Evaluation Report
proficiency test

DLA ptAUS2 (2021)

Animal Species-Screening II:

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

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

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EP-Koordinator *PT-Coordinator*	Alexandra Scharf MSc.
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (12. November 2021) 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: 12. November 2021
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung wurden nachstehende Leistungen im Unterauftrag vergeben: Qualitative Prüfung der EP-Parameter As part of the present proficency test the following services were subcontracted: Qualitative verification of the PT-Parameters
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 animal foods of donkey, beef, horse, chicken, turkey and pork were provided for qualitative determination. The parameters were present in the matrix raw meat product (salami) with contents of 4-100%.

Commercially available salamis were used as raw materials for the animal species used, which were either made from only one animal species respectively (turkey, chicken, pork and beef salami) or, in the case of horse and donkey salami, contained a further declared content of pork.
The salamis were tested for the presence of the declared and further, non-declared animal species by means of PCR analysis or immunoassay.

The corresponding amounts of meat species for the respective sample (see Table 1) have been minced.
The corresponding quantitative amounts of the meat species for the respective samples (see Tab. 1) were minced using a meat mincer and, after homogenisation, filled into portions of approx. 30 g in plastic containers.

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

Ingredients*	Sample 1	Sample 2	Sample 3.1	Sample 4
Pork meat	positive (77%)	negative	positive (4,4%)	positive (90%)
Chicken meat	negative	negative	positive (6,2%)	negative
Turkey meat	negative	negative	positive (89%)	positive (6,4%)
Horse meat	positive (7,3%)**	negative	negative	negative
Donkey meat	positive (7,3%)**	negative	negative	positive (3,3%)**
Beef meat	positive (8,1%)	positive (100%)	negative	positive***

*Animal species contents of „food item" as indicated in the column of ingredients according gravimetric mixing (with the exception of donkey meat and horse meat s.**)
**Animal species content calculated according to declaration
***Beef detectable, no spiking
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 4.0* (Chipron GmbH) (see table 2).

Table 2: Verification of detectability of the contained animal species by LCD-Array Kit MEAT 4.0 (Chipron GmbH)

chipron	LCD-Array Kit MEAT 4.0			
	Probe 1	Probe 2	Probe 3	Probe 4
Schwein / Pork	positive	negative	positive	positive
Huhn / Chicken	negative	negative	positive	negative
Pute / Turkey	positive*	negative	positive	positive
Pferd / Horse	positive	negative	negative	negative
Esel / Donkey	positive	negative	negative	positive
Rind / Cattle	positive	positive	negative**	positive

*Traces > detection limit 0.1% (w/w)
**Traces < detection limit 0.1% (w/w)

The results are in agreement with the spiking/ experimental evidence of LVU samples 1-4. In sample 1, traces of turkey (> 0.1% w/w) were detected. In sample 3, traces of beef < 0.1% w/w) were detected.

2.1.1 Stability

The sample material is salami, which have a shelf life of several months due to their high salt content (pickling salt). The sample material was sent by refrigerated shipping and the participating laboratories were instructed to store it at -18°C until testing. 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 26th week of 2021. The testing method was optional. The tests should be finished at August 27th 2021 the latest.

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

DLA ptAUS2 (2021) Animal Species-Screening II – 4 Samples qualitative: Donkey, Beef, Horse, Poultry (Chicken and Turkey) and Pork in Meat Product Salami

There are *4 different samples* possibly containing the animal products (Donkey, Beef, Horse, Pork and Poultry (Chicken and Turkey). The parameters are contained in the matrix of Salami with amounts of 1 – 100% (depending on the basic of the salami). The evaluation of results is **strictly qualitative (positive / negative).**

Note: Samples should be stored at - 18 °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 5.2 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.

All 17 participants submitted at least one result.

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		
8	negative	negative	positive	positive	-	4/4 (100%)	ETM	

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

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

Comments:

The results are in qualitative agreement with the spiking of the samples 3 and 4, as well as the consensus values of samples 2, 3 and 4 as determined by the DNA-based methods (see 4.1.2).

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		
16	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	SGS	
5	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	div	
17	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	

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

Methods:

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

Traces of turkey meat were experimentally determined in sample 1 (see page 5, table 2).

