*Proficiency Tests*
**DLA**
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cosmetics
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**Evaluation Report**
proficiency test

**DLA 12/2017**

# Allergen-Screening II:

## Crustacea, Egg, Fish, Milk, Molluscs, Mustard and Soya

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Coordinator of this PT:
Dr. Matthias Besler

## 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 12/2017 |
| *EP-Koordinator*<br>*PT-Coordinator* | Dr. Matthias Besler |
| *Status des EP-Bericht*<br>*Status of PT-Report* | Abschlussbericht / Final report (17 November 2017)<br><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: 17 November 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. |
| *Vertraulichkeit*<br>*Confidentiality* | Die Teilnehmerergebnisse sind im EP-Bericht in anonymisierter Form mit Auswertenummern benannt. Daten einzelner Teilnehmer werden ausschließlich nach vorheriger Zustimmung des Teilnehmers an Dritte weitergegeben.<br>Participant result are named anonymously with evaluation numbers in the PT report. Data of individual participants will be passed on to third parties only with prior consent of the participant. |

# 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 2-20% of the allergenic ingredients concerned.
The respective raw materials were common in commerce egg powder, milk powder and soyflour and premixes produced by DLA from commercial mustard seeds and frozen shrimps, cod and squid (s. Tab. 2). The mustard seeds were crushed, ground with addition of carrier substances and sieved (mesh 400 µm). The frozen marine foods were crushed, dried and ground with addition of carriers and sieved by means of a centrifugal mill (mesh 500 µm).
The composition 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 (30% - 88%)<br>- Sodium chloride (0,0% - 85%)<br>- Sodium sulfate (0,0% - 7,7%)<br>- Silicon dioxide (1,0% - 2,2%)<br>- Allergens (2,4% - 20% each) | 0,027 - 0,42 % |

Table 2: Added amounts of allergenic ingredients positive in mg/kg ranges** given as food item

| Ingredients * | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| *Crustaceae:* Shrimps *(Litopenaeus vannamei)*, getrocknet (Protein 63%) | negative | positive (50 - 150) | positive (25 - 75) | negative |
| *Egg:* Whole egg powder (Protein 47%) | negative | positive (50 - 150) | negative | positive (25 - 75) |
| *Fish:* Cod *(Gadus morhua)*, dried (Protein 56%) | positive (25 - 75) | negative | positive (50 - 150) | negative |
| *Milk:* Skimmed milk powder (Protein 37%) | positive (25 - 75) | negative | negative | negative |
| *Molluscs:* Squid tubes *(Illex argentinus)*, dried (Protein 34%) | negative | negative | negative | positive (50 - 150) |
| *Mustard, yellow:* Sinapis alba (Protein 31%) | negative | positive (50 - 150) | negative | negative |
| *Mustard, brown:* Brassica juncea (Protein 24%) | positive (50 - 150) | negative | negative | negative |
| *Mustard, black:* Brassica nigra (Protein 27%) | negative | negative | positive (50 - 150) | negative |
| *Soya:* Soyflour, not toasted (Protein 37%) | negative | negative | positive (50 - 150) | positive (25 - 75) |

* Protein contents according to laboratory analysis (total nitrogen, Kjeldahl)
**Allergen contents of „food item" 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® Crustaceae** | negative | positive | positive | negative |
| **AgraStrip® Egg** | negative | positive | negative | positive |
| **AgraStrip® Casein** | positive | negative | negative | negative |
| **AgraStrip® Soy** | negative | negative | positive | positive |
| **AgraStrip® Mustard** | - | - | positive | - |

* Nachweisgrenze (NWG) jeweils 2-10 mg/kg / Limit of detection (LOD) 2-10 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 82%, 100%, 89% and 98%, 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 0,79, 0,34, 0,86 and 0,65, respectively. The results of microtracer analysis are given in the documentation.

*2.1.2 Stability*

The experience with various DLA reference materials showed good storage stability with respect to the durability of the samples (spoilage) and the content of EP-parameters (allergens) in a comparable matrix and water activity ($a_W$ value <0.5). The stability of sample material is therefore given during the investigation period under consideration of given storage conditions.

## 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 30$^{th}$ week of 2017. The testing method was optional. The tests should be finished at September 22$^{nd}$ 2017 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 Crustaceae, Egg, Fish, Milk, Molluscs, Mustard (yellow/white, brown and black) and/or Soybean in a simple carrier matrix. The evaluation of results is strictly qualitative (positive / negative).*

*The following **analysis methods** can be used:*

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

***Please note the attached information on the proficiency test.***
(see documentation, section 5.3 Information on the PT)

## 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 specificities, 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.

21 out of 22 participants submitted at least one result in time. One participant submitted the results delayed.

# 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**.
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 Crustaceae

## *4.1.1 ELISA-Results: Crustaceae (Shrimps)*

### 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 | | |
| 3 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | AQ | |
| 19 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | AQ | |
| 10 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | IL | |
| 1 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 4 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 5 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 6 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 21 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |

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

**Methods:**

AQ = AgraQuant, RomerLabs

IL = Immunolab

RS-F= Ridascreen® Fast, R-Biopharm

*Comments:*

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

### *4.1.2 PCR-Results: Crustaceae (Shrimps)*

### 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 | negative | negative | 2/4 (50%) | 2/4 (50%) | SFA-ID | no positive sample detected |
| 2 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 4 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 12 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 16 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 17 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 18 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 22 | negative | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 7 | 7 | 0 |
| Number negativee | 8 | 1 | 1 | 8 |
| Percent positive | 0 | 88 | 88 | 0 |
| Percent negativee | 100 | 13 | 13 | 100 |
| Consensus value | negative | positive | positive | negative |
| Spiking | negative | positive | positive | negative |

**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 of samples. One participant could not detect the two positive samples.*

## 4.2 Proficiency Test Egg

### *4.2.1 ELISA-Results: Egg (Whole egg powder)*

**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 | | |
| 3 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | AQ | |
| 8 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | BK | |
| 9 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | BK | |
| 20 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | BK | |
| 14 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | ES | |
| 10 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | MI | |
| 19 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | MI | |
| 1 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 4 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 5 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 6 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 7 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 17 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 21 | negative | positive | negative | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |

