 1 FN	
PT224	2 satisfactory out of 2 z-scores, 1 FN	
PT225	6 satisfactory out of 6 z-scores	Optimal performance within scope
PT226	1 satisfactory out of 4 z-scores, 1 FN	
PT227	1 satisfactory out of 2 z-scores, 1 FN	
PT228	1 FN	Qualitative results
PT229	2 satisfactory out of 2 z-scores	Optimal performance within scope

* The quantitative results for PN, SMP and SOY were not taken into account, due to the high uncertainty. However, false negative results were accounted for.

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RIKILT report 2015.002

RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and reliability of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

The mission of Wageningen UR (University & Research centre) is 'To explore the potential of nature to improve the quality of life'. Within Wageningen UR, nine specialised research institutes of the DLO Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment. With approximately 30 locations, 6,000 members of staff and 9,000 students, Wageningen UR is one of the leading organisations in its domain worldwide. The integral approach to problems and the cooperation between the various disciplines are at the heart of the unique Wageningen Approach.

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