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		
8	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
1	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	CP	
3	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	CP	
13	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	CP	
6	positive	negative	positive	positive	2/4 (50%)	2/4 (50%)	GS	
10	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	RF	
2	negative	positive	positive	negative	3/4 (75%)	3/4 (75%)	SFA-4p	
15	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4p	
11	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
14	-	negative	positive	negative	3/3 (100%)	3/3 (100%)	SFA-ID	
9	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-Q	
16	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SGS	
4	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
5	positive	negative	positive	positive	2/4 (50%)	2/4 (50%)	div	Sample 4 traces
7	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
12a	negative	negative	positive	positive	3/4 (75%)	3/4 (75%)	div	
12b	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
17	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	3	1	18	3
Number negative	14	17	0	15
Percent positive	18	6	100	17
Percent negative	82	94	0	83
Consensus value	negative	negative	positive	negative
Spiking	negative	negative	positive	negative

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GS = Eurofins Genescan DNAnimal Ident

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

SFA-Q = Sure Food Animal Quant, R-Biopharm / Congen

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

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 3 (6,2% chicken meat). Three positive results each for chicken were obtained for the turkey-spiked sample 4 (6.4% turkey meat) and the unspiked sample 1 (experimentally detected traces of turkey).

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		
8	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	ASU	Sample 1 slightly above the LOD
1	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	CP	
3	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	CP	
13	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	CP	
6	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	GS	
10	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	RF	
2	negative	positive	positive	positive	2/3 (67%)	3/4 (75%)	SFA-4p	
15	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	SFA-4p	
9	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	SFA-ID	
11	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	SFA-ID	
14	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	SFA-ID	
16	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	SGS	
4	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	
5	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	div	Sample 1 traces
7	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	div	
17	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	11	1	16	16
Number negative	5	15	0	0
Percent positive	69	6	100	100
Percent negative	31	94	0	0
Consensus value	none	negative	positive	positive
Spiking	negative	negative	positive	positive

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GS = Eurofins Genescan DNAnimal Ident

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

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

Traces of turkey meat were experimentally determined in sample 1 (see page 5, table 2).

4.2 Proficiency Test Meat from Equines

4.2.1 DNA-based Results: Equines (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		
8	positive	positive	negative	positive	3/4 (75%)	3/4 (75%)	ASU	minor signals for sample 3
1	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	CP	
3	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	CP	
13	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	CP	
10	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	RF	
16	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	SGS	
4	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
7	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
17	positive	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	9	1	0	9
Number negative	0	8	9	0
Percent positive	100	11	0	100
Percent negative	0	89	100	0
Consensus value	positive	negative	negative	positive
Spiking	positive	negative	negative	positive

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

RF= RapidFinder™ ID Kit, ThermoFisher

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

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 1 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		
15	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	BP	
6	positive	negative	negative	positive	3/3 (100%)	3/4 (75%)	GS	
9	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	SFA-3p	
11	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	SFA-3p	
14	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	SFA-3p	
2	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	SFA-4p	
16	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	SGS	
5	positive	positive	negative	positive	2/3 (67%)	2/4 (50%)	div	Sample 4 traces
12	positive	positive	negative	positive	2/3 (67%)	2/4 (50%)	div	
17	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	10	2	0	3
Number negative	0	8	10	7
Percent positive	100	20	0	30
Percent negative	0	80	100	70
Consensus value	positive	negative	negative	none
Spiking	positive	negative	negative	negative

Methods:

BP = Biopremier, real time PCR

GS = Eurofins Genescan DNAnimal Ident

SFA-3P= SureFood® ANIMAL ID 3plex, R-Biopharm / Congen

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

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the results for samples 1-3 are in qualitative agreement with the spiking of sample 1 (7.3% horse meat).
For samples 4 (spiking with donkey meat) inconsistent results were obtained, thus no consensus value ≥75% could be established.

4.2.3 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		
6	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	GS	
9	positive	negative	negative	positive	3/3 (100%)	4/4 (100%)	SFA-3p	
11	positive	negative	negative	positive	3/3 (100%)	4/4 (100%)	SFA-3p	
14	positive	negative	negative	positive	3/3 (100%)	4/4 (100%)	SFA-3p	
16	positive	negative	negative	positive	3/3 (100%)	4/4 (100%)	SGS	
5	positive	negative	positive	positive	3/3 (100%)	3/4 (75%)	div	Sample 3 traces
17	positive	negative	negative	positive	3/3 (100%)	4/4 (100%)	div	

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

Methods:

GS = Eurofins Genescan DNAnimal Ident

SFA-3P= SureFood® ANIMAL ID 3plex, R-Biopharm / Congen

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the results for samples 1, 2 and 4 are in qualitative agreement with the spiking of sample 1 (7.3% donkey meat) and 4 3.3% donkey meat).

