# Evaluation Report

proficiency test

**DLA ptAUS5 (2021)**

## Crustaceae-Screening :

## Black Tiger prawn *(Penaeus monodon)*, King prawn *(Pandalus borealis)*, Crab *(Cancer pagurus)* and Scampi *(Nephrops norvegicus)*

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*Coordinator of this PT:*
*Alexandra Scharf MSc.*

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

| | |
|---|---|
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| *EP-Nummer*<br>*PT-Number* | DLA ptAUS5 (2021) |
| *EP-Koordinator*<br>*PT-Coordinator* | Alexandra Scharf MSc. |
| *Status des EP-Bericht*<br>*Status of PT-Report* | Abschlussbericht / Final report (2 March 2022)<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-Scharf (Technischer Leiter / Technical Manager)<br>*- gezeichnet / signed M. Besler-Scharf*<br>Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager)<br>*- gezeichnet / signed A. Scharf*<br>Datum / Date: 2 March 2022 |
| *Unteraufträge*<br>*Subcontractors* | Im Rahmen dieser Eignungsprüfung nachstehende Leistungen im Unterauftrag vergeben: Proteinbestimmung<br>As part of the present proficency test the following services were subcontracted: protein determination |
| *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. |

# Contents

# 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 different PT samples with possible contents of freeze-dried animal foods from Black Tiger prawn *(Penaeus monodon)*, King prawn *(Pandalus borealis)*, Crab *(Cancer pagurus) and* Scampi *(Nephrops norvegicus)* were provided for qualitative determination. The lyophilisates were present in mixtures with maltodextrin with contents of 17-20%.

The respective commodities for the crustaceans used were commercially available crustaceans (whole crustaceans or crab claws, raw). The crustaceans were stored at -20°C. They were then manually minced and lyophilized at -50°C for 78 hours. The amounts of water losses were complemented to 100% by adding maltodextrin according to the literature values (nutritional value tables, Souci, Fachmann, Kraut, 1991) (see Tab. 1). These mixtures were ground and then sieved (mesh 800 µm). The corresponding crustacean species in samples 1-4 are shown in Table 2.

After homogenization, the samples were filled into portions of approximately 15 g in metallized PET film bags.

Table 1: Composition of the DLA samples.

| **Ingredients** | **Samples 1 - 4** |
|---|---|
| Maltodextrin | 80 - 83 % |
| Crustacean contents (dry weight) | 17 – 20 % |

Tabelle 2: Crustacean species in samples 1-4.

| Ingredients | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| **Scampi** *(Nephrops norvegicus) (protein 16,9%)* | positive | negative | negative | negative |
| **King prawn** *(Pandalus borealis) (protein 16,0%)* | negative | positive | negative | negative |
| **Black Tiger prawn** *(Penaeus monodon) (protein 15,7%)* | negative | negative | positive | negative |
| **Crab** *(Cancer pagurus) (protein 14,1%)* | negative | negative | negative | positive |

* Protein contents of the PT samples (including maltodextrin) according to laboratory analysis (total nitrogen according to Kjeldahl with general factor F=6.25).

***Note:*** *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

### *2.1.1 Stability*

A water activity ($a_W$) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the $a_W$ value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity ($a_W$ value <0,5).
The $a_W$ value of the PT samples were about 0,31 (19,6°C) The stability of the sample material was thus ensured during the investigation period under the specified 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 47th week of 2021. The testing method was optional. The tests should be finished at January 21th 2022 the latest.

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

*There are 4 different samples each containing one of the following fish species:* ***Black Tiger prawn (Penaeus monodon), King prawn (Pandalus borealis), Crab (Cancer pagurus) or Scampi (Nephrops norvegicus).*** *The evaluation of results is* ***strictly qualitative (positive / negative).***

***Note:*** *Samples should be stored refrigerated (2-10 °C) upon arrival.*

*Please note the attached information on the proficiency test.*
(see documentation, section 5.2 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.

9 of 10 participants submitted at least one result. One participant did not submit any results.

