*Proficiency Tests*

**DLA**

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**Evaluation Report**
proficiency test

**DLA 13/2016**

# Allergen-Screening III:

## Cereals containing Gluten, Peanut, Lupine, Celery and Sesame

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**1st Correction 26/06/2017:**
The correction is related to the gluten determination by ELISA. One submitted result was not considered by mistake before. The result 18b was amended in the result part on page 11 and in the documetation on page 23.

**Allgemeine Informationen zur Eignungsprüfung (EP)**
**General Information on the proficiency test (PT)**

| | |
|---|---|
| *EP-Anbieter*<br>*PT-Provider* | **DLA - Dienstleistung Lebensmittel Analytik GbR**<br>Gesellschafter: Dr. Gerhard Wichmann und Dr. Matthias Besler<br><br>Waldemar-Bonsels-Weg 170,<br>22926 Ahrensburg, Germany<br><br>Tel. ++49(0)171-1954375<br>Fax. ++49(0)4102-9944976<br>eMail. proficiency-testing@dla-lvu.de |
| *EP-Nummer*<br>*PT-Number* | DLA 13/2016 |
| *EP-Koordinator*<br>*PT-Coordinator* | Dr. Matthias Besler |
| *Status des EP-Bericht*<br>*Status of PT-Report* | Abschlussbericht / Final report (13 March 2017)<br>1. Korrektur / 1st Correction (26 June 2017)<br>Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen.<br>Only the latest version/correction of the report is valid. It replaces all preceding versions. |
| *EP-Bericht Freigabe*<br>*PT-Report Authorization* | Dr. Matthias Besler (Technischer Leiter / Technical Manager)<br>- *gezeichnet / signed M. Besler*<br>Dr. Gerhard Wichmann (QM-Beauftragter / Quality Manager)<br>- *gezeichnet / signed G. Wichmann*<br>Datum / Date: 26 June 2017 |
| *Unteraufträge*<br>*Subcontractors* | Die Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern wird von DLA im Unterauftrag vergeben.<br>The analysis of the content, homogeneity and stability of PT-parameters are subcontracted by DLA. |

# Inhalt/Content

# 1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the verification and/or validation of the particular testing method [1, 5].
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 / ISO 13528:2015 [2, 3].

# 2. Realisation

## 2.1 Test material

Four PT-samples were provided for the qualitative detection of allergens in mg/kg range. To prepare the samples premixes were used at levels of about 5-10% of the allergenic ingredients concerned.
The respective raw materials were common in commerce cereal flakes, flours, nut butter, dried plant parts and seeds as well as fresh celery root, from which DLA produced allergen premixes (s. Tab. 2). If required the raw materials were crushed, dried, ground with the addition of carrier substances and sieved (mesh 400 µm) or sieved by means of a centrifugal mill (mesh 500 µm).
The composition of the basic matrix of PT samples 1-4 and of the allergen-premixes is given in table 1. The premixes were used for spiking of the PT-samples 1 to 4 (see Tab. 2).
After homogenisation the samples were portioned to approximately 20 g into metallised PET film bags.

Table 1: Composition of DLA-Samples

| **Ingredients** | **Samples 1 - 4** |
|---|---|
| Potato powder<br>(Ingredients: Potatoes, E471, E304, E223, E100) | 74 - 76 % |
| Maltodextrin | 24 - 26 % |
| Allergen-Premixes<br><br>Ingredients:<br>- Maltodextrin (88% - 93%)<br>- Sodium sulfate (0,0% - 5,5%)<br>- Silicon dioxide (2,0% - 4,1%)<br>- Allergens (5,0% - 10% each) | 0,10 - 0,50 % |

Table 2: Added amounts of allergenic ingredients positive in mg/kg ranges** given as food item (for cereals as total protein)

| Ingredients * | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| *Oat:* Oat flakes, ground (Protein 12%) | positive (25 - 75) | negative | negative | negative |
| *Rye:* Rye flour Type 1150 (Protein 9,1%) | negative | positive (25 - 75) | negative | negative |
| *Wheat:* Wheat flour Type 550 (Protein 10,5%) | negative | negative | negative | positive (25 - 75) |
| *Peanut:* commercial peanut butter (Protein 30%) | negative | negative | positive (50 - 150) | positive (25 - 75) |
| *Lupine:* Sweet lupine flour, (Protein 37%) | negative | negative | positive (50 - 150) | negative |
| *Celery:* Leafs, dried (Protein 14%) | negative | positive (50 - 150) | negative | negative |
| *Celery:* Roots, dried (Protein 8,2%) | positive (75 - 225) | negative | negative | negative |
| *Celery:* Seeds, dried (Protein 20%) | negative | negative | negative | positive (50 - 150) |
| *Sesame:* Seeds black, dried (Protein 22%) | negative | positive (50 - 150) | negative | negative |
| *Sesame:* Seeds white, dried (Protein 23%) | positive (50 - 150) | negative | positive (50 - 150) | positive (25 - 75) |

* Protein contents according to laboratory analysis of raw material (total nitrogen according to Kjeldahl)
**Allergen contents of „food item" in brackets as indicated in the column of ingredients according gravimetric mixing
***Note:*** *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

The detectability or absence of the allergens was tested by DLA using lateral flow assays. The results are in agreement with the spiking of the PT samples 1-4 (see Table 3).

Table 3: Verification of detectability of the added allergens by lateral flow assays (AgraStrip® LFD, Romer Labs®)

| Lateral Flow Device (LFD)* | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| **AgraStrip® Gluten** | negative | positive | negative | positive |
| **AgraStrip® Peanut** | negative | negative | positive | positive |
| **AgraStrip® Lupin** | negative | negative | positive | negative |
| **AgraStrip® Sesame** | positive | positive | positive | positive |

* Nachweisgrenze jeweils 5 mg/kg / Limit of detection (LOD) 5 mg/kg each

*2.1.1 Homogeneity*

The **mixture homogeneity before bottling** was examined 8-fold by **microtracer analysis**. It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1-4 showed probabilities of 66%, 96%, 97% and 81%, respectively. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. This gave a HorRat values of 0,8, 0,5, 0,5 and 0,7, respectively. The results of microtracer analysis are given in the documentation.

## 2.2 Sample shipment and information to the test

The portions of the test materials (sample 1 to 4) were sent to every participating laboratory in the 43rd week of 2016. The testing method was optional. The tests should be finished at December 9th 2016 the latest.