Two positive results were obtained for the unspiked sample 3.

4.3 Proficiency Test Beef meat

4.3.1 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		
8	positive	positive	negative	negative	-	3/4 (75%)	ETM	

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

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

Comments:

The results of samples 1, 2 and 3 are in qualitative agreement with the spiking of the samples 1 (8.1% Beef meat) and 2 (100% Beef meat), as well as the consensus values obtained by DNA-based methods (see 4.3.2).

For sample 4, in which traces of beef were experimentally determined, a negative result was obtained.

4.3.2 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		
8	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
1	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	Beef sample 4 at the LOD
3	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
13	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
6	positive	positive	positive	positive	3/4 (75%)	3/4 (75%)	GS	
10	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	RF	
2	positive	positive	negative	negative	3/4 (75%)	3/4 (75%)	SFA-4p	
11	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
14	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
9	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
16	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	SGS	
4	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
5	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	Sample 4 traces
7	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
12a	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
12b	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
17	positive	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	17	17	1	16
Number negative	0	0	16	1
Percent positive	100	100	6	94
Percent negative	0	0	94	6
Consensus value	positive	positive	negative	positive
Spiking	positive	positive	negative	positive

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GS = Eurofins Genescan DNAnimal Ident

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

SFA-Q = Sure Food Animal Quant, R-Biopharm / Congen

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

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 1, 2 and 4.

In sample 3, contents of < 0,1% beef could be experimentally determined. (see page 5, table 2).

4.4 Proficiency Test Pork meat

4.4.1 Protein-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		
8	positive	negative	positive	positive	-	4/4 (100%)	ETM	

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

Methods:
ETM = ELISA-TEK™ Cooked Meat Species Kits

Comments:
The results are in qualitative agreement with the spiking of the samples 1, 3 and 4, as well as the consensus values obtained by DNA-based methods (see 4.4.2).

4.4.2 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		
8	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
1	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	
3	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	
13	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	CP	
6	positive	positive	positive	positive	3/4 (75%)	3/4 (75%)	GS	
10	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	RF	
2	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4p	
11	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
14	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
15	positive	negative	negative	positive	3/4 (75%)	3/4 (75%)	SFA-ID	
9	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-Q	
16	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	SGS	
4	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
5	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
7	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
12	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
12	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
17	positive	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	18	1	16	18
Number negative	0	17	2	0
Percent positive	100	6	89	100
Percent negative	0	94	11	0
Consensus value	positive	negative	positive	positive
Spiking	positive	negative	positive	positive

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

GS = Eurofins Genescan DNAnimal Ident

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

SFA-Q = Sure Food Animal Quant, R-Biopharm / Congen

SGS= SGS™ All Species ID MEAT DNA Analyser Kit, ThermoFisher

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the results are in qualitative agreement with the spiking of sample 1, 3 and 4. Two negative results were obtained for the lower spiked sample 3 (4.4% pork).

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	8	09.08.21	negative	negative	positive	positive	0,5	Meat	Cooked Meat Speciation Kit; ELISA-TEK

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	8	510631		According to kit instructions	

5.1.2 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	8	09.08.21	positive	positive	negative	negative	0,5	Meat	Cooked Meat Speciation Kit; ELISA-TEK

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	8	Art. Nr.: 510611		according to test instructions	

5.1.3 Protein-based Methods: Pork

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	8	09.08.21	positive	negative	positive	positive	0,5	Meat	Cooked Meat Speciation Kit; ELISA-TEK

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	8	510621		According to kit instructions	

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
SGS	16		negative	negative	positive	positive	0,1	Meat	SGS Specie ID
div	5		positive	negative	positive	positive		DNA	house method
div	17		negative	negative	positive	positive	0,1	DNA	house method

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	
SGS	16	-	?	NucleoMag KingFisher	
div	5				
div	17			CTAB / Prot. K / FFS-Kit_Promega / Real Time PCR / 45 Cycles	

