Evaluation Report

proficiency test

DLA ptAUS2 (2020)

Animal Species-Screening II:

Donkey, Beef, Horse, Poultry (Chicken, Goose and Turkey) in Meat Product (Pork, Sausage Meat)

DLA - Proficiency Tests GmbH
Kalte Weide 21
24641 Sievershütten/Germany

proficiency-testing@dla-lvu.de *www.dla-lvu.de*

Coordinator of this PT:
Alexandra Scharf MSc.

1st Correction 14/12/2020:
A transfer error occurred in the table "DNA-based results horse" (section 4.2.2, p. 16) For participant 7 the qualitative evaluation was incorrectly given. The table was corrected accordingly.

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Kalte Weide 21, 24641 Sievershütten, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA ptAUS2 (2020)
EP-Koordinator *PT-Coordinator*	Alexandra Scharf MSc.
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (14. Dezember 2020) 1. Korrektur / 1st Correction Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 14. Dezember 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Homogenitätsprüfung der EP-Parameter, Proteinbestimmung As part of the present proficency test the following services were subcontracted: Homogeneity tests of PT-parameter(s), protein determination
Vertraulichkeit *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. 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 heated animal foods of donkey, beef, horse, chicken, goose and turkey were provided for qualitative determination. The parameters were present in the matrix of heated meat product (basis pork) with contents of 2 – 6%.

The respective raw materials for the animal species used were commercially available meat products. The corresponding amounts of meat species for the respective sample (see Table 2) have been minced.
By using a meat cutter and adding further ingredients (see Table 1), a sausage meat was produced. After homogenization, the sausage meat was filled into portions of approx. 25 g in plastic containers and then heated for one hour at 100 °C in a water bath.

Table 1: Composition of DLA-Samples

Ingredients	**Samples 1 - 4**
Water	26 - 29 %
Sodium chloride	0,26 – 0,41 %
Sodium citrate*2H_2O	0,16 – 0,31 %
Pork gelatine (100% pork)*	3,4 – 3,8 %
Total meat content	68 - 71 %

Table 2: Contents (in %) of the respective animal species in the sausage meat samples 1-4.

Ingredients*	Sample 1	Sample 2	Sample 3.1	Sample 4
Pork meat	positive (62%)	positive (64%)	positive (65%)	positive (57%)
Chicken meat	negative	positive (5,3%)	negative	positive (2,5%)
Turkey meat	positive (5,0%)	negative	negative	negative
Goose meat	negative	negative	negative	positive (5,1%)
Horse meat	negative	negative	positive**	positive (3,4%)
Donkey meat (dried)	negative	negative	positive (6,5%***)	negative
Beef meat	positive (4,0%)	negative	negative	negative

*Animal species contents of „food item" as indicated in the column of ingredients (with the exception of donkey meat s.**)according gravimetric mixing
** Horse meat detectable (see table 3), no spiking
*** The content of 6.5% donkey meat is indicated as fresh meat and has been calculated on the basis of a dry weight of 27.7% [20].
Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

All 4 samples were analyzed with the *LCD-Array Kit MEAT 5.0* (Chipron GmbH) (see table 3). Since this test does not differentiate between horse and donkey, a joint classification as *equine* was made.
To distinguish between *horse and donkey*, samples 3.1 and 4 were additionally analysed with the LCD Array Kit 4.0 (Chipron GmbH).

Table 3: Verification of detectability of the contained animal species by LCD-Array Kit MEAT 5.0 bzw. 4.0 (Chipron GmbH)

chipron	LCD-Array Kit MEAT 5.0				LCD-Array Kit MEAT 4.0	
	Probe 1	Probe 2	Probe 3.1	Probe 4	Probe 3.1	Probe 4
Schwein / Pork	positive	positive	positive	positive	positive	positive
Huhn / Chicken	negative	positive	negative	positive	negative	positive
Pute / Turkey	positive	negative	negative	negative	negative	negative
Gans / Goose	negative	negative	negative	positive	negative	positive
Equiden / Equine	negative	negative	positive	positive	-	-
Pferd / Horse	-	-	-	-	positive	positive
Esel / Donkey	-	-	-	-	positive	negative
Rind / Cattle	positive	negative	negative	negative*	negative	positive*

*LCD-Array Kit MEAT 5.0 Limit of detection for Cattle: 0.5% (w/w)
LCD-Array Kit MEAT 4.0 Limit of detection for Cattle: < 0.1% (w/w)

The results are in agreement with the spiking/ experimental evidence of LVU samples 1-4. In sample 4, traces of cattle (< 0,5% w / w) were detected.

2.1.1 Homogeneity

The homogeneity of the bottled DLA samples was determined by a 5-fold titration of chloride according to MOHR. The repeatability standard deviation for the tested samples 1-3 is less than 5 % and thus within an acceptable range.