With the cover letter along with the sample shipment the following information was given to participants:

*There are 4 different samples possibly containing the allergenic ingredients: Cereals containing Gluten, Peanut, Lupine, Celery and Sesame. The allergens are contained in a simple carrier matrix (75% potato powder / 25% maltodextrin) in the range of 50 – 250 mg/kg (Cereals could be higher and Gluten could be lower). The evaluation of results is strictly qualitative (positive / negative).*
*The following analysis methods can be used:*

*a) ELISA and Lateral Flow*
*b) PCR*

*In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.*

## 2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. The results given as positive/negative were evaluated.
Queried and documented were the indicated results and details of the test methods like specifity, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.
From 23 participants 22 submitted their results in time. One participant submitted the result delayed. Another laboratory canceled the participation in advance of sample shipment.

# 3. Evaluation

Different ELISA- and PCR-methods for the determination of allergens in foods are eventually using different antibodies and target-DNA, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different valuation of the presence and/or content of the analyte [23, 24, 25, 26]. Furthermore matrix- and/or processing of samples can have strong impact on the detectability of allergens by ELISA and PCR methods.

Therefore in the present PT the allergenic ingredients were provided for analysis in a simple matrix without further processing.

## 3.1 Agreement with consensus values from participants

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **consensus values from participants**. A consensus value is determined unless ≥ 75% positive or negative results are present for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples for which a consensus value was obtained is indicated. Behind that the agreement is expressed as the percentage in parentheses.

## 3.2 Agreement with spiking of samples

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the four PT-samples**. A consensus value is determined unless ≥ 75% positive or negative results are present for a parameter.
The assessment will be in the form that the number of matching results followed by the number of samples is indicated. Behind that the agreement is expressed as the percentage in parentheses.

## 4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

The qualitative evaluation is carried out for each parameter for ELISA and PCR methods separately. Results of lateral flow methods were valuated together with ELISA methods, because they are usually based on antibody detection.

The participant results and evaluation are tabulated as follows:

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | | | | |
| Number negative | | | | |
| Percent positive | | | | |
| Percent negative | | | | |
| Consensus value | | | | |
| Spiking | | | | |

# 4.1 Proficiency Test Gluten Containing Cereals

## *4.1.1 ELISA-Results: Gluten*

### Qualitative valuation of results

| Evaluation number | Sample 1 (Oat) | Sample 2 (Rye) | Sample 3 (none) | Sample 4 (Wheat) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 12a | positive | positive | positive | positive | 2/4 (50%) | 3/4 (75%) | AQ | |
| 12b | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | AS | |
| 6a | negative | negative | negative | positive | 3/4 (75%) | 2/4 (50%) | GX | |
| 6b | negative | negative | negative | positive | 3/4 (75%) | 2/4 (50%) | IG | |
| 2 | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | RS | |
| 4 | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | RS | |
| 9 | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | RS | |
| 14 | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | RS | |
| 15 | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | RS | |
| 16 | positive | positive | negative | positive | 3/4 (75%) | 4/4 (100%) | RS | |
| 18a | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | RS | |
| 20 | positive | positive | positive | positive | 2/4 (50%) | 3/4 (75%) | RS | |
| 3 | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | RS-F | |
| 8 | negative | positive | positive | positive | 3/4 (75%) | 2/4 (50%) | RS-F | |
| 18b | negative | positive | negative | positive | 4/4 (100%) | 3/4 (75%) | VT | |
| 22 | positive | positive | negative | negative | 2/4 (50%) | 3/4 (75%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 4 | 14 | 3 | 15 |
| Number negative | 12 | 2 | 13 | 1 |
| Percent positive | 25 | 88 | 19 | 94 |
| Percent negative | 75 | 13 | 81 | 6 |
| Consensus value | negative | positive | negative | positive |
| Spiking | positive | positive | negative | positive |

**Methods:**

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow ), RomerLabs

GX = GlutenTox Sticks (Lateral Flow ), Biomedal

RS = Ridascreen®, R-Biopharm

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

div = not indicated / other method

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking for sample 2 (with rye), sample 3 (no added cereals) and sample 4 (with wheat).*

*For sample 1 containing oat a consensus value of 75% negative results was obtained. In total 12 out of 15 results were negative for gluten in sample 1. For valuation of results, it is important to consider whether the methods used are specified as suitable for the detection of oats or not.*

### *4.1.2 PCR-Results: Gluten Containing Cereals*

#### *4.1.2.1 PCR-Results: Gluten, in general*

**Quualitative valuation of results**

| Evaluation number | Sample 1 (Oat) | Sample 2 (Rye) | Sample 3 (none) | Sample 4 (Wheat) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 2 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | SFA-ID | sample 1: oat added |
| 16 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | SFA-ID | |
| 23 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | SFA-ID | |
| 7 | negative | positive | negative | positive | 3/3 (100%) | 3/4 (75%) | div | |
| 10 | negative | positive | negative | positive | 3/3 (100%) | 3/4 (75%) | div | not detecting oat and barley |
| 18 | negative | positive | negative | positive | 3/3 (100%) | 3/4 (75%) | div | w heat/rye/barley |
| 19 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 4 | 7 | 0 | 7 |
| Number negative | 3 | 0 | 7 | 0 |
| Percent positive | 57 | 100 | 0 | 100 |
| Percent negative | 43 | 0 | 100 | 0 |
| Consensus value | none | positive | negative | positive |
| Spiking | positive | positive | negative | positive |

**Methods:**

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

div = not indicated / other method

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking for sample 2 (with rye), sample 3 (no added cereals) and sample 4 (with wheat).*

*For sample 1 containing oat no consensus value of ≥ 75% positive or negative results was obtained. All results obtained by method SFA-ID were positive. For valuation of results, it is important to consider whether the methods used are specified as suitable for the detection of oats or not.*

### *4.1.2.2 PCR-Results: Oat*

### Quualitative valuation of results

| Evaluation number | Sample 1 (Oat) | Sample 2 (Rye) | Sample 3 (none) | Sample 4 (Wheat) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 4 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | GR | |
| 18 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 19 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 3 | 0 | 0 | 0 |
| Number negative | 0 | 3 | 3 | 3 |
| Percent positive | 100 | 0 | 0 | 0 |
| Percent negative | 0 | 100 | 100 | 100 |
| Consensus value | positive | negative | negative | negative |
| Spiking | positive | negative | negative | negative |

**Methods:**
GR = SPECIALfinder Assay, real time PCR, Generon
div = not indicated / other method

*Comments:*
*The consensus values of results are in qualitative agreement with the spiking of sample 1 with oat.*