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	8	13.07.21	negative	negative	positive	negative	0,01	DNA	
CP	1		negative	negative	positive	negative	0,1	DNA	LCD Array Kit Meat 5.0, Chipron
CP	3	15.07.21	negative	negative	positive	negative	0,1	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	13	26.07.	negative	negative	positive	negative	0,5	DNA	Chipron; LCD Array Kit Meat 5.0
GS	6	22.07.21	positive	negative	positive	positive		DNA	Euroins GeneScan Technologies
RF	10	20.08.21	positive	negative	positive	negative	0,01	DNA	Thermofisher RAPIDFINDER CHICKEN ID
SFA-4p	2		negative	positive	positive	negative	0,1	DNA	SureFood® ANIMAL ID 4plex Pork/Chicken/Turkey+IAAC
SFA-4p	15	04.08.21	negative	negative	positive	negative	0,1	Meat	ANIMAL ID 4plex IAAC Pork/Chicken/Turkey R-Biopharm lot:21011
SFA-ID	11	23.08.21	negative	negative	positive	negative	0,5	DNA	R-Biopharm AG - SureFood® ANIMAL ID - Chicken IAAC
SFA-ID	14	06.07.21	-	negative	positive	negative	0,1	Meat	SureFood Animal ID Chicken IAAC Realtime Kit; Fa. Congen
SFA-Q	9	26.07.21	negative	negative	positive	negative	0,10%	Meat	SureFood Animal Quant Chicken (R-Biopharm AG)
SGS	16		negative	negative	positive	negative	0,1	Meat	SGS Specie ID
div	4		negative	negative	positive	negative		DNA	house method
div	5		positive	negative	positive	positive		DNA	house method
div	7		negative	negative	positive	negative	0,10%	Meat	
div	12a	26/07	negative	negative	positive	positive	0,5	Meat	house method (multi-plex PCR, gotag probe qPCR master mix)
div	12b	26.07.21	negative	negative	positive	negative	0,5	Meat	Realtime PCR (gotag qPCR mastermix)
div	17		negative	negative	positive	negative	0,1	DNA	house method

Other details to the Methods

ASU	8	ASU L 08.00-61 (2016-03)	Prolactin Rezeptor, L76587	Extraction: Maxw ell FFS	
CP	1	A-500-04		DNA Extraction :foodproof Sample Preparation Kit III, Biotecon (Best.Nr. S 400 06.1)	
CP	3	A-500-12	mitochondrial 16S rRNA	According to kit instructions	
CP	13	A-500-04	DNA	Extraction/ PCR/ LCD Arrray	
GS	6			CTAB-Extraxtion/ Mobispin/ RT-PCR/ 45 Cycles	
RF	10	A24393			
SFA-4p	2	S6123		SureFood® PREP Advanced Kit	
SFA-4p	15		Gallus Gallus DNA	Extraction according our protocol and PCR analysis	
SFA-ID	11	Art. No. S6115, Lot no 24071	-	"The test detects chicken (Gallus gallus). DNA preparation w ith SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (35 cycles follow ing kit setup instructions) w ith Bio-Rad CFX96"	Internal Method accredited ISO/IEC 17025:2018
SFA-ID	14	S6115		Dneasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to kit manufacturer's protocol	
SFA-Q	9	S1014		Real Time PCR	
SGS	16	-	?	NucleoMag KingFisher	
div	4				
div	5				Sample 4 traces
div	7				
div	12a	04.2-CL4/ST 3.71 (T.Matsunaga et.al, 1999)	DNA-Length: 227 bp	Sure Food Prep basic extraction / PCR / 35 cycles	Multiplex PCR
div	12b	ISO/ TS 20224-4: 2020		Sure Food Prep basic extraction / Realtime PCR (Using SYBR Green) / 45 cycles	
div	17	L08.00-61, 2016-02	TGF-beta	CTAB / Prot. K / FFS-Kit_Promega / Real Time PCR / 45 Cycles	