# 3. Evaluation

Different protein-based methods (e.g. isoelectric focusing, ELISA) and DNA-based methods for the determination of fish species in foods are eventually using different pH-gradients, 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. Furthermore, matrix and/or processing can strongly influence the detectability of fish species, especially when protein-based methods are used [19].

## 3.1 Agreement with consensus values from participants

The qualitative evaluation of the protein and DNA-based 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 if ≥ 75% positive or negative results are available 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 protein and DNA-based 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.

No protein-based results were submitted, therefore only qualitative evaluation for each parameter was performed for DNA-based methods, such as PCR and sequencing.

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 Black Tiger prawn (*Penaeus monodon*)

### Qualitative valuation of the DNA-based 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 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 7 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 4 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | NGS | |
| 9 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | NGS | |
| 1 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 2 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 3 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 5 | negative | negative | positive | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 8 | - | - | positive | - | 1/1 (100%) | 1/1 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 0 | 9 | 0 |
| Number negative | 8 | 8 | 0 | 8 |
| Percent positive | 0 | 0 | 100 | 0 |
| Percent negative | 100 | 100 | 0 | 100 |
| Consensus value | negative | negative | positive | negative |
| Spiking | negative | negative | positive | negative |

**Methods:**

ASU = ASU §64 Methode/method

NGS = Next-Generation Sequencing

div = keine genaue Angabe / andere Methode

div = not indicated / other method

*Comments:*

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

## 4.2 Proficiency Test King prawn (*Pandalus borealis*)

### Qualitative valuation of the DNA-based 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 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 7 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 4 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | NGS | |
| 9 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | NGS | |
| 1 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 2 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 3 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 5 | negative | positive | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 8 | - | positive | - | - | 1/1 (100%) | 1/1 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 9 | 0 | 0 |
| Number negative | 8 | 0 | 8 | 8 |
| Percent positive | 0 | 100 | 0 | 0 |
| Percent negative | 100 | 0 | 100 | 100 |
| Consensus value | negative | positive | negative | negative |
| Spiking | negative | positive | negative | negative |

**Methods:**

ASU = ASU §64 Methode/method

NGS = Next-Generation Sequencing

div = keine genaue Angabe / andere Methode

div = not indicated / other method

*Comments:*

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

## 4.3 Proficiency Test Scampi *(Nephrops norvegicus)*

### Qualitative valuation of the DNA-based 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 | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 7 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | ASU | |
| 4 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | NGS | |
| 9 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | NGS | |
| 1 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 2 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 3 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 5 | positive | negative | negative | negative | 4/4 (100%) | 4/4 (100%) | div | |
| 8 | positive | - | - | - | 1/1 (100%) | 1/1 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 9 | 0 | 0 | 0 |
| Number negative | 0 | 8 | 8 | 8 |
| Percent positive | 100 | 0 | 0 | 0 |
| Percent negative | 0 | 100 | 100 | 100 |
| Consensus value | positive | negative | negative | negative |
| Spiking | positive | negative | negative | negative |

**Methods:**

ASU = ASU §64 Methode/method

NGS = Next-Generation Sequencing

div = keine genaue Angabe / andere Methode

div = not indicated / other method

*Comments:*

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

## 4.4 Proficiency Test Crab *(Cancer pagurus)*

### Qualitative valuation of the DNA-based 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 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | ASU | |
| 4 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | NGS | |
| 7 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | NGS | NGS 3. Generation (Oxford Nanopore) |
| 9 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | NGS | |
| 1 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 2 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 3 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 5 | negative | negative | negative | positive | 4/4 (100%) | 4/4 (100%) | div | |
| 8 | - | - | - | positive | 1/1 (100%) | 1/1 (100%) | div | |

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Number positive | 0 | 0 | 0 | 9 |
| Number negative | 8 | 8 | 8 | 0 |
| Percent positive | 0 | 0 | 0 | 100 |
| Percent negative | 100 | 100 | 100 | 0 |
| Consensus value | negative | negative | negative | positive |
| Spiking | negative | negative | negative | positive |