### *4.1.2.3 PCR-Results: Rye*

### Qualitative valuation of results

| Evaluation number | Sample 1 (Oat) | Sample 2 (Rye) | Sample 3 (none) | Sample 4 (Wheat) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 4 | negative | negative | negative | negative | 3/4 (75%) | 3/4 (75%) | GR | no positive sample detected |
| 18 | negative | negative | negative | negative | 3/4 (75%) | 3/4 (75%) | div | no positive sample detected |
| 19 | negative | - | negative | - | 2/2 (100%) | 2/2 (100%) | div | no positive sample detected |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 0 | 0 | 0 |
| Number negative | 3 | 2 | 3 | 2 |
| Percent positive | 0 | 0 | 0 | 0 |
| Percent negative | 100 | 100 | 100 | 100 |
| Consensus value | negative | negative | negative | negative |
| Spiking | negative | positive | negative | negative |

**Methods:**
GR = SPECIALfinder Assay, real time PCR, Generon
div = not indicated / other method

*Comments:*
*None of the participants detected the addition of rye to sample 2.*

### *4.1.2.4 PCR-Results: Wheat*

### Quualitative valuation of results

| Evaluation number | Sample 1 (Oat) | Sample 2 (Rye) | Sample 3 (none) | Sample 4 (Wheat) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 4 | negative | positive | negative | positive | 3/3 (100%) | 3/4 (75%) | GR | |
| 22 | negative | negative | negative | positive | 3/3 (100%) | 4/4 (100%) | MS | |
| 19 | negative | - | negative | - | 2/2 (100%) | 2/2 (100%) | div | no positive sample detected |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 1 | 0 | 2 |
| Number negative | 3 | 1 | 3 | 0 |
| Percent positive | 0 | 50 | 0 | 100 |
| Percent negative | 100 | 50 | 100 | 0 |
| Consensus value | negative | none | negative | positive |
| Spiking | negative | negative | negative | positive |

**Methods:**

GR = SPECIALfinder Assay, real time PCR, Generon

MS = Microsynth

div = not indicated / other method

*Comments:*

*In qualitative agreement with the spiking wheat was detected by 2 participants in sample 4. For sample 2 (with rye) one positive and one negative result was reported. For valuation of results, it is important to consider whether the methods used are specified as specific for wheat alone or both wheat and rye.*

# 4.2 Proficiency Test Peanut

## *4.2.1 ELISA-Results: Peanut*

### Qualitative valuation of results

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 12a | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | AQ | |
| 12b | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | AS | |
| 20 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | BA | |
| 14 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | BC | |
| 18 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | BK | |
| 2 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | ES | |
| 6 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | NL-E | |
| 4 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 16 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 17 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 15 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | VT | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 0 | 11 | 11 |
| Number negative | 11 | 11 | 0 | 0 |
| Percent positive | 0 | 0 | 100 | 100 |
| Percent negative | 100 | 100 | 0 | 0 |
| Consensus value | negative | negative | positive | positive |
| Spiking | negative | negative | positive | positive |

**Methods:**

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow ), RomerLabs

BA = Bioavid (Lateral Flow ), R-Biopharm

BC = BioCheck ELISA

BK = BioKits, Neogen

ES = ELISA-Systems

NL-E = nutriLinia®E Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

VT = Veratox, Neogen

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking of samples.*

### *4.2.2 PCR-Results: Peanut*

## Qualitative valuation of results

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 1 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | ASU | |
| 21 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | GI | |
| 16 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | IC | |
| 9 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | MS | |
| 22 | negative | negative | positive | negative | 3/4 (75%) | 3/4 (75%) | MS | sample w ith low er amount not detected |
| 2 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 4 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 23 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 5 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 7 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 8 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 10 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 18 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 19 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 0 | 14 | 13 |
| Number negative | 14 | 14 | 0 | 1 |
| Percent positive | 0 | 0 | 100 | 93 |
| Percent negative | 100 | 100 | 0 | 7 |
| Consensus value | negative | negative | positive | positive |
| Spiking | negative | negative | positive | positive |

**Methods:**

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen, Coring System Diagnostix

IC = Food Allergen Detection PCR Kit, real Time PCR, InCura

MS = Microsynth

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

div = not indicated / other method

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking of samples.*

# 4.3 Proficiency Test Lupine

## *4.3.1 ELISA-Results: Lupine*

### Qualitative valuation of results

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 12a | positive | positive | positive | positive | 1/4 (25%) | 1/4 (25%) | AQ | |
| 12b | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | AS | |
| 18 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | ES | |
| 6 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | NL-E | |
| 4 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 16 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 17 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 1 | 1 | 7 | 1 |
| Number negative | 6 | 6 | 0 | 6 |
| Percent positive | 14 | 14 | 100 | 14 |
| Percent negative | 86 | 86 | 0 | 86 |
| Consensus value | negative | negative | positive | negative |
| Spiking | negative | negative | positive | negative |

**Methods:**

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow ), RomerLabs

ES = ELISA-Systems

NL-E = nutriLinia®E Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking of samples.*

### *4.3.2 PCR-Results: Lupine*

## Qualitative valuation of results

| Evaluation number | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 1 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 10 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 21 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | GI | |
| 16 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | IC | |
| 9 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | MS | |
| 22 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | MS | |
| 2 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 23 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 5 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 7 | positive | negative | positive | negative | 3/4 (75%) | 3/4 (75%) | div | |
| 8 | negative | positive | positive | negative | 3/4 (75%) | 3/4 (75%) | div | |
| 18 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 19 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 1 | 1 | 13 | 0 |
| Number negative | 12 | 12 | 0 | 13 |
| Percent positive | 8 | 8 | 100 | 0 |
| Percent negative | 92 | 92 | 0 | 100 |
| Consensus value | negative | negative | positive | negative |
| Spiking | negative | negative | positive | negative |

**Methods:**

ASU = ASU §64 Methode/method

GI = GEN-IAL First Allergen, Coring System Diagnostix

IC = Food Allergen Detection PCR Kit, real Time PCR, InCura

MS = Microsynth

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

div = not indicated / other method

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking of samples.*

## 4.4 Proficiency Test Celery

### *4.4.1 ELISA-Results: Celery*

*Comments:*
*None of the participants used the ELISA method for determination of celery.*

### *4.4.2 PCR-Results: Celery*

### Qualitative valuation of results

| Evaluation number | Sample 1 (Root) | Sample 2 (Leafs) | Sample 3 (none) | Sample 4 (Seed) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 1 | negative | positive | negative | positive | 3/3 (100%) | 3/4 (75%) | ASU | |
| 8 | negative | negative | negative | negative | 1/3 (33%) | 1/4 (25%) | ASU | no positive sample detected |
| 10 | negative | positive | negative | positive | 3/3 (100%) | 3/4 (75%) | ASU | |
| 18 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | ASU | |
| 19 | negative | positive | negative | positive | 3/3 (100%) | 3/4 (75%) | ASU | |
| 21 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | GI-4 | |
| 16 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | IC | |
| 9 | negative | positive | negative | negative | 2/3 (67%) | 2/4 (50%) | MS | |
| 22 | positive | positive | positive | positive | 2/3 (67%) | 3/4 (75%) | MS | |
| 2 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | SFA-ID | |
| 4 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | SFA-ID | |
| 20 | positive | negative | positive | positive | 1/3 (33%) | 2/4 (50%) | SFA-ID | |
| 23 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | SFA-ID | |
| 5 | positive | positive | negative | negative | 2/3 (67%) | 3/4 (75%) | div | |
| 12 | negative | positive | negative | negative | 2/3 (67%) | 2/4 (50%) | div | |
| 13 | positive | positive | negative | positive | 3/3 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 10 | 14 | 2 | 12 |
| Number negative | 6 | 2 | 14 | 4 |
| Percent positive | 63 | 88 | 13 | 75 |
| Percent negative | 38 | 13 | 88 | 25 |
| Consensus value | none | positive | negative | positive |
| Spiking | positive | positive | negative | positive |