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	8	13.07.21	positive	negative	positive	positive	0,01	DNA	
CP	1		positive	negative	positive	positive	0,1	DNA	LCD Array Kit Meat 5.0, Chipron
CP	3	15.07.21	positive	negative	positive	positive	0,1	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	13	26.07.	positive	negative	positive	positive	0,5	DNA	Chipron; LCD Array Kit Meat 5.0
GS	6	22.07.21	positive	negative	positive	positive		DNA	Euroins GeneScan Technologies
RF	10	20.08.21	positive	negative	positive	positive	0,01	DNA	Thermofisher RAPIDFINDER TURKEY ID
SFA-4p	2		negative	positive	positive	positive	0,1	DNA	SureFood® ANIMAL ID 4plex Pork/Chicken/Turkey+IAAC
SFA-4p	15	04.08.21	negative	negative	positive	positive	0,1	Meat	ANIMAL ID 4plex IAAC Pork/Chicken/Turkey R-Biopharm lot:21011
SFA-ID	9	20.07.21	positive	negative	positive	positive	0,10%	Meat	SureFood Animal ID Turkey IAAC (R-Biopharm AG)
SFA-ID	11	23.08.21	positive	negative	positive	positive	0,5	DNA	R-Biopharm AG - SureFood® ANIMAL ID - Turkey IAAC
SFA-ID	14	06.07.21	positive	negative	positive	positive	0,1	Meat	SureFood Animal ID Turkey IAAC Realtime Kit; Fa. Congen
SGS	16		negative	negative	positive	positive	0,1	Meat	SGS Specie ID
div	4		negative	negative	positive	positive		DNA	house method
div	5		positive	negative	positive	positive		DNA	house method
div	7		positive	negative	positive	positive	0,10%	Meat	
div	17		negative	negative	positive	positive	0,1	DNA	house method

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	8	ASU L 08.00-61 (2016-03) mod.	TF-GB3 X6009	Extraction: Maxw ell FFS	Sample 1 very slightly above the LOD. The sequence of the sonde from the ASU has been changed: CGT TCT GAG GCT ACA CAG TAA CTT TCC C
CP	1	A-500-04		DNA Extraxtion:foodproof Sample Preparation Kit III, Biotecon (Best.Nr. S 400 06.1)	
CP	3	A-500-12	mitochondrial 16S rRNA	according to kit instructions	
CP	13	A-500-04	DNA	Extraction/ PCR/ LCD Arrray	
GS	6			CTAB-Extraktion/ Mobispin/ RT-PCR/ 45 Cycles	
RF	10	A24394			
SFA-4p	2	S6123		SureFood® PREP Advanced Kit	
SFA-4p	15		Meleagris Gallopavo DNA	Extraction according our protocol and PCR analysis	
SFA-ID	9	S6116		Real Time PCR	
SFA-ID	11	Art. No. S6116, Lot no 23410	-	"The test detects turkey DNA (Meleagris gallopavo). DNA preparation w ith SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (35 cycles follow ing kit setup instructions) w ith Bio-Rad CFX96"	Internal Method accredited ISO/IEC 17025:2018
SFA-ID	14	S6116		Dneasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to kit manufacturer's protocol	
SGS	16	-	?	NucleoMag KingFisher	
div	4				
div	5				Sample 1 traces
div	7				
div	17	L08.00-61, 2016-02	Prolactine	Real Time PCR/ 45 Cycles	

5.1.7 DNA-based Methods: Equines

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	8	08.07.21	positive	positive	negative	positive	0,001	DNA	
CP	1		positive	negative	negative	positive	0,1	DNA	LCD Array Kit Meat 5.0, Chipron
CP	3	15.07.21	positive	negative	negative	positive	0,1	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	13	26.07.	positive	negative	negative	positive	0,5	DNA	Chipron; LCD Array Kit Meat 5.0
RF	10	20.08.21	positive	negative	negative	positive	0,01	DNA	Thermofisher RAPIDFINDER EQUINE ID
SGS	16		positive	negative	negative	positive	0,1	Meat	SGS Specie ID
div	4		positive	negative	negative	positive		DNA	house method
div	7		positive	negative	negative	positive	0,10%	Meat	
div	17		positive	negative	negative	positive	0,1	DNA	house method

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	8	ASU L 06.26/27-2 (2007-12)	Cytochrom b-Gene	Extraction: Maxw ell FFS; Restriction analyses w ith MboI and DdI	minor signals present in PCR and restriction analysis in sample 3.1
CP	1	A-500-04		DNA Extraction:foodproof Sample Preparation Kit III, Biotecon (Best.Nr. S 400 06.1)	
CP	3	A-500-12	mitochondrial 16S rRNA	according testkit	
CP	13	A-500-04	DNA	Extraction/ PCR/ LCD Arrray	
RF	10	A15570			
SGS	16	-	?	NucleoMag KingFisher	
div	4				
div	7				
div	17	L08.00-61, 2016-02	cytochrom b	CTAB / Prot. K / FFS-Kit_Promega / Real Time PCR / 45 Cycles	