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

**Methods:**

AQ = AgraQuant, RomerLabs

BK = BioKits, Neogen

ES = ELISA-Systems

MI = Morinaga Institute ELISA

RS-F= Ridascreen® Fast, R-Biopharm

*Comments:*

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

### *4.2.2 PCR-Results: Egg (Whole egg powder)*

## 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 | | |
| 12 | negative | positive | negative | positive | - | 4/4 (100%) | div | |

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

**Methods:**

div = not indicated / other method

*Comments:*

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

# 4.3 Proficiency Test Fish

## *4.3.1 ELISA-Results: Fish (Cod)*

### 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 | | |
| 3 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | AQ | |
| 19 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | AQ | |
| 4 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | BC | |
| 20 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | IL | |

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

**Methods:**

AQ = AgraQuant, RomerLabs

BC = BioCheck ELISA

IL = Immunolab

*Comments:*

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

### *4.3.2 PCR-Results: Fish (Cod)*

## 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 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 2 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 4 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 12 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 16 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 18 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 5 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 10 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 15 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 17 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 21 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 22 | positive | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |

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

**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 of samples.*

# 4.4 Proficiency Test Milk

## *4.4.1 ELISA-Results: Milk, Casein, beta-Lactoglobulin*

### 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 | | |
| 3 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | AQ | Milk |
| 9 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | AQ | Milk |
| 10a | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | AQ | Casein |
| 13 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | AT | Lateral Flow |
| 10b | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | ES | β-Lactoglobulin |
| 19 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | ES | Milk |
| 10c | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | MI | Casein |
| 10d | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | MI | β-Lactoglobulin |
| 5 | positive | positive | negative | negative | 3/4 (75%) | 3/4 (75%) | RS-F | Milk |
| 6 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | RS-F | Milk |
| 11 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | RS-F | Milk |
| 17 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | RS-F | Milk |
| 20 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | RS-F | Milk |
| 21 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | RS-F | Milk |
| 7 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | VT | Milk |
| 8 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | VT | Milk |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 16 | 1 | 0 | 0 |
| Number negative | 0 | 15 | 16 | 16 |
| Percent positive | 100 | 6 | 0 | 0 |
| Percent negative | 0 | 94 | 100 | 100 |
| Consensus value | positive | negative | negative | negative |
| Spiking | positive | negative | negative | negative |

**Methods:**

AQ = AgraQuant, RomerLabs

AT = AlerTox (LFD), Biomedal

ES = ELISA-Systems

MI = Morinaga Institute 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. There was one positive result for sample 2.*

### *4.4.2 PCR-Results: Milk (Skimmed milk powder)*

*Comments:*
*PCR methods were no applied by the participants.*

# 4.5 Proficiency Test Molluscs

## *4.5.1 ELISA-Results: Molluscs (Squid)*

### 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 | | |
| 19 | negative | positive | positive | positive | 2/2 (100%) | 2/4 (50%) | ET | |
| 10 | negative | negative | negative | positive | 2/2 (100%) | 4/4 (100%) | IL | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 1 | 1 | 2 |
| Number negative | 2 | 1 | 1 | 0 |
| Percent positive | 0 | 50 | 50 | 100 |
| Percent negative | 100 | 50 | 50 | 0 |
| Consensus value | negative | - | - | positive |
| Spiking | negative | negative | negative | positive |

**Methods:**

ET = Elution Technologies ELISA Kit

IL = Immunolab

*Comments:*

*The results of participant no. 10 are in qualitative agreement with the spiking of samples. The positive results for samples 2 and 3 from participant no. 19 are possibly due to a cross-reactivity to crustaceae in these samples.*

### *4.5.2 PCR-Results: Molluscs (Squid)*

## 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 | | |
| 6 | positive | negative | negative | positive | - | 3/4 (75%) | IC | |
| 1 | negative | negative | negative | negative | - | 3/4 (75%) | SFA-ID | no positive sample detected |
| 2 | negative | negative | negative | positive | - | 4/4 (100%) | SFA-ID | |
| 12 | negative | negative | negative | positive | - | 4/4 (100%) | SFA-ID | |
| 16 | negative | negative | negative | negative | - | 3/4 (75%) | SFA-ID | no positive sample detected |
| 17 | negative | negative | negative | negative | - | 3/4 (75%) | SFA-ID | no positive sample detected |
| 18 | negative | negative | negative | positive | - | 4/4 (100%) | SFA-ID | |
| 5 | negative | negative | negative | positive | - | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 1 | 0 | 0 | 5 |
| Number negative | 7 | 8 | 8 | 3 |
| Percent positive | 13 | 0 | 0 | 63 |
| Percent negative | 88 | 100 | 100 | 38 |
| Consensus value | negative | negative | negative | none |
| Spiking | negative | negative | negative | positive |

**Methods:**

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

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

div = not indicated / other method

*Comments:*

*Five participants detected the positive sample 4 by PCR, while three participants could not detect it. Thus no consensus value of ≥75% positive results was obtained for sample 4.*

# 4.6 Proficiency Test Mustard

## 4.6.1 ELISA-Results: Mustard

### 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 | | |
| 3 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | AQ | |
| 8 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | AQ | |
| 1 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | ES | |
| 21 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | NL-E | |
| 7 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 10 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | VT | |
| 14 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | VT | |
| 19 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | VT | |

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

**Methods:**

AQ = AgraQuant, RomerLabs

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.*

### *4.6.2 PCR-Results: Mustard*

### Qualitative valuation of results

### *4.6.2.1 Mustard, in general*

| 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 | | |
| 9 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 17 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 21 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 1 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 7 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 18 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 5 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 10 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 12 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 22 | positive | positive | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 10 | 10 | 10 | 0 |
| Number negative | 0 | 0 | 0 | 10 |
| Percent positive | 100 | 100 | 100 | 0 |
| Percent negative | 0 | 0 | 0 | 100 |
| Consensus value | positive | positive | positive | negative |
| Spiking | positive | positive | positive | negative |