**Methods:**

ASU = ASU §64 Methode/method

GI-4= GEN-IAL First Allergen Tetra, Coring System Diagnostix

IC = Food Allergen Detection PCR Kit, real Time PCR, InCura

MS = Microsynth

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

div = not indicated / other method

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking for sample 2 (with celery leafs), sample 3 (no added celery) and sample 4 (with celery seeds).*

*For sample 1 containing celery root no consensus value of ≥ 75% positive or negative results was obtained. All results obtained by the methods GI-4, IC and SFA-ID were positive. For valuation of results, it is important to consider whether the methods used are specified as suitable for the detection of celery root and which limit of detection could be reached.*

# 4.5 Proficiency Test Sesame

## *4.5.1 ELISA-Results: Sesame*

### Qualitative valuation of results

| Evaluation number | Sample 1 (white) | Sample 2 (black) | Sample 3 (white) | Sample 4 (white) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 12a | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | AQ | |
| 12b | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | AS | |
| 20 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | BA | |
| 14 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | BC | |
| 11 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | BK | |
| 18 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | BK | |
| 2 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | ES | |
| 15 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | ES | |
| 16 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | ES | |
| 6 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | NL-E | |
| 4 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 13 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 17 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 13 | 13 | 13 | 13 |
| Number negative | 0 | 0 | 0 | 0 |
| Percent positive | 100 | 100 | 100 | 100 |
| Percent negative | 0 | 0 | 0 | 0 |
| Consensus value | positive | positive | positive | positive |
| Spiking | positive | positive | positive | positive |

**Methods:**

AQ = AgraQuant, RomerLabs

AS = AgraStrip (Lateral Flow ), RomerLabs

BA = Bioavid (Lateral Flow ), R-Biopharm

BC = BioCheck ELISA

BK = BioKits, Neogen

ES = ELISA-Systems

NL-E = nutriLinia®E Allergen-ELISA

RS-F= Ridascreen® Fast, R-Biopharm

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking of samples. None of the participants differentiated between black and white sesame.*

### *4.5.2 PCR-Results: Sesame*

## Qualitative valuation of results

| Evaluation number | Sample 1 (white) | Sample 2 (black) | Sample 3 (white) | Sample 4 (white) | Qualitative Valuation | Qualitative Valuation | Method | Remarks |
|---|---|---|---|---|---|---|---|---|
| | pos/neg | pos/neg | pos/neg | pos/neg | Agreement with consensus value | Agreement with spiking of samples | | |
| 10 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | ASU | |
| 21 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | GI-4 | |
| 16 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | IC | |
| 9 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | MS | |
| 22 | negative | negative | negative | negative | 0/4 (0%) | 0/4 (0%) | MS | keine Positiveprobe identifiziert |
| 2 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 23 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 5 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 8 | positive | positive | negative | positive | 3/4 (75%) | 3/4 (75%) | div | |
| 18 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 19 | positive | positive | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 10 | 10 | 9 | 10 |
| Number negative | 1 | 1 | 2 | 1 |
| Percent positive | 91 | 91 | 82 | 91 |
| Percent negative | 9 | 9 | 18 | 9 |
| Consensus value | positive | positive | positive | positive |
| Spiking | positive | positive | positive | positive |

**Methods:**

ASU = ASU §64 Methode/method

GI-4= GEN-IAL First Allergen Tetra, Coring System Diagnostix

IC = Food Allergen Detection PCR Kit, real Time PCR, InCura

MS = Microsynth

SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen

div = not indicated / other method

*Comments:*

*The consensus values of results are in qualitative agreement with the spiking of samples. None of the participants differentiated between black and white sesame.*

# 5. Documentation

## 5.1 Details by the participants

Note: Information given in German was translated by DLA to the best of our knowledge (without guarantee of correctness).

### 5.1.1 ELISA: Gluten

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| AQ | 12a | positive | positive | positive | positive | 2 | Food | AgraQuant, RomerLabs |
| AS | 12b | negative | positive | negative | positive | 20 | Food | AgraStrip, RomerLabs |
| GX | 6a | negative | negative | negative | positive | 3 | | GlutenTox Sticks Plus |
| IG | 6b | negative | negative | negative | positive | 5 | Gluten | Ingezim Gluten-INGENASA |
| RS | 2 | negative | positive | negative | positive | 5 | Gluten | Ridascreen, r-Biopharm |
| RS | 4 | negative | positive | negative | positive | 5 | Gluten | Ridascreen, r-Biopharm |
| RS | 9 | negative | positive | negative | positive | 1,5 | Gluten | Ridascreen, r-Biopharm |
| RS | 14 | negative | positive | negative | positive | 5 | Gluten | Ridascreen, r-Biopharm |
| RS | 15 | negative | positive | negative | positive | | Gliadin | Ridascreen, r-Biopharm |
| RS | 16 | positive | positive | negative | positive | 3 | Gluten | Ridascreen, r-Biopharm |
| RS | 18a | negative | positive | negative | positive | 3 | Gluten | Ridascreen, r-Biopharm |
| RS | 20 | 2,7 | 40 | 2,8 | 57,5 | 2 | Gluten | Ridascreen, r-Biopharm |
| RS-F | 3 | negative | positive | negative | positive | < 10 | Gluten | Ridascreen Fast, r-Biopharm |
| RS-F | 8 | negative | 167 | 14 | 41 | 4 | Gluten | Ridascreen Fast, r-Biopharm |
| VT | 18b | negative | positive | negative | positive | 8 | Gluten | Veratox Allergen, Neogen |
| div | 22 | positive | positive | negative | negative | 10 | Gliadin | in house |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Antibody | e.g. Extraction Solution / Time / Temperature | |
| AQ | 12a | COKAL0200 | | | |
| AS | 12b | COKAL0200AS | | | |
| GX | 6a | KT-5340 | | | |
| IG | 6b | | | Extraction solution+Ethnol 80%/120 min/RT | |
| RS | 2 | | | As Per Kit Instructions | |
| RS | 4 | R7001 | As Per Kit Instructions | As Per Kit Instructions | |
| RS | 9 | R7001 | R5 | As Per Kit Instructions; addition of milk pow der (1:2 to sample amount) | 43,1 mg/kg; sample 4: 44,9 mg/kg |
| RS | 14 | | | 60°C extraction | |
| RS | 15 | R7001 | | | |
| RS | 16 | | | | |
| RS | 18a | R7001 | R5, Prolamine from w he-at, rye and barley | As Per Kit Instructions | |
| RS | 20 | R7001 | | Cockail solutio and ethanol solution 70 % | |
| RS-F | 3 | 11186 | | RIDA Extraction solution | |
| RS-F | 8 | R7002 | R5 | | |
| VT | 18b | 8480 | R5, Prolamins from w heat, rye and barley | As Per Kit Instructions | |
| div | 22 | | | in house MeOH- Extraction | |