2.1.2 Stability

The sample material is sausage meat, which has been heated to 100°C for 1 h after production and bottling. The storage stability or shelf life of the samples (microbial spoilage) is thus guaranteed during the examination 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 27th week of 2020. In the 29th week, sample 3.1 was sent as a replacement for sample 3. The testing method was optional. The tests should be finished at September 11th 2020 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 heated animal products (Donkey, Beef, Horse, Poultry (Chicken, Goose and Turkey)). The parameters are present in the matrix heated meat product (pork base) with contents of 1-10%. The evaluation of results is ***strictly qualitative (positive / negative).***

Note: *Samples should be stored refrigerated (2-10 °C) upon arrival. Before analysis, the entire sample quantity should be homogenized, since components such as fat can separate during the production/processing of the samples.*

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.

All 17 participants submitted at least one result in time.

3. Evaluation

Different protein and DNA-based methods for the determination of animal species 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. Furthermore, matrix and/or processing as well as the type of meat component used (musculature or internal organs such as liver) can strongly influence the detectability of animal species, especially by the use of ELISA methods [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.

The qualitative evaluation is carried out for each parameter for protein and DNA-based methods separately.

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 Poultry meat

4.1.1 Protein-based Results: Poultry (in general)

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		
10	positive	positive	negative	positive	3/3 (100%)	4/4 (100%)	ETM	
1	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	ETM3	
4	positive	positive	negative	positive	3/3 (100%)	4/4 (100%)	ETM3	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	3	3	0	2
Number negative	0	0	3	1
Percent positive	100	100	0	67
Percent negative	0	0	100	33
Consensus value	positive	positive	negative	none
Spiking	positive	positive	negative	positive

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

ETM3= ELISA-TEK™ Cooked Meat 3 Species Kit: beef, pork, poultry

Comments:

The consensus values of the results for samples 1, 2 and 3 are in qualitative agreement with the spiking of the samples.
Inconsistent results were obtained for the sample 4, spiked with chicken and goose, and therefore no consensus value ≥75% could be established.

4.1.2 DNA-based Results: Poultry (in general)

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	2/2 (100%)	2/4 (50%)	CP	
5	positive	positive	negative	positive	2/2 (100%)	4/4 (100%)	CP	positive if goose, chicken or/and turkey is detected
13	positive	positive	negative	positive	2/2 (100%)	4/4 (100%)	GS	
12	positive	positive	negative	positive	2/2 (100%)	4/4 (100%)	NGS	
17	positive	positive	negative	positive	2/2 (100%)	4/4 (100%)	NGS	
6	positive	positive	positive	negative	2/2 (100%)	2/4 (50%)	SFA-ID	
16	positive	positive	negative	positive	2/2 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	7	7	2	5
Number negative	0	0	5	2
Percent positive	100	100	29	71
Percent negative	0	0	71	29
Consensus value	positive	positive	none	none
Spiking	positive	positive	negative	positive

Methods:

CP = Chipron LCD Array Kit MEAT 5.0

GS = Eurofins Genescan DNAnimal Ident

NGS = Next-Generation Sequencing

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the results for samples 1 and 2 are in qualitative agreement with the spiking of the samples.
For the unspiked samples 3 and sample 4 (addition of goose) inconsistent results were obtained, thus no consensus value ≥75% could be established.

Participant 3 obtained a positive result for sample 3 and a negative result for sample 4, which may be due to an interchanging of the two samples.

4.1.3 DNA-based Results: Chicken

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	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
2	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	ASU	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
3	negative	positive	positive	negative	2/4 (50%)	2/4 (50%)	CP	
5	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
9a	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI	
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI-4	
9b	negative	positive	-	positive	3/3 (100%)	3/3 (100%)	MS	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	NGS	
17	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	NGS	
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	no differentiation between goose and chicken
16	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	15	1	13
Number negative	15	0	13	2
Percent positive	0	100	7	87
Percent negative	100	0	93	13
Consensus value	negative	positive	negative	positive
Spiking	negative	positive	negative	positive

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GI = GEN-IAL First Allergen

GI-4= GEN-IAL First Allergen Tetra

MS = Microsynth

NGS = Next-Generation Sequencing

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

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. For the lower spiked sample 4 (2,5% chicken compared to 5,3% chicken in sample 2) two negative results were obtained.

Participant 3 obtained a positive result for sample 3 and a negative result for sample 4, which may be due to an interchanging of the two samples.

4.1.4 DNA-based Results: Turkey

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	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
11	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
5	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
9a	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	GI	
13	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	GI	
9b	positive	negative	-	negative	3/3 (100%)	3/3 (100%)	MS	
12	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
17	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
4	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	SFA-ID	
8	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
16	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	15	0	0	1
Number negative	0	15	14	14
Percent positive	100	0	0	7
Percent negative	0	100	100	93
Consensus value	positive	negative	negative	negative
Spiking	positive	negative	negative	negative

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GI = GEN-IAL First Allergen

MS = Microsynth

NGS = Next-Generation Sequencing

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

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.