**Methods:**

ASU = ASU §64 Methode/method

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.*

### *4.6.2.2 Mustard, yellow (Sinapis alba)*

| 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 | | |
| 8 | positive | positive | negative | negative | 4/4 (100%) | 3/3 (100%) | ASU | |
| 9 | positive | positive | negative | negative | 4/4 (100%) | 3/3 (100%) | ASU | |
| 5 | positive | positive | negative | negative | 4/4 (100%) | 3/3 (100%) | div | |
| 16 | positive | positive | negative | negative | 4/4 (100%) | 3/3 (100%) | div | |
| 22 | positive | positive | negative | negative | 4/4 (100%) | 3/3 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 5 | 5 | 0 | 0 |
| Number negative | 0 | 0 | 5 | 5 |
| Percent positive | 100 | 100 | 0 | 0 |
| Percent negative | 0 | 0 | 100 | 100 |
| Consensus value | positive | positive | negative | negative |
| Spiking | (negative) | positive | negative | negative |

**Methods:**
ASU = ASU §64 Methode/method
div = not indicated / other method

### *4.6.2.3 Mustard, brown and black (Brassica juncea / nigra)*

| 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 | | |
| 8 | negative | negative | positive | negative | 4/4 (100%) | 3/3 (100%) | ASU | brauner und schwarzer Senf |
| 16 | negative | negative | positive | negative | 4/4 (100%) | 3/3 (100%) | div | brauner und schwarzer Senf |
| 22 | negative | negative | positive | negative | 4/4 (100%) | 3/3 (100%) | div | brauner und schwarzer Senf |

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

**Methods:**
ASU = ASU §64 Methode/method
div = not indicated / other method

*Comments (4.6.2.2 and 4.6.2.3):*

*Five participants tested for mustard species by PCR. Sinapis alba was detected in samples 1 and 2 by all of them. Only sample 2 was spiked with Sinapis alba. Possibly there are cross-reactivities [30, 31] and/or sample 1 contains parts of Sinapis alba.*

*Three participants detected Brassica species in sample 3 (containing Brassica nigra). Brassica juncea was not detected in sample 1. Possibly sample 1 contains no Brassica species.*

*Due to the results for sample 1 it was excluded from qualitative valuation with respect agreement with the spiking.*

# 4.7 Proficiency Test Soya

## *4.7.1 ELISA-Results: Soya (Soyflour)*

### 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 | | |
| 3 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | AQ | |
| 13 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | AT | Lateral Flow |
| 1 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | ES | |
| 7 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | IL | |
| 17 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | IL | |
| 10a | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | MI | |
| 19 | negative | negative | positive | negative | 3/4 (75%) | 3/4 (75%) | MI | |
| 4a | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 5 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 8 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 9 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 20 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 21 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | RS-F | |
| 4b | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | VT | |
| 10b | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | VT | |
| 14 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | VT | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positivee | 0 | 0 | 16 | 15 |
| Number negative | 16 | 16 | 0 | 1 |
| Percent positivee | 0 | 0 | 100 | 94 |
| Percent negative | 100 | 100 | 0 | 6 |
| Consensus value | negative | negative | positive | positive |
| Spiking | negative | negative | positive | positive |

**Methods:**

AQ = AgraQuant, RomerLabs

AT = AlerTox (LFD), Biomedal

ES = ELISA-Systems

IL = Immunolab

MI = Morinaga Institute 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. For sample 4 with a lower content of soya one negative result was submitted.*

### *4.7.2 PCR-Results: Soya (Soyflour)*

### 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 | | |
| 17 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | ASU | |
| 1 | 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 | |
| 7 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | SFA-ID | |
| 5 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 8 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 9 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 10 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 12 | negative | negative | positive | negative | 3/4 (75%) | 3/4 (75%) | div | |
| 16 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 22 | negative | negative | positive | positive | 4/4 (100%) | 4/4 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 0 | 11 | 10 |
| Number negative | 11 | 11 | 0 | 1 |
| Percent positive | 0 | 0 | 100 | 91 |
| Percent negative | 100 | 100 | 0 | 9 |
| Consensus value | negative | negative | positive | positive |
| Spiking | negative | negative | positive | positive |

**Methods:**

ASU = ASU §64 Methode/method

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. For sample 4 with a lower content of soya one negative result was submitted.*

# 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: Crustaceae

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 3 | 14.09.17 | negative | positive | positive | negative | 0,02 | Tropomyosin | AQ = AgraQuant, RomerLabs |
| AQ | 19 | 11.08.17 | negative | positive | positive | negative | 0,02 | tropomyosin | AQ = AgraQuant, RomerLabs |
| IL | 10 | 11.8. | negative | positive | positive | negative | 0,02 | Tropomyosin from crustacea | IL = Immunolab |
| RS-F | 1 | | negative | positive | positive | negative | 2 | Crustaceae | RS-F= Ridascreen® Fast, R-Biopharm (Second generation) |
| RS-F | 4 | 03.08.17 | negative | positive | positive | negative | 20 | Food item, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 5 | | negative | positive | positive | negative | 2 | Protein, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 6 | 30.08.17 | negative | positive | positive | negative | 2,0 mg/kg | CRUSTACEAN PROTEIN | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 21 | 10.08.17 | negative | positive | positive | negative | 2 | Food item, total | RS-F= 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. Extractionbuffer / Time / Temperature | |
| AQ | 3 | | | | |
| AQ | 19 | | | | |
| IL | 10 | CRU-E01 | Crustaceae Tropomyosin | As per kit instructions | |
| RS-F | 1 | | | | |
| RS-F | 4 | R7312 | As per instructions | | |
| RS-F | 5 | | | | |
| RS-F | 6 | R 7312 | CRUSTACEAN PROTEIN | ONE BUFFER EXTRACTION ( 60°C) | |
| RS-F | 21 | R7312 | mostly Tropomyosin | Extraction buffer/10min/60°C | |