### 5.1.2 ELISA: Peanut

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| AQ | 12a | negative | negative | positive | positive | 0,1 | Food | AgraQuant, RomerLabs |
| AS | 12b | negative | negative | positive | positive | 1 | Food | AgraStrip, RomerLabs |
| BA | 20 | negative | negative | positive | positive | | Food item, total | bioavid |
| BC | 14 | negative | negative | positive | positive | 1 | Whole peanut | BioCheck |
| BK | 18 | negative | negative | positive | positive | 0,5 | Food item, total | BioKits Assay Kit, Neogen |
| ES | 2 | negative | negative | positive | positive | 1 | Protein | ELISA-Systems, Residue Assay |
| NL-E | 6 | negative | negative | positive | positive | 3 | Food item, total | nutriLinia E ELISA, Transia |
| RS-F | 4 | negative | negative | positive | positive | 2,5 | Food item, total | Ridascreen Fast, r-Biopharm |
| RS-F | 16 | negative | negative | positive | positive | 1,5 | | Ridascreen Fast, r-Biopharm |
| RS-F | 17 | negative | negative | positive | positive | 0,13 | Peanut | Ridascreen Fast, r-Biopharm |
| VT | 15 | negative | negative | positive | positive | | Food item, total | Veratox Allergen, Neogen |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Antibody | e.g. Extraction Solution / Time / Temperature | |
| AQ | 12a | COKAL0148 | | | |
| AS | 12b | COKAL0110AS | | | |
| BA | 20 | | | aqueous extraction | |
| BC | 14 | Biocheck Peanut Check | | 60° extraction | |
| BK | 18 | 902048Q | Conarachin-A | As Per Kit Instructions | peanut |
| ES | 2 | | | As Per Kit Instructions | same results with Ridascreen Fast |
| NL-E | 6 | | | Extraction solution/15 min/60°C | |
| RS-F | 4 | R6202 | As Per Kit Instructions | As Per Kit Instructions | |
| RS-F | 16 | | | | |
| RS-F | 17 | R6202 | Antibodies against peanut protein | Allergen extraction buffer 10 min 60°C | |
| VT | 15 | 8430 | | | |

### 5.1.3 ELISA: Lupine

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| AQ | 12a | positive | positive | positive | positive | 0,2 | Food | AgraQuant, RomerLabs |
| AS | 12b | negative | negative | positive | negative | 10 | Food | AgraStrip, RomerLabs |
| ES | 18 | negative | negative | positive | negative | 0,25 | | ELISA-Systems, Residue Assay |
| NL-E | 6 | negative | negative | positive | negative | 2 | Food item, total | nutriLinia E ELISA, Transia |
| RS-F | 4 | negative | negative | positive | negative | 1 | Lupin Protein | Ridascreen Fast, r-Biopharm |
| RS-F | 16 | negative | negative | positive | negative | 0,6 | | Ridascreen Fast, r-Biopharm |
| RS-F | 17 | negative | negative | positive | negative | 0,7 | Protein | Ridascreen Fast, r-Biopharm |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Antibody | e.g. Extraction Solution / Time / Temperature | |
| AQ | 12a | COKAL1548 | | | |
| AS | 12b | COKAL1510AS | | | |
| ES | 18 | ESLFP-48 | Lupine flour proteins | As Per Kit Instructions | Lupine flour protein |
| NL-E | 6 | | | Extraction solution/15 min/60°C | |
| RS-F | 4 | R6102 | As Per Kit Instructions | As Per Kit Instructions | |
| RS-F | 16 | | | | |
| RS-F | 17 | R6102 | Antibodies specific for protein including y-conglutin and against all food and feed relevant european sweet lupine species (Lupinus albus, luteus and angustifolius) | Allergen extraction buffer 10 min 60°C | |

### 5.1.4 ELISA: Sesame

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| AQ | 12a | positive | positive | positive | positive | 0,2 | | AgraQuant, RomerLabs |
| AS | 12b | positive | positive | positive | positive | 5 | Food | AgraStrip, RomerLabs |
| BA | 20 | positive | positive | positive | positive | | Food item, total | bioavid |
| BC | 14 | positive | positive | positive | positive | 2 | Whole sesame | BioCheck |
| BK | 11 | positive | positive | positive | positive | 6,25 | Food item, total | BioKits Assay Kit, Neogen |
| BK | 18 | positive | positive | positive | positive | 1,5 | Food item, total | BioKits Assay Kit, Neogen |
| ES | 2 | positive | positive | positive | positive | 0,5 | Protein | ELISA-Systems, Residue Assay |
| ES | 15 | positive | positive | positive | positive | | Food item, total | ELISA-Systems, Residue Assay |
| ES | 16 | positive | positive | positive | positive | 0,25 | | ELISA-Systems, Residue Assay |
| NL-E | 6 | positive | positive | positive | positive | 2 | Food item, total | nutriLinia E ELISA, Transia |
| RS-F | 4 | positive | positive | positive | positive | 2,5 | Food item, total | Ridascreen Fast, r-Biopharm |
| RS-F | 13 | positive | positive | positive | positive | 2,5 | | Ridascreen, r-Biopharm |
| RS-F | 17 | positive | positive | positive | positive | 0,2 | Sesame | Ridascreen Fast, r-Biopharm |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Antibody | e.g. Extraction Solution / Time / Temperature | |
| AQ | 12a | COKAL1948 | | | |
| AS | 12b | COKAL1910AS | | | |
| BA | 20 | | | aqueous extraction | |
| BC | 14 | Biocheck Sesame Check | | 60° extraction | |
| BK | 11 | 902070X | Sesame protein | Samples extracted in Biokits extraction buffer by shaking at 150rpm in orbital incubator at room temperature for 15 minutes | |
| BK | 18 | 902070X | Sesame proteins | As Per Kit Instructions | Sesame |
| ES | 2 | | | As Per Kit Instructions | |
| ES | 15 | ESSESRD-48 | | | |
| ES | 16 | | | | |
| NL-E | 6 | | | Extraction solution/15 min/60°C | |
| RS-F | 4 | R7202 | As Per Kit Instructions | As Per Kit Instructions | |
| RS-F | 13 | ELISA | | | |
| RS-F | 17 | R7202 | specific against sesame protein | Allergen extraction buffer 10 min 60°C | |