4.1.5 DNA-based Results: Goose

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		
2	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	CP	no positive sample detected
3	negative	negative	positive	negative	2/4 (50%)	2/4 (50%)	CP	
5	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	CP	
9	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	CP	
13	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	GS	
12	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	NGS	
17	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	NGS	
7	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
8	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
11	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
16	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	1	9
Number negative	11	11	10	2
Percent positive	0	0	9	82
Percent negative	100	100	91	18
Consensus value	negative	negative	negative	positive
Spiking	negative	negative	negative	positive

Methods:

CP = Chipron LCD Array Kit MEAT 5.0

GS = Eurofins Genescan DNAnimal Ident

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 sample 4 (5,1% goose meat). One participant could not detect any of the samples as positive using method CP.

Participant 3 obtained a positive result for sample 3 and a negative result for sample 4, which may be due to an interchanging of the two samples.

4.2 Proficiency Test Horse meat

4.2.1 Protein-based Results: Horse

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		
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ETM	
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ETM	

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

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

Comments:

The consensus values of results are in qualitative agreement with the spiking or experimentally determined contents of the samples 3 and 4.

4.2.2 DNA-based Results: Horse

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
2	negative	negative	-	positive	3/3 (100%)	3/3 (100%)	ASU	no reliable differentiation between horse and donkey, Sample 3: questionable
11a	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	sample 1 and 2: weak bands, possibly traces of horse or mule
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	no differentiation between horse and donkey
5	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	
9a	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	no differentiation between horse and donkey
7	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	GI	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	GI	
15	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	GR	
9b	-	-	positive	positive	2/2 (100%)	2/2 (100%)	MS	
12	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	NGS	
17	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	NGS	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
12	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-IDH	
8	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	div	
11b	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	samples 1 and 2: late amplicons, possibly traces of horse or mule
14	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	div	
16	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	1	14	19
Number negative	18	17	4	0
Percent positive	0	6	78	100
Percent negative	100	94	22	0
Consensus value	negative	negative	positive	positive
Spiking	negative	negative	positive	positive

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GI = GEN-IAL First Allergen

GR = VERYFINDER EQUINE, real time PCR, Generon

MS = Microsynth

NGS = Next-Generation Sequencing

SFA-ID= SureFood® ANIMAL ID Horse/Donkey, R-Biopharm/ Congen

SFA-IDH= SureFood Allergen ID Horse, R-Biopharm/Congen

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking or experimentally determined contents of the samples.
For samples 3 (addition of donkey meat and experimental evidence of horse meat) inconsistent results were obtained. Three participants point out that it is not possible to distinguish between horse and donkey reliably with the ASU or CP method used.

4.3 Proficiency Test Donkey meat

4.3.1 Protein-based Results: Donkey

No results were submitted for the parameter donkey using protein-based methods.

4.3.2 DNA-based Results: Donkey

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	-	-	2/2 (100%)	2/2 (100%)	CP	no reliable differentiation between horse and donkey
9	negative	negative	positive	positive	3/4 (75%)	3/4 (75%)	CP	no reliable differentiation between horse and donkey
7	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	GI	
13	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	GI	
2	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	ISO	
12	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	NGS	
17	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	NGS	no positive sample identified
4	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
6	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
8	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
11	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
14	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	div	no positive sample identified
16	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	10	1
Number negative	13	13	2	11
Percent positive	0	0	83	8
Percent negative	100	100	17	92
Consensus value	negative	negative	positive	negative
Spiking	negative	negative	positive	negative

Methods:

CP = Chipron LCD Array Kit MEAT 5.0
GI = GEN-IAL First Allergen
ISO = ISO/TS Methode/ method
NGS = Next-Generation Sequencing
SFA-ID= SureFood® ANIMAL ID Horse/Donkey, R-Biopharm/ Congen
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 sample 3.
Two participants were unable to detect any of the samples as positive using the NGS method or an unspecified method (div).

For samples 4 (no addition of donkey meat but spiking with horse meat) a positiv result was obtained. This participant and another one point out that it is not possible to differentiate between horse and donkey with the used method CP meat 5.0.

4.4 Proficiency Test Beef meat

4.4.1 Protein-based Results: Beef

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		
10	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	ETM	
1	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	ETM3	
4	positive	negative	negative	positive	3/3 (100%)	3/4 (75%)	ETM3	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	3	0	0	1
Number negative	0	3	3	2
Percent positive	100	0	0	33
Percent negative	0	100	100	67
Consensus value	positive	negative	negative	none
Spiking	positive	negative	negative	negative

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

ETM3= ELISA-TEK™ Cooked Meat 3 Species Kit: beef, pork, poultry

Comments:

The consensus values of the results for samples 1, 2 and 3 are in qualitative agreement with the spiking of the samples.
For the unspiked sample 4 inconsistent results were obtained, thus no consensus value ≥75% could be established.