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
BP	15	04.08.21	positive	negative	negative	negative	0,1	Meat	Horse DNA detection KIT Biopremier lot:022106
GS	6	15.07.21	positive	negative	negative	positive		DNA	Euroins GeneScan Technologies
SFA-3p	9	15.07.21	positive	negative	negative	negative	0,10%	Meat	SureFood Animal ID 3plex Horse/Donkey (R-Biopharm AG)
SFA-3p	11	23.08.21	positive	negative	negative	negative	0,5	DNA	R-Biopharm AG - SureFood® ANIMAL ID 3plex - Horse/Donkey+IAAC
SFA-3p	14	06.07.21	positive	negative	negative	negative	0,1	Meat	SureFood Animal ID 3plex Horse/Donkey+IAAC Realtime Kit; Fa. Congen
SFA-4p	2		positive	negative	negative	negative	0,1	DNA	SureFood® ANIMAL ID 4plex Beef/Horse/Pork+IAAC
SGS	16		positive	negative	negative	negative	0,1	Meat	SGS Specie ID
div	5		positive	positive	negative	positive		DNA	house method
div	12	03.08.21	positive	positive	negative	positive	0,5	Meat	Realtime PCR (gotag probe qPCR mastermix)
div	17		positive	negative	negative	negative	0,1	DNA	house method

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	
BP	15		Equus Caballus DNA	Extraction according to protocol and subsequent PCR analysis	
GS	6			CTAB-Extraktion/ Mobispin/ RT-PCR/ 45 Cycles	
SFA-3p	9	S6119		Real Time PCR	
SFA-3p	11	Art. No. S6119, Lot 23500	-	"The test detects horse DNA (Equus caballus). DNA preparation w ith SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (35 cycles follow ing kit setup instructions) w ith Bio-Rad CFX96"	Internal Method accredited ISO/IEC 17025:2018
SFA-3p	14	S6119		Dneasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to kit manufacturer's protocol	
SFA-4p	2	S6126		SureFood® PREP Advanced Kit	
SGS	16	-	?	NucleoMag KingFisher	
div	5				Sample 4 traces
div	12	ISO/ TS 20224-6: 2020		Sure Food Prep Basis Extraction / Realtime PCR (Using Tag-man Probe)/ 45 Cycles	
div	17	L08.00-61, 2016-02	cytochrom b	CTAB / Prot. K / FFS-Kit_Promega / Real Time PCR / 45 Cycles	

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
GS	6	15.07.21	positive	negative	positive	positive		DNA	Euroins GeneScan Technologies
SFA-3p	9	15.07.21	positive	negative	negative	positive	0,10%	Meat	SureFood Animal ID 3plex Horse/Donkey (R-Biopharm AG)
SFA-3p	11	23.08.21	positive	negative	negative	positive	0,5	DNA	R-Biopharm AG - SureFood® ANIMAL ID 3plex - Horse/Donkey+IAAC
SFA-3p	14		positive	negative	negative	positive	0,1	Meat	SureFood Animal ID 3plex Horse/Donkey+IAAC Realtime Kit; Fa. Congen
SGS	16		positive	negative	negative	positive	0,1	Meat	SGS Specie ID
div	5		positive	negative	positive	positive		DNA	house method
div	17		positive	negative	negative	positive	0,1	DNA	house method

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	
GS	6			CTAB-Extraction/ Mobispin/ RT-PCR/ 45 Cycles	
SFA-3p	9	S6119		Real Time PCR	
SFA-3p	11	Art. No. S6119, Lot 23500	-	"The test detects donkey DNA (Equus asinus). DNA preparation w ith SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (35 cycles follow ing kit setup instructions) w ith Bio-Rad CFX96"	Internal Method accredited ISO/IEC 17025:2018
SFA-3p	14	S6119		Dneasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to kit manufacturer's protocol	
SGS	16	-	?	NucleoMag KingFisher	
div	5				Sample 3 traces
div	17	Literaturmethode	cytochrom b	CTAB / Prot. K / FFS-Kit_Promega / Real Time PCR / 45 Cycles	