**Methods:**

ASU = ASU §64 Methode/method

NGS = Next-Generation Sequencing

div = keine genaue Angabe / andere Methode

div = not indicated / other method

*Comments:*

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

# 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 DNA-based Methods: Black Tiger prawn (Penaeus monodon)*

*Primary data*

| Meth. Abbr. | 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 | % | e.g. food/ protein | Test-Kit + Manufacturer |
| ASU | 6 | | negative | negative | positive | negative | | DNA | rbiopharm |
| ASU | 7 | 16.12.21 | negative | negative | positive | negative | | | BigDye Terminator v1.1 Cycle Sequencing Kit, AppliedBiosystems |
| NGS | 4 | 23.12.21 | negative | negative | positive | negative | | DNA | NGS |
| NGS | 9 | | negative | negative | positive | negative | | DNA | NGS - In house |
| div | 1 | 18.01. | negative | negative | positive | negative | 5 | DNA | Cytochrome oxidase, literature method |
| div | 2 | 09.11.21 | negative | negative | positive | negative | | DNA | Home made PCR + sequencing |
| div | 3 | 07.01.22 | negative | negative | positive | negative | | DNA | in-house method sequencing |
| div | 5 | 29.12.21 | negative | negative | positive | negative | 3 | copy number | real-time PCR |
| div | 8 | | - | - | positive | - | | | house method crustacean sequencing |

*Other details to the Methods*

| Meth. Abbr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No./ASU-No. | Target-Sequence / -DNA | e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles | |
| ASU | 6 | ASU L 12.01-3, Juli 2012 | 16S rRNA | DNA-Extraction via rbiopharm Kit SureFood® PREP Basic Art. Nr. S1052 / Gelelektrophoresis / PCR w ith 35 Cycles | |
| ASU | 7 | 4337450 | | DNeasy Mericon Food Kit, Qiagen; 16s und COI PCR | Sanger sequencing; Official collection of test methods according to §64 LFGB L 12.01-3 July 2012 and Geller, J., Meyer, C., Parker, M. and H.Haw k (2013): Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources; DOI:10.1111/1755-0998.12138 |
| NGS | 4 | | Animalia Kingdom | | |
| NGS | 9 | | | | |
| div | 1 | | | CTAB (1 hr, 60°C) and CTAB-Präzipitation+Chloroformextr. + precipitation | |
| div | 2 | | 16S | Maxw ell RSC Pure Food GMO and Authentification Kit – Produits de la mer + PCR + sequencing | |
| div | 3 | DL8836 | spec. sequence for crustaceans | SureFood® Prep Basic | K01 |
| div | 5 | house method | 16S-rRNA | CTAB-Extraction, magnetic-bead Clean-Up, real-time PCR | |
| div | 8 | | | | |

## *5.1.2 DNA-based Methods: King prawn (Pandalus borealis)*

*Primary data*

| Meth. Abbr. | 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 | % | e.g. food/ protein | Test-Kit + Manufacturer |
| ASU | 6 | | negative | positive | negative | negative | | DNA | rbiopharm |
| ASU | 7 | 16.12.21 | negative | positive | negative | negative | | | BigDye Terminator v1.1 Cycle Sequencing Kit, AppliedBiosystems |
| NGS | 4 | 23.12.21 | negative | positive | negative | negative | | DNA | NGS |
| NGS | 9 | | negative | positive | negative | negative | | DNA | NGS - In house |
| div | 1 | 18.01. | negative | positive | negative | negative | 5 | DNA | Cytochrome oxidase, literature method |
| div | 2 | 09.11.21 | negative | positive | negative | negative | | DNA | Home made PCR + sequencing |
| div | 3 | 07.01.22 | negative | positive | negative | negative | | DNA | in-house method sequencing |
| div | 5 | 11.01.22 | negative | positive | negative | negative | 3,3 | copy number | real-time PCR |
| div | 8 | | - | positive | - | - | | | Hausmethode Krustentiersequenzierung |