In sample 4, contents of < 0,5% beef could be experimentally determined. (see page 5, table 3).

4.4.2 DNA-based Results: Beef

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	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
11	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
5	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
9a	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
13	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	GI	
7	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	GI-4	
9b	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	MS	
12	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
17	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
6	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
4	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	SFA-ID	sample 4 weakly positive
8	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	div	
16	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	16	0	1	3
Number negative	0	16	15	13
Percent positive	100	0	6	19
Percent negative	0	100	94	81
Consensus value	positive	negative	negative	negative
Spiking	positive	negative	negative	negative

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GI = GEN-IAL First Allergen

GI-4= GEN-IAL First Allergen Tetra

MS = Microsynth

NGS = Next-Generation Sequencing

SFA-4p = Sure Food Allergen 4plex, R-Biopharm / Congen

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

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 samples.

In sample 4, contents of < 0,5% beef could be experimentally determined. (see page 5, table 3).

4.5 Proficiency Test Pork meat

4.5.1 Protein-based Results: Pork

No results were submitted for the parameter pork using protein-based methods.

4.5.2 DNA-based Results: Pork

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		
11	positive	positive	positive	positive	-	4/4 (100%)	ASU	

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

Methods:

ASU = ASU §64 Methode/method

Comments:
The results are in qualitative agreement with the basis "pork meat" 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 Protein-based Methods: Poultry

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ETM	10	06.08.	positive	positive	negative	positive	3	Meat and other tissues e.g. intestines	ELISA-TEK Cooked meat species Kit Elisa Technologies
ETM3	1	14.08.20	positive	positive	negative	negative	0.5	Protein	ELISA-TEK™ Cooked Meat 3 Species Kit: beef, pork, poultry, r-biopharm
ETM3	4	23.07.20	positive	positive	negative	positive	1	Protein	Cooked Meat Beef Pork Poultry; ELISA, Technologies (r-biopharm)

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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	10	510631	animal species-specific	0,9% NaCl solution; 15min 95-100°C water bath	
ETM3	1	510603	According to kit instructions	According to kit instructions	
ETM3	4	510603		According to test instructions	noticeably low absorbance values, especially sample 2 at the cut-off; No differentiation between chicken and turkey possible using ELISA

5.1.2 Protein-based Methods: Horse

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ETM	4	23.07.20	negative	negative	positive	positive	1	Protein	Cooked Meat Horse; ELISA Technologies (r-biopharm)
ETM	10	06.08.	negative	negative	positive	positive	1	Meat and other tissues e.g. intestines	ELISA-TEK Cooked meat species Kit Elisa Technologies

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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	4	510651		according to test instructions	
ETM	10	510651	animal species-specific	0,9% NaCl solution; 15min 95-100°C water bath	

5.1.3 Protein-based Methods: Beef

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ETM	10	06.08.	positive	negative	negative	negative	1	Meat and other tissues e.g. intestines	ELISA-TEK Cooked meat species Kit Elisa Technologies
ETM3	1	14.08.20	positive	negative	negative	negative	0.5	Protein	ELISA-TEK™ Cooked Meat 3 Species Kit: beef, pork, poultry - r-biopharm
ETM3	4	23.07.20	positive	negative	negative	positive	1	Protein	Cooked Meat Beef Pork Poultry; ELISA Technolo-gies (r-biopharm)

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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	10	510611	animal species-specific	0,9% NaCl solution; 15min 95-100°C water bath	
ETM3	1	510603	According to kit instructions	According to kit instructions	
ETM3	4	510603		According to test instructions	low absorbance value in sample 4 (0.15), but above cut-off

5.1.4 DNA-based Methods: Poultry

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
CP	3	13./14.07.	positive	positive	positive	negative	0,001	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	5		positive	positive	negative	positive			DNA chip : chipron
GS	13	03.08.20	positive	positive	negative	positive	0,001	DNA	DNAinmal Screen Bird, GeneScan
NGS	12		positive	positive	negative	positive	1	DNA	NGS - internal method
NGS	17		positive	positive	negative	positive	0,3	DNA	All Species ID, SGS MOLECULAR
SFA-ID	6	21.07.20	positive	positive	positive	negative	0,1	Meat	Congen/R-Biopharm: SureFood® Animal ID Poultry IAAC
div	16		positive	positive	negative	positive	0,01	Meat	