### 5.1.2 ELISA: Egg

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 3 | 11.08.17 | negative | positive | negative | positive | 0,4 | Egg white protein | AQ = AgraQuant, RomerLabs |
| BK | 8 | 30.08.17 | negative | positive | negative | positive | 0,05 | Ovomucoid | BK |
| BK | 9 | 14.09. | negative | positive | negative | positive | 0,5 | Egg white protein | BK = BioKits, Neogen |
| BK | 20 | 03.08.17 | negative | positive | negative | positive | 0,5 | Egg white powder | BK = BioKits, Neogen |
| ES | 14 | 08.08.17 | neg | pos | neg | pos | 0,05 | Egg powder | Enhanced Egg Residue, ELISA Systems |
| MI | 10 | 10.8. | negative | positive | negative | positive | 0,31 | Whole egg powder | MI = Morinaga Institute ELISA |
| MI | 19 | 09.08.17 | negative | positive | negative | positive | 0,3 | Protein, total | MI = Morinaga Institute ELISA |
| RS-F | 1 | | negative | positive | negative | positive | 0,1 | Whole egg powder | R6402 RIDASCREEN FAST Egg Protein R-Biopharm |
| RS-F | 4 | 03.08.17 | negative | positive | negative | positive | 0,13 | Egg white protein | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 5 | | negative | positive | negative | positive | 0,1 | Protein, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 6 | 23.08.17 | negative | positive | negative | positive | 0,10 mg/kg | Whole egg powder | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 7 | 15.09.17 | negative | positive | negative | positive | | Whole egg powder | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 17 | 07.08.17 | negative | positive | negative | positive | 0,1 | Whole egg powder | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 21 | 31.07.17 | negative | positive | negative | positive | 0,1 | Whole egg powder | RS-F= 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. Extractionbuffer / Time / Temperature | |
| AQ | 3 | | | | |
| BK | 8 | 902072 | Ovomucoid | | |
| BK | 9 | 902072T | anti-Ovomucoid | | |
| BK | 20 | 902072T | Gal d1 | High salt Tris/115 min/room temperature | |
| ES | 14 | ESEGG-48 / Lot EG-G16-288 | Ovomucoid-antibody | Extraction: Room temperature PBS extraction buffer / 15 min @ 60C in shaking w aterbath / centrifugation Determination: 4 parameter curve | |
| MI | 10 | M2111 | Ovalbumin | As per kit instructions | |
| MI | 19 | | | | |
| RS-F | 1 | | | | |
| RS-F | 4 | R6402 | As per instructions | | |
| RS-F | 5 | | | | |
| RS-F | 6 | R 6402 | OVOALBUMINE, OVO-MUCOID | ONE BUFFER EXTRACTION ( 60°C) | |
| RS-F | 7 | | | As per kit instructions | |
| RS-F | 17 | R6402 | | | |
| RS-F | 21 | R6402 | Ovalbumin/Ovomucoid | Extraction buffer/10min/60°C | |

### 5.1.3 ELISA: Fish

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 3 | 17.08.17 | positive | negative | positive | negative | 4 | Protein, total | AQ = AgraQuant, RomerLabs |
| AQ | 19 | 25.08.17 | positive | negative | positive | negative | 4 | cod | AQ = AgraQuant, RomerLabs |
| BC | 4 | 03.08.17 | positive | negative | positive | negative | 5 | other: please fill in! | BC = BioCheck ELISA |
| IL | 20 | 05.09.17 | positive | negative | positive | negative | 4 | other: please fill in! | IL = Immunolab |

*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. Extractionbuffer / Time / Temperature | |
| AQ | 3 | | | | |
| AQ | 19 | | | | |
| BC | 4 | R6010 | As per instructions | | LOD = Fish (Cod) |
| IL | 20 | FIS-E01 | Fish proteins | Tris/60 min/room temperature | mg cod/kg |

### 5.1.4 ELISA: Milk

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 3 | 14.08.17 | positive | negative | negative | negative | 0,4 | Protein, total | AQ = AgraQuant, RomerLabs |
| AQ | 9 | 30.08. | positive | negative | negative | negative | 0,8 | Milk powder | AQ = AgraQuant, RomerLabs |
| AQ | 10a | 22.8. | positive | negative | negative | negative | 0,2 | Casein | AQ = AgraQuant, RomerLabs |
| AT | 13 | 13.09.17 | positive | negative | negative | negative | 0,05 | Casein | AlerTox Casein |
| ES | 10b | 23.8. | positive | negative | negative | negative | 0,1 | β-Lactoglobulin | ES = ELISA-Systems |
| ES | 19 | 17.08.17 | positive | negative | negative | negative | 1 | Skimmed milk powder | ES = ELISA-Systems |
| MI | 10c | 3.8. | positive | negative | negative | negative | 0,25 | Casein | MI = Morinaga Institute ELISA |
| MI | 10d | 10.8. | positive | negative | negative | negative | 0,03 | β-Lactoglobulin | MI = Morinaga Institute ELISA |
| RS-F | 5 | | positive | positive | negative | negative | 0,7 | Protein, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 6 | 28.08.17 | positive | negative | negative | negative | 0,19 mg/kg | BETALACTOGLOBULIN | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 11 | | positive | negative | negative | negative | 0,7 | Milk protein | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 17 | 16.08.17 | positive | negative | negative | negative | 0,7 | Protein, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 20 | 02.08.17 | positive | negative | negative | negative | 3 | other: please fill in! | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 21 | 03.08.17 | positive | negative | negative | negative | 0,7 | Milk powder | RS-F= Ridascreen® Fast, R-Biopharm |
| VT | 7 | 25.08.17 | positive | negative | negative | negative | | Skimmed milk powder | VT = Veratox, Neogen |
| VT | 8 | 04.09.17 | positive | negative | negative | negative | 2,5 | Skimmed milk powder | VT |

*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. Extractionbuffer / Time / Temperature | |
| AQ | 3 | | | | |
| AQ | 9 | COKAL1200 | anti-Casein | | |
| AQ | 10a | COKAL 1200 | Casein | As per kit instructions | Casein |
| AT | 13 | 80350 | | Water an buffer solution/15min-60°C/15min centrifuge | Lateral Flow |
| ES | 10b | ESMRDBLG | β-Lactoglobulin | As per kit instructions | β-Lactoglobulin |
| ES | 19 | | | | |
| MI | 10c | M2116 | Casein | As per kit instructions | Casein |
| MI | 10d | M2112 | β-Lactoglobulin | As per kit instructions | β-Lactoglobulin |
| RS-F | 5 | | | | |
| RS-F | 6 | R 4902 | COW, SHEEP, GOAT, BUFFALO MILK | TWO BUFFER EXTRACTION (100°C, 60°C) | |
| RS-F | 11 | R4652 | see kit instructions | exactly according to test kit instructions | Quantitative result for 1 (Mean of multiple determination): 6,2 mg/kg |
| RS-F | 17 | R4652 | | | |
| RS-F | 20 | R4612 | casein | Extractor 2+A-AEP/60min/room temperature | mg casein/kg |
| RS-F | 21 | R4652 | Casein / ß-Lactoglobulin | Extractor 2/10min/100°C/ Extraktionspuffer mit Additi-ve/10min/ 60°C | |
| VT | 7 | | | As per kit instructions | |
| VT | 8 | 8470 | | | |