*Other details to the Methods*

| Meth. Abbr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No./ASU-No. | Target-Sequence / -DNA | e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles | |
| ASU | 6 | ASU L 12.01-3, Juli 2012 | 16S rRNA | DNA-Extraction via rbiopharm Kit SureFood® PREP Basic Art. Nr. S1052 / Gelelektrophoresis / PCR w ith 35 Cycles | |
| ASU | 7 | 4337450 | | DNeasy Mericon Food Kit, Qiagen; 16s und COI PCR | Sanger sequencing; Official collection of test methods according to §64 LFGB L 12.01-3 July 2012 and Geller, J., Meyer, C., Parker, M. and H.Haw k (2013): Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources; DOI:10.1111/1755-0998.12139 |
| NGS | 4 | | Animalia Kingdom | | |
| NGS | 9 | | | | |
| div | 1 | | | CTAB (1 hr, 60°C) and CTAB-Präzipitation+Chloroformextr. + precipitation | |
| div | 2 | | 16S | Maxw ell RSC Pure Food GMO and Authentification Kit – Produits de la mer + PCR + sequencing | |
| div | 3 | DL8836 | spec. sequence for crustaceans | SureFood® Prep Basic | K01 |
| div | 5 | house method | 16S-rRNA | CTAB-Extraction, magnetic-bead Clean-Up, real-time PCR | |
| div | 8 | | | | |

### *5.1.3 DNA-based Methods: Scampi (Nephrops norvegicus)*

*Primary data*

| Meth. Abbr. | 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 | % | e.g. food/ protein | Test-Kit + Manufacturer |
| ASU | 6 | | positive | negative | negative | negative | | DNA | rbiopharm |
| ASU | 7 | 16.12.21 | positive | negative | negative | negative | | | BigDye Terminator v1.1 Cycle Sequencing Kit, AppliedBiosystems |
| NGS | 4 | 23.12.21 | positive | negative | negative | negative | | DNA | NGS |
| NGS | 9 | | positive | negative | negative | negative | | DNA | NGS - In house |
| div | 1 | 18.01. | positive | negative | negative | negative | 5 | DNA | Cytochrome oxidase, literature method |
| div | 2 | 09.11.21 | positive | negative | negative | negative | | DNA | Home made PCR + sequencing |
| div | 3 | 07.01.22 | positive | negative | negative | negative | | DNA | in-house Methode Sequenzierung |
| div | 5 | 29.12.21 | positive | negative | negative | negative | 2,5 | copy number | real-time PCR |
| div | 8 | | positive | - | - | - | | | house method crustacean sequencing |

*Other details to the Methods*

| Meth. Abbr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No./ASU-No. | Target-Sequence / -DNA | e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles | |
| ASU | 6 | ASU L 12.01-3, Juli 2012 | 16S rRNA | DNA extraction via rbiopharm Kit SureFood® PREP Basic Art. No. S1052 / Gel electrophoresis / PCR with 35 cycles | |
| ASU | 7 | 4337450 | | DNeasy Mericon Food Kit, Qiagen; 16s und COI PCR | Sanger sequencing; Official collection of test methods according to §64 LFGB L 12.01-3 July 2012 and Geller, J., Meyer, C., Parker, M. and H.Hawk (2013): Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources; DOI:10.1111/1755-0998.12139 |
| NGS | 4 | | Animalia Kingdom | | |
| NGS | 9 | | | | |
| div | 1 | | | CTAB (1 hr, 60°C) and CTAB precipitation+chloroform ext. +precipitation | |
| div | 2 | | 16S | idem | |
| div | 3 | DL8836 | spec. sequence for crustaceans | SureFood® Prep Basic | K01 |
| div | 5 | Hausmethode | 16S-rRNA | CTAB-Extraction, magnetic-bead Clean-Up, real-time PCR | |
| div | 8 | | | | |

### *5.1.4 DNA-based Methods: Crab (Cancer pagurus)*

*Primary data*

| Meth. Abbr. | 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 | % | e.g. food/ protein | Test-Kit + Manufacturer |
| ASU | 6 | | negative | negative | negative | positive | | DNA | rbiopharm |
| NGS | 4 | 23.12.21 | negative | negative | negative | positive | | DNA | NGS |
| NGS | 7 | 07.01.22 | negative | negative | negative | positive | | | Ligation Sequencing Kit + Min ION (Oxford Nanopore Technologies) |
| NGS | 9 | | negative | negative | negative | positive | | DNA | NGS - In house |
| div | 1 | 18.01. | negative | negative | negative | positive | 5 | DNA | Cytochrome oxidase, literature method |
| div | 2 | 09.11.21 | negative | negative | negative | positive | | DNA | Home made PCR + sequencing |
| div | 3 | 07.01.22 | negative | negative | negative | positive | | DNA | in-house method sequencing |
| div | 5 | 11.01.22 | negative | negative | negative | positive | 2,7 | copy number | real-time PCR |
| div | 8 | | - | - | - | positive | | | House method crustacean sequencing |