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	
CP	3	A-500-12	mitochondrial 16S rRNA	Implementation according to kit instructions!	
CP	5				Poultry was set as 'positive' if goose, chicken or/and turkey is 'positive'.
GS	13	5422211410		CTAB, FFS-Kit Promega Maxwell	
NGS	12			Next Generation Sequencing - NGS - Ion Torrent Platform	
NGS	17			Marchery-Nagel NucleoMag	
SFA-ID	6	S6125	Phasianidae, Numididae, Anatidae, Columbidae	SureFood Prep Basic	K01
div	16				

5.1.5 DNA-based Methods: Chicken

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	29.07.20	negative	positive	negative	positive	0,01	DNA	ASU/§64 Method
ASU	2	23.07.20	negative	positive	negative	negative	0,1	rel. DNA content	
ASU	11	8.+14.;20. 7.20	negative	positive	negative	positive	0,1	Sum of amplifiable DNA in 50 ng DNA	biomers
CP	3	13./14.07.	negative	positive	positive	negative	0,001	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	5		negative	positive	negative	positive			DNA chip : chipron
CP	9a		negative	positive	negative	positive	100-250 fg		
GI	13	03.08.20	negative	positive	negative	positive	0,001	DNA	First-Chicken PCR Kit , Gen-ial
GI-4	7	13.07.20	negative	positive	negative	positive	0,1	DNA	First-Animal Tetra /GEN-IAL
MS	9b		negative	positive	-	positive		Please select!	
NGS	12		negative	positive	negative	positive	1	DNA	NGS - internal method
NGS	17		negative	positive	negative	positive	0,3	DNA	All Species ID, SGS MOLECULAR
SFA-ID	4	23.07.20	negative	positive	negative	positive	0,1	Meat	SureFood Animal ID Chicken IAAC Realtime Kit; Fa. Congen
div	8	30.08.20	negative	positive	negative	positive	1	DNA	
div	14		negative	positive	negative	positive		Please select!	
div	16		negative	positive	negative	positive	0,01	Meat	

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	1	ASU L 08.00-61 (2016-03)	TF-GB3 X6009	Extraction with Maxwell FFS Kit, Real Time PCR with QuantiNova Multiplex Mastermix (Qiagen) 40 cycles	
ASU	2	ASU L 08.00-61			
ASU	11	ASU L 08.00-61	TF-GB3-gene chicken	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
CP	3	A-500-12	mitochondrial 16S rRNA	Implementation according to kit instructions!	
CP	5				
CP	9a				
GI	13	5207083		CTAB, FFS-Kit Promega Maxwell	
GI-4	7	ANIT1 0050		real-time PCR , 40 cycles	
MS	9b				
NGS	12			Next Generation Sequencing - NGS - Ion Torrent Platform	
NGS	17			Marchery-Nagel NucleoMag	
SFA-ID	4			DNeasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to the kit manufacturer's protocol	
div	8				
div	14				No distinction is made between goose and chicken
div	16				

5.1.6 DNA-based Methods: Turkey

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	29.07.20	positive	negative	negative	negative	0,01	DNA	ASU/§64 Method
ASU	2	23.07.20	positive	negative	negative	negative	0,1	rel. DNA content	
ASU	11	8.+14.;20. 7.20	positive	negative	negative	negative	0,1	Sum pf amplifiable DNA in 50 ng DNA	biomers
CP	3	13./14.07.	positive	negative	negative	negative	0,001	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	5		positive	negative	negative	negative			DNA chip : chipron
CP	9a		positive	negative	negative	negative	100-250 fg		
GI	7	13.07.20	positive	negative	negative	negative	0,1	DNA	First-Animal Tetra /GEN-IAL
GI	13	03.08.20	positive	negative	negative	negative	0,001	DNA	First-Turkey PCR Kit , Gen-ial
MS	9b		positive	negative	-	negative		Please select!	
NGS	12		positive	negative	negative	negative	1	DNA	NGS - internal method
NGS	17		positive	negative	negative	negative	0,3	DNA	All Species ID, SGS MOLECULAR
SFA-ID	4	23.07.20	positive	negative	negative	positive	0,1	Meat	SureFood Animal ID Turkey IAAC Realtime Kit; Fa. Congen
div	8	30.08.20	positive	negative	negative	negative	1	DNA	
div	14		positive	negative	negative	negative		Please select!	
div	16		positive	negative	negative	negative	0,01	Meat	

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	1	ASU L 08.00-61 (2016-03) mod.	Prolactin Receptor L76587	Extraction with Maxwell FFS Kit, Real Time PCR with QuantiNova Multiplex Mastermix (Qiagen) 40 cycles	Modified primer and probe
ASU	2	ASU L 08.00-61			
ASU	11	ASU L 08.00-61	Prolactin Receptor gene turkey	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
CP	3	A-500-12	mitochondrial 16S rRNA	Implementation according to kit instructions!	
CP	5				
CP	9a				
GI	7	ANIT1 0050		real-time PCR , 40 cycles	
GI	13	5207087		CTAB, FFS-Kit Promega Maxwell	
MS	9b				
NGS	12			Next Generation Sequencing - NGS - Ion Torrent Platform	
NGS	17			Marchery-Nagel NucleoMag	
SFA-ID	4			DNeasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to the kit manufacturer's protocol	
div	8				
div	14				
div	16				