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	8	14.07.21	positive	positive	negative	positive	0,01	DNA	
CP	1		positive	positive	negative	positive	0,1	DNA	LCD Array Kit Meat 5.0, Chipron
CP	3	15.07.21	positive	positive	negative	positive	0,1	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	13	26.07.	positive	positive	negative	positive	0,5	DNA	Chipron; LCD Array Kit Meat 5.0
GS	6	15.07.21	positive	positive	positive	positive		DNA	Euroins GeneScan Technologies
RF	10	20.08.21	positive	positive	negative	positive	0,01	DNA	Thermofisher RAPIDFINDER BEEF ID
SFA-4p	2		positive	positive	negative	negative	0,1	DNA	SureFood® ANIMAL ID 4plex Beef/Horse/Pork+IAAC
SFA-ID	11	23.08.21	positive	positive	negative	positive	0,5	DNA	R-Biopharm AG - SureFood® ANIMAL ID - Beef IAAC
SFA-ID	14	06.07.21	positive	positive	negative	positive	0,1	meat	SureFood Animal ID Beef IAAC Realtime Kit; Fa. Congen
SFA-Q	9	27.07.21	positive	positive	negative	positive	0,04%	meat	SureFood Animal Quant Beef (R-Biopharm AG)
SGS	16		positive	positive	negative	positive	0,1	meat	SGS Specie ID
div	4		positive	positive	negative	positive		DNA	house method
div	5		positive	positive	negative	positive		DNA	house method
div	7		positive	positive	negative	positive	0,10%	meat	
div	12a	26/07	positive	positive	negative	positive	0,5	meat	House method (multi-plex PCR, gotag probe qPCR master mix)
div	12b	26.07.21	positive	positive	negative	positive	0,5	meat	Realtime PCR (gotag qPCR mastermix)
div	17		positive	positive	negative	positive	0,1	DNA	house method

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	8	ASU L 08.00-61 (2016-03)	B-Actin-Gene EH 170825	Extraction: Maxw ell FFS	
CP	1	A-500-04		DNA Extraction:foodproof Sample Preparation Kit III, Biotecon (Best.Nr. S 400 06.1)	Beef sample 4 at the LOD
CP	3	A-500-12	mitochondrial 16S rRNA	according to kit instruction	
CP	13	A-500-04	DNA	Extraction/ PCR/ LCD Arrray	
GS	6			CTAB-Extraxtion/ Mobispin/ RT-PCR/ 45 Cycles	
RF	10	A24391			
SFA-4p	2	S6126		SureFood® PREP Advanced Kit	
SFA-ID	11	Art. No. S6113, Lot No 14469	-	"The test detects beef DNA (Bos taurus). DNA preparation w ith SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (35 cycles follow ing kit setup instructions) w ith Bio-Rad CFX96"	Internal Method accredited ISO/IEC 17025:2018
SFA-ID	14	S6113		Dneasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to kit manufacturer's protocol	
SFA-Q	9	S1010		Real Time PCR	
SGS	16	-	?	NucleoMag KingFisher	
div	4				
div	5				Sample 4 traces
div	7				
div	12a	04.2-CL4/ST 3.71 (T.Matsunaga et.al, 1999)	DNA-Length: 274 bp	Sure Food Prep Basis Extraction / PCR / 35 Cycles	Multiplex PCR
div	12b	ISO/ TS 20224-1: 2020		Sure Food Prep Basis Extraction / Realtime PCR (Using SYBR Green) / 45 Cycles	
div	17	L08.00-61, 2016-02	beta actin	CTAB / Prot. K / FFS-Kit_Promega / Real Time PCR / 45 Cycles	

5.1.11 DNA-based Methods: Pork

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	8	13.07.21	positive	negative	positive	positive	0,01	DNA	
CP	1		positive	negative	positive	positive	0,1	DNA	LCD Array Kit Meat 5.0, Chipron
CP	3	15.07.21	positive	negative	positive	positive	0,1	DNA	LCD Array Kit, Meat 5.0; Fa. Chipron
CP	13	26.07.	positive	negative	negative	positive	0,5	DNA	Chipron; LCD Array Kit Meat 5.0
GS	6	05.08.21	positive	positive	positive	positive		DNA	Euroins GeneScan Technologies
RF	10	20.08.21	positive	negative	positive	positive	0,01	DNA	Thermofisher RAPIDFINDER PORK ID
SFA-4p	2		positive	negative	positive	positive	0,5	DNA	SureFood® ANIMAL ID 4plex Beef/Horse/Pork+IAAC
SFA-ID	11	23.08.21	positive	negative	positive	positive	0,5	DNA	R-Biopharm AG - SureFood® ANIMAL ID - Pork IAAC
SFA-ID	14	06.07.21	positive	negative	positive	positive	0,1	meat	SureFood Animal ID Pork IAAC Realtime Kit; Fa. Congen
SFA-ID	15	04.08.21	positive	negative	negative	positive	0,1	meat	ANIMAL ID 4plex IAAC Pork/Chicken/Turkey R-Biopharm lot:21011
SFA-Q	9	13.08.21	positive	negative	positive	positive	0,04%	meat	SureFood Animal Quant Pork (R-Biopharm AG)
SGS	16		positive	negative	positive	positive	0,1	meat	SGS Specie ID
div	4		positive	negative	positive	positive		DNA	house method
div	5		positive	negative	positive	positive		DNA	house method
div	7		positive	negative	positive	positive	0,10%	meat	
div	12a	26/07	positive	negative	positive	positive	0,5	meat	in-house method (multi-plex PCR, gotag probe qPCR master mix)
div	12b	26.07.21	positive	negative	positive	positive	0,5	meat	Realtime PCR (gotag qPCR mastermix)
div	17		positive	negative	positive	positive	0,1	DNA	house method