## 5.1.5 PCR: Gluten Containing Cereals

### 5.1.5.1 PCR: Gluten, in general

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| SFA-ID | 2 | positive | positive | negative | positive | 0,4 | Allergen-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 16 | positive | positive | negative | positive | 0,4 | Allergen DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 23 | positive | positive | negative | positive | 0,4 | Cereal-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| div | 7 | negative | positive | negative | positive | 25 | Allergen-DNA | in house method |
| div | 10 | negative | positive | negative | positive | 20 | Food item, total | ASU §64 |
| div | 18 | negative | positive | negative | positive | 4 | Allergen-DNA | |
| div | 19 | positive | positive | negative | positive | | | in house method |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| SFA-ID | 2 | | | As Per Kit Instructions | sample 1 oat added |
| SFA-ID | 16 | | | | |
| SFA-ID | 23 | S3106 | | Real Time PCR | |
| div | 7 | - | PRP8 | CTAB, magnetic beads | |
| div | 10 | ASU No. not available yet | high molecular weight (HMW) Glutenin Gen B1-1 from wheat and 1-R from rye | CTAB/QIAQuick, s. e.g. ASU L 08.00-59 | oat and barley could not be detected; Method: § 64 in press; Reference: Detection and quantitation of wheat and/or rye by real-time PCR. Literature: Zeltner D, Glomb MA, Maede D (2009) Real-time PCR systems for the detection of the gluten-containing cereals wheat, spelt, kamut, rye, barley and oat. Eur Food Res Technol 228:321-330 |
| div | 18 | Eur F Res Tech 212 (2001) 228ff., mod | | CTAB/Proteinase K/Promega Wizard DNA CleanUp/Gelelektrophorese/45 Cycles | Wheat/Rye/Barley |
| div | 19 | | | Silica-columns, Real-Time PCR, 45 cycles | |

### 5.1.5.2 PCR: Oat

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| GR | 4 | positive | negative | negative | negative | 10 | Food item, total | Generon Oats Assay |
| div | 18 | positive | negative | negative | negative | 10-20 | Allergen-DNA | in house method |
| div | 19 | positive | negative | negative | negative | | | in house method |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| GR | 4 | PAV11A | As Per Kit Instructions | As Per Kit Instructions | Generon Oats Assay |
| div | 18 | | | CTAB/Proteinase K/Promega Wizard DNA CleanUp/Gelelektrophorese/45 Cycles | |
| div | 19 | | | Silica-columns, Real-Time PCR, 45 cycles | |

### 5.1.5.3 PCR: Rye

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| GR | 4 | negative | negative | negative | negative | 10 | Food item, total | Generon Rye Assay |
| div | 18 | negative | negative | negative | negative | 10-20 | Allergen-DNA | in house method |
| div | 19 | negative | - | negative | - | | | in house method |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| GR | 4 | PAV10A | As Per Kit Instructions | As Per Kit Instructions | Generon Rye Assay |
| div | 18 | | | CTAB/Proteinase K/Promega Wizard DNA CleanUp/Gelelektrophorese/45 Cyclen | |
| div | 19 | | | Silica-columns, Real-Time PCR, 45 cycles | Rye and w heat is not differentiated |

### 5.1.5.4 PCR: Wheat

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| GR | 4 | negative | positive | negative | positive | 1 | Food item, total | Generon Wheat Assay |
| MS | 22 | negative | negative | negative | positive | 100 | Food item, total | Microsynth |
| div | 19 | negative | - | negative | - | | | in house method |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| GR | 4 | PGE29A | As Per Kit Instructions | As Per Kit Instructions | Generon Wheat Assay |
| MS | 22 | | | Wizard/Rotorgene Realtime/45 | |
| div | 19 | | | Silica-columns, Real-Time PCR, 45 cycles | Rye and w heat is not differentiated |

### 5.1.6 PCR: Peanut

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| ASU | 1 | negative | negative | positive | positive | | | ASU §64 |
| GI | 21 | negative | negative | positive | positive | | | GEN-IAL First-Peanut (Erdnuss)/Coring |
| IC | 16 | negative | negative | positive | positive | 1 | Allergen DNA | Incura |
| MS | 9 | negative | negative | positive | positive | 0,005% | Allergen-DNA | Microsynth |
| MS | 22 | negative | negative | positive | negative | 100 | Food item, total | Microsynth |
| SFA-ID | 2 | negative | negative | positive | positive | 1,5 | Allergen-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 4 | negative | negative | positive | positive | 1 | Food item, total | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 23 | negative | negative | positive | positive | 1,5 | Peanut-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| div | 5 | negative | negative | positive | positive | 0,008 | Allergen DNA | in-house method |
| div | 7 | negative | negative | positive | positive | 25 | Allergen-DNA | in-house method |
| div | 8 | negative | negative | positive | positive | 0,01% | Allergen-DNA | in-house method |
| div | 10 | negative | negative | positive | positive | 5 | Food item, total | Köppel et al (2010) Eur. Food Res. Technol. 230: 367-374. |
| div | 18 | negative | negative | positive | positive | 40 | Allergen-DNA | in-house method |
| div | 19 | negative | negative | positive | positive | | | in-house method |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| ASU | 1 | L 44.00-11 | peanut | Extraction with Wizard DNA Clean-Up System | |
| GI | 21 | | | Real-time PCR | |
| IC | 16 | | | | |
| MS | 9 | | peanut-DNA | Macherey Nagel Nucleo Spin Food optimized: increased sample weight, buffer change (washing with Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, decontamination step with UNG; own thermoprofile; Inhibition control | Reference material: ground peanuts |
| MS | 22 | | | Wizard/Rotorgene Realtime/45 | |
| SFA-ID | 2 | | | As Per Kit Instructions | |
| SFA-ID | 4 | S3103 | As Per Kit Instructions | As Per Kit Instructions | |
| SFA-ID | 23 | S3103 | | Real Time PCR | |
| div | 5 | | | | |
| div | 7 | | Ara d2 | CTAB, magnetic beads | |
| div | 8 | Scaravelli et al., 2008 | | RealTime PCR | |
| div | 10 | | Cor A 1 | CTAB/QIAQuick, s. z.B. ASU L 08.00-59 | |
| div | 18 | | | CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real-time PCR/45 Cycles | |
| div | 19 | | | Silica-columns, Real-Time PCR, 45 cycles | |