5.1.7 DNA-based Methods: Goose

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
CP	2	28.07.20	negative	negative	negative	negative	0,1	ng/PCR	Chipron MEAT 5.0 LCD-Array Kit
CP	3	13./14.07.	negative	negative	positive	negative	0,001	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	5		negative	negative	negative	positive			DNA chip : chipron
CP	9		negative	negative	negative	positive	100-250 fg		
GS	13	03.08.20	negative	negative	negative	positive	0,001	DNA	DNAnimal Ident RT Goose, GeneScan
NGS	12		negative	negative	negative	positive	1	DNA	NGS - internal method
NGS	17		negative	negative	negative	positive	0,3	DNA	All Species ID, SGS MOLECULAR
div	7	26.08.20	negative	negative	negative	positive	0,01	DNA	house method
div	8	30.08.20	negative	negative	negative	positive	1	DNA	
div	11	08.+15.+20.07.2020	negative	negative	negative	positive	0,1	Sum amplifiable DNA in 50 ng DNA	biomers
div	16		negative	negative	negative	positive	0,01	Meat	

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	
CP	2				
CP	3	A-500-12	mitochondrial 16S rRNA	Implementation according to kit instructions!	
CP	5				
CP	9				
GS	13	5422220810		CTAB, FFS-Kit Promega Maxwell	
NGS	12			Next Generation Sequencing - NGS - Ion Torrent Platform	
NGS	17			Marchery-Nagel NucleoMag	
div	7			Endpunkt-PCR 35 cycles / gel electrophoresis	
div	8				
div	11	Fleischwirtschaft 2/2007, S. 86 ff	Cyt-b-gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	16				

5.1.8 DNA-based Methods: Horse

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	28.07.20	negative	negative	positive	positive	0,001	DNA	ASU/§64 Method
ASU	2	28.07.20	negative	negative	questiona-ble	positive	0,1	ng/PCR	
ASU	11a	22.07.20	negative	negative	positive	positive	0,1	Sum of amplifiable DNA in 20 ng DNA	biomers
CP	3	13./14.07.	negative	negative	positive	positive	0,001	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	5		negative	negative	positive	positive			DNA chip : chipron
CP	9a		negative	negative	positive	positive	100-250 fg		
GI	7	13.07.20	negative	positive	positive	positive	0,01	DNA	First-duplex Donkey/Horse /GEN-IAL
GI	13	03.08.20	negative	negative	positive	positive	0,001	DNA	First-Duplex Donkey/Horse PCR Kit, Gen-ial
GR	15		negative	negative	positive	positive	0,001	DNA	VERYFINDER EQUINE Generon
MS	9b		-	-	positive	positive		Please select!	AllHorse, Microsynth
NGS	12		negative	negative	negative	positive	1	DNA	NGS - internal method
NGS	17		negative	negative	negative	positive	0,3	DNA	All Species ID, SGS MOLECULAR
SFA-3p	6	21.07.20	negative	negative	positive	positive	0,1	Meat	Congen/R-Biopharm: SureFood® Animal ID 3plex Horse/Donkey + IAAC
SFA-ID	4	23.07.20	negative	negative	positive	positive	0,1	Meat	SureFood Animal ID Horse&Donkey IAAC Realtime Kit; Fa. Congen
SFA-ID	12		negative	negative	positive	positive	0,1	DNA	SureFood® ANIMAL ID Horse IAAC (Congen)
div	8	30.08.20	negative	negative	negative	positive	1	DNA	
div	11b	08.+15.+20.07.2020	negative	negative	positive	positive	0,1	Sum of amplifiable DNA in 50 ng DNA	biomers
div	14		negative	negative	negative	positive		Bitte auswählen!	
div	16		negative	negative	positive	positive	0,01	Meat	