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	8	ASU L 08.00-61 (2016-03)	B-Actin-Gene DQ452569	Extraktion: Maxwell FFS	
CP	1	A-500-04		DNS Extraktion:foodproof Sample Preparation Kit III, Biotecon (Best.Nr. S 400 06.1)	
CP	3	A-500-12	Mitochondrial 16S rRNA	according to testkit instruction	
CP	13	A-500-04	DNA	Extraction/ PCR/ LCD Arrray	
GS	6			CTAB-Extraktion/ Mobispin/ RT-PCR/ 45 Cycles	
RF	10	A24392			
SFA-4p	2	S6126		SureFood® PREP Advanced Kit	
SFA-ID	11	Art. No. S6114, Lot no 24131	-	"The test detects pork DNA (Sus scrofa). DNA preparation with SureFood® PREP Advanced (Principle according to protocol 2: Lysis at 65°C - Pre-filtration and setting of optimal binding conditions - Binding of the nucleic acids on a Spin Filter - Purification of the bound nucleic acids - Drying of the Spin Filter - First Elution of nucleic acids from the Spin Filter - Repeated setting of optimal binding conditions - Second binding of the nucleic acids on a Spin Filter - Second purification of the bound nucleic acids - Drying of the Spin Filter - Elution of nucleic acids from the Spin Filter for analysis) and real-time PCR (35 cycles following kit setup instructions) with Bio-Rad CFX96"	Internal Method accredited ISO/IEC 17025:2018
SFA-ID	14	S6114		Dneasy Mericon Food Kit; Qiagen; Real Time PCR 35 cycles according to kit manufacturer's protocol	
SFA-ID	15		Sus Scrofa DNA	Extraction according our protocol and PCR analysis	
SFA-Q	9	S1011		Real Time PCR	
SGS	16	-	?	NucleoMag KingFisher	
div	4				
div	5				
div	7				
div	12a	04.2-CL4/ST 3.71 (T.Matsunaga et.al, 1999)	DNA length: 398bp	Sure Food Prep basic extraction / PCR / 35 cycles	Multiplex PCR
div	12b	ISO/ TS 20224-3: 2020		Sure Food Prep basic extraction / Realtime PCR (Using SYBR Green) / 45 cycles	
div	17	L08.00-61, 2016-02	beta actin	CTAB / Prot. K / FFS-Kit_Promega / Real Time PCR / 45 Cycles	

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 ptAUS2 (2021)***
PT name	***Animal Species-Screening II – 4 Samples qualitative: Donkey, Beef, Horse, Pork and Poultry (Chicken and Turkey) in Meat Product Salami***
Sample matrix	*Samples 1-4:* *Salami products (some of them smoked)/ ingredients: various meat species, palm fat, salt, spices, maltodextrin, dextrose, sodium ascorbate, sodium nitrite, beech wood smoke.*
Number of samples and sample amount	*4 different Samples 1-4: 30 g each*
Storage	*Samples 1-4: frozen < -18°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative: Donkey, Beef, Horse, Pork and Poultry (Chicken and Turkey)* *Samples 1-4: appr. 1-100%*
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 August 27th 2021.***
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
		AUSTRIA
		ITALY
		Germany
		ITALY
		Germany
		ITALY
		FRANCE
		Germany
		AUSTRIA
		Germany
		SWITZERLAND
		Germany
		Germany
		Vietnam
		Germany
		Germany
		Great Britain

[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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