### 5.1.7 PCR: Lupine

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| ASU | 1 | negative | negative | positive | negative | | | ASU §64 |
| ASU | 10 | negative | negative | positive | negative | 5 | Food item, total | ASU §64 |
| GI | 21 | negative | negative | positive | negative | | | GEN-IAL First-Lupine /Co-ring |
| IC | 16 | negative | negative | positive | negative | 1 | Allergen DNA | Incura |
| MS | 9 | negative | negative | positive | negative | 0,01% | Allergen-DNA | Microsynth |
| MS | 22 | negative | negative | negative | negative | 100 | Food item, total | Microsynth |
| SFA-ID | 2 | negative | negative | positive | negative | 0,4 | Allergen-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 23 | negative | negative | positive | negative | 0,4 | Lupin-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| div | 5 | negative | negative | positive | negative | 0,008 | Allergen DNA | in-house method |
| div | 7 | positive | negative | positive | negative | 25 | Allergen-DNA | in-house method |
| div | 8 | negative | positive | positive | negative | 0,01% | Allergen-DNA | in-house method |
| div | 18 | negative | negative | positive | negative | 10 | Allergen-DNA | in-house method |
| div | 19 | negative | negative | positive | negative | | | in-house method |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| ASU | 1 | L 18.00-22 | Lupine | Extraction with Wizard DNA Clean-Up System | |
| ASU | 10 | L 08.00-58 | s. ASU | CTAB/QIAQuick, s. e.g. ASU L 08.00-59 | result given as Lupinus albus |
| GI | 21 | | | Real-time PCR | |
| IC | 16 | | | | |
| MS | 9 | | Lupine-DNA | Macherey Nagel Nucleo Spin Food optimized: increased sample weight, buffer change (washing with Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, decontamination step with UNG; own thermoprofile; Inhibition control | Reference material: spiked sausage |
| MS | 22 | | | Wizard/Rotorgene Realtime/45 | |
| SFA-ID | 2 | | | As Per Kit Instructions | |
| SFA-ID | 23 | S3111 | | Real Time PCR | |
| div | 5 | | | | |
| div | 7 | - | IST | CTAB, magnetic beads | |
| div | 8 | Demmel et al., 2008 | | RealTime PCR | |
| div | 18 | | | CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real-time PCR/45 Cyclen | |
| div | 19 | | | Silica-columns, Real-Time PCR, 45 cycles | |

## 5.1.8 PCR: Celery

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| ASU | 1 | negative | positive | negative | positive | | | ASU §64 |
| ASU | 8 | negative | negative | negative | negative | 0,01% | Allergen-DNA | |
| ASU | 10 | negative | positive | negative | positive | 5 | Food item, total | ASU §64 |
| ASU | 18 | positive | positive | negative | positive | 4 | Allergen-DNA | ASU §64 |
| ASU | 19 | negative | positive | negative | positive | | | ASU §64 |
| GI-4 | 21 | positive | positive | negative | positive | | | GEN-IAL First-Allergen Tetra I (celery, sesame, mustard)/Coring |
| IC | 16 | positive | positive | negative | positive | 10 | Allergen DNA | Incura |
| MS | 9 | negative | positive | negative | negative | 0,01% | Allergen-DNA | Microsynth |
| MS | 22 | positive | positive | positive | positive | 100 | Food item, total | Microsynth |
| SFA-ID | 2 | positive | positive | negative | positive | 0,4 | Allergen-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 4 | positive | positive | negative | positive | 1 | Food item, total | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 20 | positive | negative | positive | positive | | Food item, total | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 23 | positive | positive | negative | positive | 0,4 | Celery-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| div | 5 | positive | positive | negative | negative | 0,008 | Allergen DNA | in-house method |
| div | 12 | negative | positive | negative | negative | 100 | Food | in-house method |
| div | 13 | positive | positive | negative | positive | | Allergen DNA | |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| ASU | 1 | L 08.00-56 | Celery | Extraction with Wizard DNA Clean-Up System | |
| ASU | 8 | ASU §64 | | RealTime PCR | |
| ASU | 10 | L 08.00-56 | s. ASU | CTAB/QIAQuick, s. e.g. ASU L 08.00-59 | result given as celery seed |
| ASU | 18 | L 08.00-56, mod. | | CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real-time PCR/45 Cycles | |
| ASU | 19 | L08.00-56 | | Silica-columns, Real-Time PCR, 45 cycles | |
| GI-4 | 21 | | | Real-time PCR | |
| IC | 16 | | | | |
| MS | 9 | | Celery-DNA | Macherey Nagel Nucleo Spin Food optimized: increased sample weight, buffer change (washing with Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, decontamination step with UNG; own thermoprofile; Inhibition control | Reference material: dried celery root |
| MS | 22 | | | Wizard/Rotorgene Realtime/45 | |
| SFA-ID | 2 | | | As Per Kit Instructions | |
| SFA-ID | 4 | S3105 | As Per Kit Instructions | As Per Kit Instructions | |
| SFA-ID | 20 | | | QIAgen DNA Extraction | |
| SFA-ID | 23 | S3105 | | Real Time PCR | |
| div | 5 | | | | |
| div | 12 | | | Real Time PCR | |
| div | 13 | PB-22/LM wyd.1 z dn. 15.11.2016 | | | |

## 5.1.9 PCR: Sesame

*Primary data*

| Meth. Abr. | Evaluation number | Result Sample 1 | Result Sample 2 | Result Sample 3 | Result Sample 4 | Limit of detection | Limit of detection given as | Method |
|---|---|---|---|---|---|---|---|---|
| | | qualitative | qualitative | qualitative | qualitative | mg/kg | e.g. food / food protein | Test-Kit + Provider |
| ASU | 10 | positive | positive | positive | positive | 5 | Food item, total | ASU §64 |
| GI-4 | 21 | positive | positive | positive | positive | | | GEN-IAL First-Allergen Tetra I (celery, sesame, mustard)/Coring |
| IC | 16 | positive | positive | positive | positive | 2 | Allergen DNA | Incura |
| MS | 9 | positive | positive | positive | positive | 0,005% | Allergen-DNA | Microsynth |
| MS | 22 | negative | negative | negative | negative | 100 | Food item, total | Microsynth |
| SFA-ID | 2 | positive | positive | positive | positive | 0,4 | Allergen-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| SFA-ID | 23 | positive | positive | positive | positive | 0,4 | Sesame-DNA | Sure Food Allergen ID, Congen / r-Biopharm |
| div | 5 | positive | positive | positive | positive | 0,008 | Allergen DNA | in-house method |
| div | 8 | positive | positive | negative | positive | 0,01% | Allergen-DNA | in-house method |
| div | 18 | positive | positive | positive | positive | 40 | Allergen-DNA | in-house method |
| div | 19 | positive | positive | positive | positive | | | in-house method |