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	1	ASU L 06.26/27-2 (2007-12)	Cytochrom b	Extraction with Maxwell FFS Kit, 50ng DNA for PCR, HOT Star-Taq polymerase (Qiagen) Amplification (40 cycles), digestion with MboI and DdeI, gel electrophoresis	
ASU	2	ASU L 06.26/27-2			Questionable result for the donkey-positive sample, as differentiation between horse and donkey is not reliable possible with the method ASU used.
ASU	11a	ASU L-06.26/27-2	Cyt. b	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	Sample 1 + 2: weak bands: traces of horese < 0,1% or mule DNA?
CP	3	A-500-12	mitochondrial 16S rRNA	Implementation according to kit instructions!	With the method used, the animal species donkey and horse cannot be distinguished! The positive results for the horse species in samples 3 and 4 on the chip cannot be differentiated - i.e. no distinction between donkey and horse possible!
CP	5				
CP	9a		Equus caballus, Equus asinus (Equus sp.)		It is impossible to differentiate between donkey and horse
GI	7	PHDOH 0050		real-time PCR , 40 cycles	
GI	13	5207181		CTAB, FFS-Kit Promega Maxwell	
GR	15				
MS	9b	1206		CTAB-Extraction	
NGS	12			Next Generation Sequencing - NGS - Ion Torrent Platform	
NGS	17			Marchery-Nagel NucleoMag	
SFA-3p	6	S6119	Equus caballus	SureFood Prep Basic	K01
SFA-ID	4			DNeasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to the kit manufacturer's protocol	
SFA-ID	12			Real time PCR	
div	8				
div	11b	Eur Food Res Technol (2009) 230: 125-133	Cyt. b Gen	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	Sample 1 + 2: late amplicons: traces of horse or mule DNA?
div	14				
div	16				

5.1.9 DNA-based Methods: Donkey

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
CP	3	13./14.07.	negative	negative	-	-	0,001	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	9		negative	negative	positive	positive	100-250 fg	DNA	Chipron MEAT 5.0 LCD-Array Kit
GI	7	13.07.20	negative	negative	positive	negative	0,01	DNA	First-duplex Donkey/Horse /GEN-IAL
GI	13	03.08.20	negative	negative	positive	negative	0,001	DNA	First-Duplex Donkey/Horse PCR Kit, Gen-ial
ISO	2	22.07.20	negative	negative	positive	negative	0,1	rel. DNA content	
NGS	12		negative	negative	positive	negative	1	DNA	NGS - internal method
NGS	17		negative	negative	negative	negative	0,3	DNA	All Species ID, SGS MOLECULAR
SFA-3p	6	21.07.20	negative	negative	positive	negative	0,1	Meat	Congen/R-Biopharm: SureFood® Animal ID 3plex Horse/Donkey + IAAC
SFA-ID	4	23.07.20	negative	negative	positive	negative	0,1	Meat	SureFood Animal ID Horse&Donkey IAAC Realtime Kit; Fa. Congen
div	8	30.08.20	negative	negative	positive	negative	1	DNA	
div	11	08.+20.07. 2020	negative	negative	positive	negative	0,1	Sum of amplifiable DNA in 50 ng DNA	biomers
div	14		negative	negative	negative	negative		Please select!	
div	16		negative	negative	positive	negative	0,01	Meat	

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	
CP	3	A-500-12	mitochondrial 16S rRNA	Implementation according to kit instructions!	With the method used, the animal species donkey and horse cannot be distinguished! The positive results for the animal species horse in samples 3 and 4 on the chip cannot be differentiated - therefore no statement about the animal species donkey is possible here!
CP	9	A-500-04/-12	Equus caballus, Equus asinus (Equus sp.)	CTAB-Extraktion	Target sequence specific for donkey and horse
GI	7	PHDOH 0050		real-time PCR , 40 cycles	PCR sample 3.1 at 20.8.2020
GI	13	5207181		CTAB, FFS-Kit Promega Maxwell	
ISO	2	ISO/TS 20224-7:2020 (Entwurf)	captures donkeys, mules, mules and plains zebra		
NGS	12			Next Generation Sequencing - NGS - Ion Torrent Platform	
NGS	17			Marchery-Nagel NucleoMag	LOD 130 in 43320 pg
SFA-3p	6	S6119	Equus asinus	SureFood Prep Basic	K01
SFA-ID	4			DNeasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to the kit manufacturer's protocol	
div	8				
div	11	International Journal of Food Properties, 17:3, 629-638	mitochondr. NADH dehydrogenase gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	14				
div	16				

5.1.10 DNA-based Methods: Beef

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 detecti-on given as	Method
		Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manufacturer
ASU	1	29.07.20	positive	negative	negative	negative	0,01	DNA	ASU/§64 Method
ASU	2	23.07.20	positive	negative	negative	negative	0,1	rel. DNA content	
ASU	11	8.+14.;20. 7.20	positive	negative	negative	negative	0,1	Sum of amplifiable DNA in 50 ng DNA	biomers
CP	3	13./14.07.	positive	negative	negative	negative	0,001	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	5		positive	negative	negative	negative			DNA chip : chipron
CP	9a		positive	negative	negative	negative	100-250 fg		
GI	13	03.08.20	positive	negative	negative	negative	0,001	DNA	First-Beef PCR Kit , Gen-ial
GI-4	7	13.07.20	positive	negative	negative	positive	0,1	DNA	First-Animal Tetra /GEN-IAL
MS	9b		positive	negative	negative	negative		Please select!	
NGS	12		positive	negative	negative	negative	1	DNA	NGS - internal method
NGS	17		positive	negative	negative	negative	0,3	DNA	All Species ID, SGS MOLECULAR
SFA-4p	6	30.07.20	positive	negative	negative	negative	0,1	Meat	Congen/R-Biopharm: SureFood® Animal ID 4plex Beef/Horse/Pork+IAAC
SFA-ID	4	23./29.07. 20	positive	negative	negative	positive	0,1	Meat	SureFood Animal ID Beef IAAC Realtime Kit; Fa. Congen
div	8	30.08.20	positive	negative	negative	negative	1	DNA	
div	14		positive	negative	positive	negative		Please select!	
div	16		positive	negative	negative	positive	0,01	Meat	