*Other details to the Methods*

| Meth. Abbr. | Evaluation number | Method-No. / Test-Kit No. | Specifity | Remarks to the Method (Extraction and Determination) | Further Remarks |
|---|---|---|---|---|---|
| | | Article-No./ASU-No. | Target-Sequence / -DNA | e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles | |
| ASU | 6 | ASU L 12.01-3, Juli 2012 | 16S rRNA | DNA extraction via rbiopharm Kit SureFood® PREP Basic Art. No. S1052 / Gel electrophoresis / PCR w ith 35 cycles | |
| NGS | 4 | | Animalia Kingdom | | |
| NGS | 7 | SQK-LSK109 | | DNeasy Mericon Food Kit, Qiagen; 16s and COI PCR | NGS 3. Generation (Oxford Nanopore) |
| NGS | 9 | | | | |
| div | 1 | | | CTAB (1 hr, 60°C) and CTAB precipitation+chloroform ext. +precipitation | |
| div | 2 | | 16S | idem | |
| div | 3 | DL8836 | spec. Sequence for crustacean | SureFood® Prep Basic | K01 |
| div | 5 | house method | 16S-rRNA | CTAB-Extraction, magnetic-bead Clean-Up, real-time PCR | |
| div | 8 | | | | |

## 5.2 Information on the Proficiency Test (PT)

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

| | |
|---|---|
| *PT number* | ***DLA ptAUS5 (2021)*** |
| *PT name* | ***Crustaceae-Screening – 4 Samples qualitative: Black Tiger prawn (Penaeus monodon), King prawn (Pandalus borealis), Crab (Cancer pagurus), Scampi (Nephros novegicus) in Crustacean Product (freeze-dried Mixtures, one Species per Sample)*** |
| *Sample matrix* | *Samples 1-4:*<br>*Crustacean powder/ ingredients: freeze-dried crustacea, maltodextrin (amount of crustacean corresponds to 100% fresh crustacea)* |
| *Number of samples and sample amount* | *4 different Samples 1-4: 15 g each* |
| *Storage* | *Samples 1-4: cooled 2 - 10°C* |
| *Intentional use* | *Laboratory use only (quality control samples)* |
| *Parameter* | *Qualitative: Black Tiger prawn (Penaeus monodon), King prawn (Pandalus borealis), Crab (Cancer pagurus), Scampi (Nephros novegicus)*<br>*Samples 1-4: one species per sample* |
| *Methods of analysis* | *The analytical methods are optional* |
| *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* | *posititv / negativ (limit of detection %)* |
| *Number of digits* | *at least 2* |
| *Result submission* | *The result submission file should be sent by e-mail to:*<br>***pt@dla-lvu.de*** |
| *Last Deadline* | ***the latest January 21st 2022.*** |
| *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* | *Alexandra Scharf M.Sc.* |

** Control of mixture homogeneity and qualitative testings are carried out by DLA. Any 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 |
|---|---|---|
| | | Germany |
| | | USA |
| | | Germany |
| | | Germany |
| | | GREAT BRITAIN |
| | | Germany |
| | | Germany |
| | | SWITZERLAND |
| | | Germany |
| | | PORTUGAL |

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

# 7. Index of references


1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methoden-validierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and preci-sion) of measurement methods and results
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19. Lebensmittelchemische Gesellschaft [LChG der GDCh] „Stellungnahme der AG zu: Methoden zur Differenzierung von Tierarten in Lebensmitteln – Status quo / "Statement of the WG on: Methods for the Differentiation of Animal Species in Foods - Status quo, (2016), Food Chemistry Society of the GDCh