### 5.1.5 ELISA: Molluscs

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 |
| ET | 19 | 25.08.17 | negative | positive | positive | positive | 10 | Protein, total | ET = Elution Technologies ELISA Kit |
| IL | 10 | 4.8. | negative | negative | negative | positive | 0,03 | Tropomyosin from Mollusks | IL = Immunolab |

*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. Extractionbuffer / Time / Temperature | |
| ET | 19 | | | | |
| IL | 10 | MOL-E01 | Mollusk Tropomyosin | As per kit instructions | |

### 5.1.6 ELISA: Mustard

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 3 | 03.08.17 | positive | positive | positive | negative | 2 | Food item, total | AQ = AgraQuant, RomerLabs |
| AQ | 8 | 21.09.17 | positive | positive | positive | negative | 0,5 | Mustard protein | AQ |
| ES | 1 | | positive | positive | positive | negative | 1 | Mustard protein | ESMUS-48 Mustard Seed Protein Residue ELISA SYSTEMS |
| NL-E | 21 | 04.08.17 | positive | positive | positive | negative | 1 | Food item, total | NL-E = nutriLinia®E Allergen-ELISA |
| RS-F | 7 | 03.08.17 | positive | positive | positive | negative | | Mustard powder | RS-F= Ridascreen® Fast, R-Biopharm |
| VT | 10 | 7.8. | positive | positive | positive | negative | 2,5 | Food item, total | VT = Veratox, Neogen |
| VT | 14 | 24.08.17 | pos | pos | pos | neg | 1 | Mustard | Veratox Allergen, Neogen |
| VT | 19 | 21.08.17 | positive | positive | positive | negative | 2,5 | mustard | VT = Veratox, 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. Extractionbuffer / Time / Temperature | |
| AQ | 3 | | | | |
| AQ | 8 | COKAL 2148 | | | |
| ES | 1 | | | | |
| NL-E | 21 | NC6007 | Mustard proteins | Extraction buffer/15min/60°C | |
| RS-F | 7 | | | As per kit instructions | |
| VT | 10 | 8400 | Mustard proteins from white/black and brown mustard | As per kit instructions | |
| VT | 14 | Product 8400 / Lot 237321 | | | not tested |
| VT | 19 | | | | |

## 5.1.7 ELISA: Soya

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 3 | 11.08.17 | negative | negative | positive | positive | 0,04 | Protein, total | AQ = AgraQuant, RomerLabs |
| AT | 13 | 23.08.17 | negative | negative | positive | positive | 0,016 | Soy protein | AlerTox Elisa Soy |
| ES | 1 | | negative | negative | positive | positive | 2,5 | Soy protein | ESSOYPRD-48 Soy Protein Residue ELISA SYSTEMS |
| IL | 7 | 20.09.17 | negative | negative | positive | positive | | Soya-Trypsin-Inhibitor | IL = Immunolab |
| IL | 17 | 15.08.17 | negative | negative | positive | positive | 0,016 | STI (soy trypsin Inhibitor | IL = Immunolab |
| MI | 10a | 3.8. | negative | negative | positive | positive | 0,31 | Protein, total | MI = Morinaga Institute ELISA |
| MI | 19 | 16.08.17 | negative | negative | positive | negative | 0,3 | Protein, total | MI = Morinaga Institute ELISA |
| RS-F | 4a | 03.08.17 | negative | negative | positive | positive | 2,5 | Food item, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 5 | | negative | negative | positive | positive | 0,24 | Protein, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 8 | 14.09.17 | negative | negative | positv | positive | 2,5 | Soyprotein | RS-F |
| RS-F | 9 | 16.08. | negative | negative | positive | positive | 2,5 | Protein, total | RS-F= Ridascreen® Fast, R-Biopharm |
| RS-F | 20 | 01.08.17 | negative | negative | positive | positive | 2,5 | Protein, total | RS = Ridascreen®, R-Biopharm |
| RS-F | 21 | 09.08.17 | negative | negative | positive | positive | 0,24 | Food item, total | RS-F= Ridascreen® Fast, R-Biopharm |
| VT | 4b | 07.08.17 | negative | negative | positive | positive | 2,5 | other: please fill in! | VT = Veratox, Neogen |
| VT | 10b | 28.8. | negative | negative | positive | positive | 2,5 | soy flour | VT = Veratox, Neogen |
| VT | 14 | 03.08.17 | neg | neg | Pos | Pos | 0,96 | soy flour | 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. Extractionbuffer / Time / Temperature | |
| AQ | 3 | | | | |
| AT | 13 | 200450 | | Water an buffer solution/15min-60°C/15min centrifuge | Lateral Flow |
| ES | 1 | | | | |
| IL | 7 | | | As per kit instructions | |
| IL | 17 | SOJ-E01 | | | |
| MI | 10a | M2117 | Beta-Conglycinin | As per kit instructions | |
| MI | 19 | | | | |
| RS-F | 4a | R7102 | As Per Instructions | | |
| RS-F | 5 | | | | |
| RS-F | 8 | R7102 | | | |
| RS-F | 9 | R7102 | anti-Soy protein | | |
| RS-F | 20 | R7102 | soya proteins | AEP+Extractor 3/55min/room temperature | |
| RS-F | 21 | R7102 | soya proteins | Extractor 3 + Extraction buffer/10min/100°C | |
| VT | 4b | 8410 | As per instructions | | LOD = Soya Flour |
| VT | 10b | 8410 | heat resistent markers of soybean | As per kit instructions | soy flour |
| VT | 14 | Product 8410 / Lot 243638 | | Extraction: 60C pre-heated PBS / 15 min @ 60C in shaking w aterbath / centrifugation<br>Determination: 4 parameter curve | |

### 5.1.8 PCR: Crustacea

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 1 | | negative | negative | negative | negative | 0,4 | Allergen DNA | SureFood® ALLERGEN Crustaceans Art.-No. S3112 Congen |
| SFA-ID | 2 | 22.09.17 | negative | positive | positive | negative | 50 | Food item, total | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 4 | 04.08.17 | negative | positive | positive | negative | 1 | Food item, total | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 12 | | negative | positive | positive | negative | 5 | food/food | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 16 | | negative | positive | positive | negative | ≤0,4≤0,4≤0,4 | | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 17 | 15.08.17 | negative | positive | positive | negative | 0,4 | Allergen DNA | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 18 | | negative | positive | positive | negative | 2 | Please select! | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| div | 22 | 16.08.17 | negative | positive | positive | negative | | | Endpunkt-PCR und Sequenzierung |