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	1	ASU L 08.00-61 (2016-03)	Beta-Actin EH170825	Extraction with Maxwell FFS Kit, Real Time PCR with QuantiNova Multiplex Mastermix (Qiagen) 40 cycles	
ASU	2	ASU L 08.00-61			
ASU	11	ASU L 08.00-61	beta actin gene beef	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
CP	3	A-500-12	mitochondrial 16S rRNA	Implementation according to kit instructions!	
CP	5				
CP	9a				
GI	13	10004677		CTAB, FFS-Kit Promega Maxwell	
GI-4	7	ANIT1 0050		real-time PCR , 40 cycles	
MS	9b				
NGS	12			Next Generation Sequencing - NGS - Ion Torrent Platform	
NGS	17			Marchery-Nagel NucleoMag	
SFA-4p	6	S6126	Bos taurus	SureFood Prep Basic	K01
SFA-ID	4			DNeasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to the kit manufacturer's protocol	Sample 4 first batch negative, repeated batch with 2 purifications both weakly positive (ct value 29)
div	8				
div	14				
div	16				Sample 4 in traces

5.1.11 DNA-based Methods: Pork and other

Primary data

Parameter	Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detecti-on given as	Method
other Methods			Day/ Month	positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food/ protein	Test-Kit + Manu-facturer
Pork	ASU	11	8.+14.;20. 7.20	positive	positive	positive	positive	0,1	Sum of amplifiable DNA in 50 ng DNA	biomers
Myostatin	ASU	11	08.+15.+20.07.2020	positive	positive	positive	positive	0,1	Sum of amplifiable DNA in 50 ng DNA	biomers

Other details to the Methods

Parameter	Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
other Methods			Article-No. / ASU-No.			
Pork	ASU	11	ASU L 08.00-61	beta actin gene pork	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
Myostatin	ASU	11	ASU L 06.00-69	Myostatin gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	

5.2 Homogeneity

5.2.1 Mixture homogeneity after bottling

Homogeneity test based on the determination of chloride by titration according to MOHR.

Homogeneity test Sample 1

Replicate measurements	mg/100g
1	212,4
2	223,0
3	223,0
4	198,2
5	212,4

General average	213,8	
Reapeatability standard deviation	10,20	4,8%

Homogeneity test Sample 2

Replicate measurements	mg/100g
1	260,0
2	261,5
3	260,3
4	254,5
5	268,5

General average	261,0	
Reapeatability standard deviation	5,01	1,9%

Homogeneity test Sample 3

Replicate measurements	mg/100g
1	225,9
2	218,9
3	232,9
4	232,7
5	219,3

General average	225,9	
Reapeatability standard deviation	6,85	3,0%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA ptAUS2 (2020)***
PT name	***Animal Species-Screening II – 4 Samples qualitative: Donkey, Beef, Horse, Poultry (Chicken, Goose and Turkey) in Meat Product (Pork) (Sausage Meat)***
Sample matrix	*Samples 1-4:* *sausage meat (heated)/ ingredients: pork, water, gelatine (pork), salt, sodium citrate and further meat species*
Number of samples and sample amount	*4 different Samples 1-4: 25 g each*
Storage	*Samples 1-4: cooled 2 - 10°C (long term frozen < -18°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*qualitative: Donkey, Beef, Horse, Poultry (Chicken, Goose and Turkey)* *Samples 1-4: appr. 1-10%*
Methods of analysis	*The analytical methods are optional*
Notes to analysis	*The analysis of PT samples should be performed like a routine laboratory analysis.* *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.* *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:* ***pt@dla-lvu.de***
Last Deadline	***the latest Septemer 11th 2020***
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

[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

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 interlaboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and precision) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estimates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messungen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Checking procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Requirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific proteins in foods, CAC/GL 74-2010
19. Lebensmittelchemische Gesellschaft [LChG der GDCh] „Stellungnahme der AG zu: Methoden zur Differenzierung von Tierarten in Lebensmitteln – Status quo, (2016), Food Chemistry Society of the GDCh]
20. Carcass analysis and meat composition of the donkey; A.A. Aganga, A.O. Aganga, T. Thema, K.O. Obocheleng; Pakistan Journal of Nutrition, 2(3), 138-147 (2003)