*Other details to the Methods*

| Meth. Abr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No. / ASU-No. | Target-DNA | e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles | |
| ASU | 10 | L 18.00-22 | s. ASU | CTAB/QIAQuick, s. e.g. ASU L 08.00-59 | L 18.00-22 |
| GI-4 | 21 | | | Real-time PCR | |
| IC | 16 | | | | |
| MS | 9 | | Sesame-DNA | Macherey Nagel Nucleo Spin Food optimized: increased sample weight, buffer change (washing with Lysis Buffer) RNase-step, Chloroform-step, 2xCQW; RealTime PCR with 45 cycles, decontamination step with UNG; own thermoprofile; Inhibition control | |
| MS | 22 | | | Wizard/Rotorgene Realtime/45 | |
| SFA-ID | 2 | | | As Per Kit Instructions | |
| SFA-ID | 23 | S3108 | | Real Time PCR | S3108 |
| div | 5 | | | | |
| div | 8 | Köppel et al., 2010 | Oleosin | RealTime PCR | Köppel et al., 2010 |
| div | 18 | | | CTAB/Proteinase K/Promega Wizard DNA CleanUp/Real-time PCR/45 Cycles | |
| div | 19 | | | Silica-columns, Real-Time PCR, 45 cycles | |

## 5.2 Homogeneity

### 5.2.1 Mixture homogeneity before bottling

**Microtracer Homogeneity Test**
**DLA 13-2016 Sample 1**

| | | |
|---|---|---|
| Weight whole sample | 1,01 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 62,3 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 5,19 | 166 | 64,0 |
| 2 | 5,27 | 165 | 62,6 |
| 3 | 5,12 | 172 | 67,2 |
| 4 | 5,03 | 177 | 70,4 |
| 5 | 5,10 | 164 | 64,3 |
| 6 | 5,05 | 168 | 66,5 |
| 7 | 5,24 | 147 | 56,1 |
| 8 | 4,95 | 166 | 67,1 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 166 | Particles |
| Standard deviation | 10,9 | Particles |
| $\chi^2$ (CHI-Quadrat) | 4,99 | |
| **Probability** | **66** | % |
| Recovery rate | 104 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 64,8 | mg/kg |
| Standard deviation | 4,25 | mg/kg |
| rel. Standard deviaton | 6,56 | % |
| Horwitz standard deviation | 8,54 | % |
| **HorRat-value** | **0,77** | |
| Recovery rate | 104 | % |

**Microtracer Homogeneity Test**
**DLA 13-2016 Sample 2**

| | | |
|---|---|---|
| Weight whole sample | 1,01 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 47,5 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 5,16 | 142 | 55,0 |
| 2 | 5,24 | 156 | 59,5 |
| 3 | 5,13 | 145 | 56,5 |
| 4 | 5,05 | 153 | 60,6 |
| 5 | 5,18 | 153 | 59,1 |
| 6 | 5,07 | 152 | 60,0 |
| 7 | 5,14 | 137 | 53,3 |
| 8 | 5,24 | 151 | 57,6 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 149 | Particles |
| Standard deviation | 6,64 | Particles |
| $\chi^2$ (CHI-Quadrat) | 2,08 | |
| **Probability** | **96** | % |
| Recovery rate | 121 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 57,7 | mg/kg |
| Standard deviation | 2,58 | mg/kg |
| rel. Standard deviaton | 4,47 | % |
| Horwitz standard deviation | 8,69 | % |
| **HorRat-value** | **0,51** | |
| Recovery rate | 121 | % |

**Microtracer Homogeneity Test**
**DLA 13-2016 Sample 3**

| | | |
|---|---|---|
| Weight whole sample | 1,00 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 45,2 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 5,01 | 126 | 50,3 |
| 2 | 5,18 | 125 | 48,3 |
| 3 | 5,05 | 130 | 51,5 |
| 4 | 5,05 | 123 | 48,7 |
| 5 | 5,09 | 136 | 53,4 |
| 6 | 5,19 | 138 | 53,2 |
| 7 | 5,15 | 123 | 47,8 |
| 8 | 4,63 | 122 | 52,7 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 128 | Particles |
| Standard deviation | 5,79 | Particles |
| $\chi^2$ (CHI-Quadrat) | 1,83 | |
| **Probability** | **97** | % |
| Recovery rate | 112 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 50,7 | mg/kg |
| Standard deviation | 2,30 | mg/kg |
| rel. Standard deviaton | 4,53 | % |
| Horwitz standard deviation | 8,86 | % |
| **HorRat-value** | **0,51** | |
| Recovery rate | 112 | % |

**Microtracer Homogeneity Test**
**DLA 13-2016 Sample 4**

| | | |
|---|---|---|
| Weight whole sample | 1,01 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 59,5 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 5,15 | 178 | 69,1 |
| 2 | 5,05 | 182 | 72,1 |
| 3 | 5,16 | 180 | 69,8 |
| 4 | 5,09 | 188 | 73,9 |
| 5 | 5,09 | 179 | 70,3 |
| 6 | 5,12 | 183 | 71,5 |
| 7 | 5,04 | 158 | 62,7 |
| 8 | 4,63 | 175 | 75,6 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 178 | Particles |
| Standard deviation | 9,71 | Particles |
| $\chi^2$ (CHI-Quadrat) | 3,71 | |
| **Probability** | **81** | % |
| Recovery rate | 119 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 70,6 | mg/kg |
| Standard deviation | 3,85 | mg/kg |
| rel. Standard deviaton | 5,46 | % |
| Horwitz standard deviation | 8,43 | % |
| **HorRat-value** | **0,65** | |
| Recovery rate | 119 | % |

# 6. Index of participant laboratories

| Teilnehmer / Participant | Ort / Town | Land / Country |
|---|---|---|
| | | GREAT BRITAIN |
| | | SPAIN |
| | | Germany |
| | | ITALY |
| | | Germany |
| | | Germany |
| | | Germany |
| | | Germany |
| | | AUSTRIA |
| | | Germany |
| | | POLAND |
| | | SWITZERLAND |
| | | Germany |
| | | Germany |
| | | Germany |
| | | AUSTRIA |
| | | GREAT BRITAIN |
| | | FRANCE |
| | | Germany |
| | | Germany |
| | | GREAT BRITAIN |
| | | AUSTRIA |
| | | CANADA |

*[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]*

*[The address data of the participants were deleted for publication of the evaluation report.]*

# 7. Index of references


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