*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 | 1 | | | | |
| SFA-ID | 2 | | | In house extraction plus Qiagen Dneasy Kit. Real Time PCR. | |
| SFA-ID | 4 | S3112 | As per instructions | | |
| SFA-ID | 12 | | unknown | Extraction: NucleoSpin Food (Macherey Nagel)/Real Time PCR / 35 cycles | |
| SFA-ID | 16 | S3112 | | | |
| SFA-ID | 17 | S3112 | | | |
| SFA-ID | 18 | | | | |
| div | 22 | L12.01-3 | | | |

### 5.1.9 PCR: Egg

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 |
| div | 12 | | negative | positive | negative | positive | 0,001 | ADN/ADN | 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 | |
| div | 12 | | cytochromeb/ovalbumin/vitellogenin | Extraction: NucleoSpin Food (Macherey Nagel)/Real Time PCR / 45 cycles | |

## 5.1.10 PCR: Fish

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 1 | | positive | negative | positive | negative | 0,4 | Allergen DNA | SureFood® ALLERGEN Fish Art.-No. S3110 Congen |
| SFA-ID | 2 | 22.09.17 | positive | negative | positive | negative | 10 | Food item, total | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 4 | 04.08.17 | positive | negative | positive | negative | 1 | Food item, total | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 12 | | positive | negative | positive | negative | 5 | food/food | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 16 | | positive | negative | positive | negative | ≤0,4≤0,4 | | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 18 | | positive | negative | positive | negative | 0,8 | Please select! | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| div | 5 | | positive | negative | positive | negative | 0,008 | Allergen DNA | other: please fill in! |
| div | 10 | 3.8. | positive | negative | positive | negative | 40 | Allergen-DNA | in house method |
| div | 15 | 06.09.17 | positive | negative | positive | negative | 74,9 | Allergen-DNA | in house method |
| div | 17 | 15.08.17 | positive | negative | positive | negative | 0,4 | Allergen DNA | Housemethod |
| div | 21 | 18.08.17 | positive | negative | positive | negative | 5 | Food item, total | Sun et al.; J. AOAC Int. Vol. 92 (1), 2009 |
| div | 22 | 01.09.17 | positive | negative | positive | negative | | | End point PCR and sequencing |

*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 | 1 | | | | |
| SFA-ID | 2 | | | In house extraction plus Qiagen Dneasy Kit. Real Time PCR. | |
| SFA-ID | 4 | S3110 | As Per Instructions | | |
| SFA-ID | 12 | | unknown | Extraction: NucleoSpin Food (Macherey Nagel)/Real Time PCR / 35 cycles | |
| SFA-ID | 16 | S3110 | | | |
| SFA-ID | 18 | | | | |
| div | 5 | | | | |
| div | 10 | | | CTAB, Proteinase K, Promega Wizard DNA Ck-leanUp, Real-time PCR 45 Zyklen | |
| div | 15 | | | CTAB Extraction; Real-Time PCR (45 Cycles) | |
| div | 17 | | | | |
| div | 21 | in house method | Parvalbumin | CTAB,/Prot. K/Cleanup: DNeasy Mericon Food Kit / Real Time PCR/45 cycles | |
| div | 22 | L10.00-12 | | | |

### 5.1.11 PCR: Milk

*No PCR methods were applied by the participants.*

### 5.1.12 PCR: Molluscs

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 |
| IC | 6 | 18.09.17 | positive | negative | negative | positive | 1 COPY OF APLOID GENOME = 1,6 pg | Allergen DNA | IC = Food Allergen Detection PCR Kit, real Time PCR, InCura |
| SFA-ID | 1 | | negative | negative | negative | negative | | Allergen DNA | SureFood® ALLERGEN Molluscs Art.-No. S3113 Congen |
| SFA-ID | 2 | 22.09.17 | negative | negative | negative | positive | 50 | Food item, total | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 12 | | negative | negative | negative | positive | 5 | food/food | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 16 | | negative | negative | negative | negative | ≤0,4≤0,4 | | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 17 | 15.08.17 | negative | negative | negative | negative | 0,4 | Allergen DNA | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 18 | | negative | negative | negative | positive | 0,8 | | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| div | 5 | | negative | negative | negative | positive | 0,008 | Allergen DNA | other: please fill in! |

*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 | |
| IC | 6 | IC-02-1008 | BIVALVE 228 BP, CEPHALOPODE 150 BP, GASTEROPODE 157 BP | FOOD GRES DNA KIT INCURA IC-02-0095 | |
| SFA-ID | 1 | | | | |
| SFA-ID | 2 | | | In house extraction plus Qiagen Dneasy Kit. Real Time PCR. | |
| SFA-ID | 12 | | unknown | Extraction: NucleoSpin Food (Macherey Nagel)/Real Time PCR / 35 cycles | |
| SFA-ID | 16 | S3113 | | | |
| SFA-ID | 17 | S3113 | | | |
| SFA-ID | 18 | | | | |
| div | 5 | | | | |

## 5.1.13 PCR: Mustard, in general

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 9 | | positive | positive | positive | negative | | | ASU = ASU §64 Methode/method |
| ASU | 17 | 07.08.17 | positive | positive | positive | negative | 4 | Allergen DNA | ASU = ASU §64 Methode/method |
| ASU | 21 | 18.08.17 | positive | positive | positive | negative | 2 | Food item, total | ASU = ASU §64 Methode/method |
| SFA-ID | 1 | | positive | positive | positive | negative | 0,4 | Allergen DNA | SureFood® ALLERGEN Mustard Art.-No. S3109 Congen |
| SFA-ID | 7 | 01.08.17 | positive | positive | positive | negative | | Allergen-DNA | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 18 | | positive | positive | positive | negative | 5 | Please select! | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| div | 5 | | positive | positive | positive | negative | 0,008 | Allergen DNA | other: please fill in! |
| div | 10 | 3.8. | positive | positive | positive | negative | 1 | Allergen-DNA | in-house method |
| div | 12 | | positive | positive | positive | negative | 0,001 | ADN/ADN | in-house method |
| div | 22 | 16.08.17 | positive | positive | positive | negative | | | real-time PCR |

*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 | 9 | L 08.00 65 | | | |
| ASU | 17 | ASU L 08.00-59 | | | |
| ASU | 21 | ASU §64 LFGB L 08.00-59 | | CTAB,/Prot. K/Cleanup: DNeasy Mericon Food Kit / Real Time PCR/45 cycles | |
| SFA-ID | 1 | | | | |
| SFA-ID | 7 | | | As per kit instructions | |
| SFA-ID | 18 | | | | |
| div | 5 | | | | |
| div | 10 | | | CTAB, Proteinase K, Promega Wizard DNA CleanUp, Real-time PCR 45 Cycles | |
| div | 12 | Mustorp ycol, 2008 | sinA | Extraction: NucleoSpin Food (Macherey Nagel)/Real Time PCR / 45 cycles | |
| div | 22 | L08.00-64 | | | |

## 5.1.14 PCR: Mustard, Sinapis alba

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 8 | 13.09.17 | positive | positive | negative | negative | 5 pg | Allergen-DNA | ASU |
| ASU | 9 | | positive | positive | negative | negative | | | ASU = ASU §64 Methode/method |
| div | 5 | | positive | positive | negative | negative | 0,008 | Allergen DNA | other: please fill in! |
| div | 16 | | positive | positive | negative | negative | < 5 copies | | in house method |
| div | 22 | | positive | positive | negative | negative | | | real-time PCR |

*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 | 8 | L 08.00-59:2013-01, modified | | Mericon Food Kit (Qiagen) | |
| ASU | 9 | L 08.00 65 | | | |
| div | 5 | | | | |
| div | 16 | | | | |
| div | 22 | | | | |

## 5.1.15 PCR: Mustard, Brassica juncea / Brassica nigra

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 8 | 13.09.17 | negative | negative | positive | negative | 1 pg | Allergen-DNA | ASU |
| div | 16 | | negative | negative | positive | negative | < 5 copies | | in house method |
| div | 22 | | negative | negative | positive | negative | | | real-time PCR |

*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 | 8 | L 08.00-64:2016-10, modified | | Mericon Food Kit (Qiagen) | Detection of brown and black mustard, no differentiation possible |
| div | 16 | | | | Detection of brown and black mustard, no differentiation possible |
| div | 22 | | | | Detection of brown and black mustard, no differentiation possible |

## 5.1.16 PCR: Soya

*Primary data*

| Meth. Abr. | Evaluation number | Date of analysis | 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 | 17 | 07.08.17 | negative | negative | positive | positive | 10 | Allergen DNA | ASU = ASU §64 Methode/method |
| SFA-ID | 1 | | negative | negative | positive | positive | 0,4 | Allergen DNA | SureFood® ALLERGEN Soya Art.-No. S3101 Congen |
| SFA-ID | 4 | 03.08.17 | negative | negative | positive | positive | 1 | Food item, total | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| SFA-ID | 7 | 01.08.17 | negative | negative | positive | positive | | Allergen-DNA | SFA-ID = Sure Food Allergen ID, R-Biopharm / Congen |
| div | 5 | | negative | negative | positive | positive | 0,02 | Allergen DNA | other: please fill in! |
| div | 8 | 13.09.17 | negative | negative | positive | positive | 10 pg | Allergen-DNA | DIN EN ISO |
| div | 9 | | negative | negative | positive | positive | | | in-house method |
| div | 10 | 3.8. | negative | negative | positive | positive | 40 | Allergen-DNA | Eur F Res Tech 216 (2003) 412 ff., mod. |
| div | 12 | | negative | negative | positive | negative | 0,001 | ADN/ADN | in-house method |
| div | 16 | | negative | negative | positive | positive | < 10 copies | | in-house method |
| div | 22 | 05.09.17 | negative | negative | positive | positive | | | real-time PCR |

*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 | 17 | ASU L 08.00-59 | | | |
| SFA-ID | 1 | | | | |
| SFA-ID | 4 | S3101 | As per Instructions | | |
| SFA-ID | 7 | | | As per kit instructions | |
| div | 5 | | | | |
| div | 8 | DIN EN ISO 21570, Annex C2, August 2013, modified | | Mericon Food Kit (Qiagen) | |
| div | 9 | | | | |
| div | 10 | | | CTAB, Proteinase K, Promega Wizard DNA CleanUp, Real-time PCR 45 Cycles | |
| div | 12 | Koppel y col, 2010 | Le1 | Extraction: NucleoSpin Food (Macherey Nagel)/Real Time PCR / 45 cycles | |
| div | 16 | | Lectin | | |
| div | 22 | L00.00-105 | | | |

## 5.2 Homogeneity

### 5.2.1 Mixture homogeneity before bottling

**Microtracer Homogeneity Test**
**DLA 12-2017 Sample 1**

| | | |
|---|---|---|
| Weight whole sample | 1,00 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 40,5 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 5,02 | 91 | 36,3 |
| 2 | 5,02 | 92 | 36,7 |
| 3 | 5,05 | 109 | 43,2 |
| 4 | 5,00 | 102 | 40,8 |
| 5 | 5,02 | 90 | 35,9 |
| 6 | 5,05 | 96 | 38,0 |
| 7 | 5,00 | 106 | 42,4 |
| 8 | 5,02 | 97 | 38,6 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 97,9 | Particles |
| Standard deviation | 7,11 | Particles |
| $\chi^2$ (CHI-Quadrat) | 3,62 | |
| **Probability** | **82** | % |
| Recovery rate | 96 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 39,0 | mg/kg |
| Standard deviation | 2,83 | mg/kg |
| rel. Standard deviaton | 7,27 | % |
| Horwitz standard deviation | 9,22 | % |
| **HorRat-value** | **0,79** | |
| Recovery rate | 96 | % |

**Microtracer Homogeneity Test**
**DLA 12-2017 Sample 2**

| | | |
|---|---|---|
| Weight whole sample | 1,00 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 34,7 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 5,06 | 85 | 33,6 |
| 2 | 5,03 | 84 | 33,4 |
| 3 | 5,10 | 91 | 35,7 |
| 4 | 5,02 | 86 | 34,3 |
| 5 | 5,09 | 89 | 35,0 |
| 6 | 5,02 | 82 | 32,7 |
| 7 | 5,00 | 85 | 34,0 |
| 8 | 5,02 | 88 | 35,1 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 86,2 | Particles |
| Standard deviation | 2,78 | Particles |
| $\chi^2$ (CHI-Quadrat) | 0,63 | |
| **Probability** | **100** | % |
| Recovery rate | 99 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 34,2 | mg/kg |
| Standard deviation | 1,00 | mg/kg |
| rel. Standard deviaton | 2,91 | % |
| Horwitz standard deviation | 9,39 | % |
| **HorRat-value** | **0,31** | |
| Recovery rate | 99 | % |

**Microtracer Homogeneity Test**
**DLA 12-2017 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 | 22,7 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 4,97 | 53 | 21,3 |
| 2 | 5,05 | 64 | 25,3 |
| 3 | 4,97 | 52 | 20,9 |
| 4 | 4,99 | 60 | 24,0 |
| 5 | 5,04 | 61 | 24,2 |
| 6 | 5,01 | 64 | 25,5 |
| 7 | 5,03 | 52 | 20,7 |
| 8 | 4,97 | 56 | 22,5 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 57,7 | Particles |
| Standard deviation | 4,93 | Particles |
| $\chi^2$ (CHI-Quadrat) | 2,95 | |
| **Probability** | **89** | % |
| Recovery rate | 102 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 23,1 | mg/kg |
| Standard deviation | 1,97 | mg/kg |
| rel. Standard deviaton | 8,55 | % |
| Horwitz standard deviation | 9,98 | % |
| **HorRat-value** | **0,86** | |
| Recovery rate | 102 | % |

**Microtracer Homogenitätstest**
**DLA 12-2017 Sample 4**

| | | |
|---|---|---|
| Weight whole sample | 1,00 | kg |
| Microtracer | FSS-rot lake | |
| Particle size | 75 – 300 | µm |
| Weight per particle | 2,0 | µg |
| Addition of tracer | 23,1 | mg/kg |

**Result of analysis**

| Sample | Weight [g] | Particle number | Particles [mg/kg] |
|---|---|---|---|
| 1 | 5,00 | 53 | 21,2 |
| 2 | 5,02 | 54 | 21,5 |
| 3 | 5,00 | 51 | 20,4 |
| 4 | 4,97 | 54 | 21,7 |
| 5 | 5,00 | 45 | 18,0 |
| 6 | 5,02 | 50 | 19,9 |
| 7 | 5,02 | 56 | 22,3 |
| 8 | 5,12 | 51 | 19,9 |

| **Poisson distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Degree of freedom | 7 | |
| Mean | 51,8 | Particles |
| Standard deviation | 3,44 | Particles |
| $\chi^2$ (CHI-Quadrat) | 1,60 | |
| **Probability** | **98** | % |
| Recovery rate | 89 | % |

| **Normal distribution** | | |
|---|---|---|
| Number of samples | 8 | |
| Mean | 20,6 | mg/kg |
| Standard deviation | 1,37 | mg/kg |
| rel. Standard deviaton | 6,64 | % |
| Horwitz standard deviation | 10,1 | % |
| **HorRat-value** | **0,65** | |
| Recovery rate | 89 | % |

## 5.3 Information on the Proficiency Test (PT)

Before the PT the participants received the following information in the sample cover letter:

| | |
|---|---|
| *PT number* | ***DLA12-2017*** |
| *PT name* | ***Allergen-Screening II - 4 Samples qualitative: Crustaceae, Egg, Fish, Milk, Molluscs, Mustard*** *(yellow/white, brown and black)****, Soybean*** |
| *Sample matrix* | *Samples 1-4:*<br>*Carrier matrix / ingredients: potato powder (appr. 75%), maltodextrin (appr. 25%), other food additives and allergenic foods* |
| *Number of samples and sample amount* | *4 different Samples 1-4: 20 g each* |
| *Storage* | *Samples A + B: room temperature (long term cooled 2 - 10°C)* |
| *Intentional use* | *Laboratory use only (quality control samples)* |
| *Parameter* | *Qualitative:*<br>***Crustaceae, Egg, Fish, Milk, Molluscs, Mustard*** *(yellow/white, brown and black)****, Soybean***<br>*Samples 1-4: appr. 25 - 250 mg/kg* |
| *Methods of analysis* | *The analytical methods ELISA (+ Lateral Flow) and PCR can be applied for qualitative determinations.* |
| *Notes to analysis* | *The analysis of PT samples should be performed like a routine laboratory analysis.*<br>*In general we recommend to homogenize a representative sample amount before analysis according to good laboratory practice, especially in case of low sample weights.* |
| *Result sheet* | *One result each should be determined for Samples 1-4.*<br>*The results should be filled in the result submission file.* |
| *Units* | *posititve / negative (limit of detection mg/kg)* |
| *Number of digits* | *at least 2* |
| *Result submission* | *The result submission file should be sent by e-mail to:*<br>***pt@dla-lvu.de*** |
| *Deadline* | ***the latest 22th September 2017*** |
| *Evaluation report* | *The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.* |
| *Coordinator and contact person of PT* | *Matthias Besler, PhD* |

* Control of mixture homogeneity and qualitative testings are carried out by DLA. Testing of the content, homogeneity and stability of PT parameters is subcontracted by DLA.

# 6. Index of participant laboratories

| Teilnehmer / Participant | Ort / Town | Land / Country |
|---|---|---|
| | | SPAIN |
| | | SPAIN |
| | | CANADA |
| | | ITALY |
| | | Germany |
| | | SPAIN |
| | | Germany |
| | | ITALY |
| | | Germany |
| | | Germany |
| | | Germany |
| | | Germany |
| | | ITALY |
| | | Germany |
| | | Germany |
| | | Germany |
| | | Germany |
| | | GREAT BRITAIN |
| | | FRANCE |
| | | GREAT BRITAIN |
| | | GREAT BRITAIN |
| | | 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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2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
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19. DIN EN ISO 15842:2010 Lebensmittel – Nachweis von Lebensmittelallergenen – Allgemeine Betrachtungen und Validierung von Verfahren / Foodstuffs - Detection of food allergens - General considerations and validation of

methods
20. Ministry of Health and Welfare, JSM, Japan 2006
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