Evaluation Report

proficiency test

DLA 44/2019

Animal Species-Screening II:

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

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

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Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (26 November 2019) 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: 25 November 2019
Unteraufträge *Subcontractors*	Falls im Rahmen der Eignungsprüfung eine Prüfung der Gehalte, Homogenität und Stabilität von EP-Parametern durchgeführt wurde, hat DLA diese im Unterauftrag vergeben. In case the analysis of the content, homogeneity and stability of PT-parameters was part of the proficiency test, the determinations were subcontracted by DLA.
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.

Content

1. Introduction

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

2. Realisation

2.1 Test material

Four different samples with possible contents of heated animal foods of donkey, beef, horse, chicken and turkey were provided for qualitative determination. The parameters were present in the matrix of heated meat product (basis pork) with contents of 1 – 10%.

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	0,3 – 0,4 %
Sodium citrate*2H_2O	0,3 – 0,4 %
Pork gelatine (100% pork)*	3,4 – 3,8 %
Total meat content	69 - 74 %

*No gelatine has been added to sample 1.

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

Ingredients*	Sample 1	Sample 2	Sample 3	Sample 4
Pork meat	positive (69%)	positive (65%)	positive (62%)	positive (64%)
Horse meat	positive (4,9%)	negative	negative	negative
Donkey meat (dried)	negative	positive (6,5%**)	negative	negative
Turkey meat	negative	negative	positive (5,0%)	negative
Chicken meat	negative	negative	negative	positive (5,3%)
Beef meat	negative	negative	positive (4,0%)	negative

*Animal species contents of „food item" as indicated in the column of ingredients (with the exception of donkey meat s.**)according gravimetric mixing
** 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.*

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 all four samples 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 26th week of 2019. The testing method was optional. The tests should be finished at August 9th 2019 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 meat from **donkey, beef, horse, chicken and turkey**. The parameters are present in the matrix meat product **(pork base)** with contents of **1 - 10%**.

The evaluation of results is **strictly qualitative (positive / negative).**

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. 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 10 participants submitted their results in time.

3. Evaluation

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

3.2 Agreement with spiking of samples

The qualitative evaluation of the ELISA and PCR results of each participant was based on the agreement of the indicated results (positive or negative) with the **spiking of the four PT-samples**.
The assessment will be in the form that the number of matching results followed by the number of samples is indicated. Behind that the agreement is expressed as the percentage in parentheses.

4. Results

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

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

The participant results and evaluation are tabulated as follows:

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

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

4.1 Proficiency Test Poultry meat

4.1.1 ELISA-Results: Poultry meat

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		
5	negative	negative	positive	positive	-	4/4 (100%)	ETM	
9	negative	negative	positive	positive	-	4/4 (100%)	ETM3	

	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	none	none	none	none
Spiking	negative	negative	positive	positive

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

ETM3= ELISA-TEK™ Cooked Meat 3 Species Kit

Comments:

The results are in qualitative agreement with the spiking of samples 3 and 4.

A consensus value for consistent results independent of the spiking of the samples is not specified until at least 4 results are available.

4.1.2 PCR-Results: Poultry meat

Qualitative valuation of results

Chicken:

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	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
7	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	ASU	
3	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	BS	
6	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	CP	
2	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	MS	
1	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	RF	
10	negative	negative	positive	negative	2/4 (50%)	2/4 (50%)	SFA-4P	
9	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
5	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	
8	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Anzahl positive	0	0	1	9
Anzahl negative	10	10	9	1
Prozent positive	0	0	10	90
Prozent negative	100	100	90	10
Konsenswert	negative	negative	negative	positive
Dotierung	negative	negative	negative	positive

Methods:

ASU = ASU §64 Methode/method
BS= Qualyfast® MEAT ID, Bioside
CP = Chipron LCD Array Kit MEAT 5.0
MS = Microsynth
RF= RapidFinder™ ID Kit, ThermoFisher
SFA-4P= SureFood® ANIMAL ID 4plex, R-Biopharm / Congen
SFA-ID= SureFood Animal ID, R-Biopharm / Congen
div = not indicated / other method

Comments:
The consensus values of results are in qualitative agreement with the spiking of sample 4.

Participant 10 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.

Turkey:

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	negative	4/4 (100%)	4/4 (100%)	ASU	
7	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
6	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	CP	
2	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	MS	
1	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	RF	
10	negative	negative	negative	positive	2/4 (50%)	2/4 (50%)	SFA-4P	
9	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
5	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
8	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

BS= Qualyfast® MEAT ID, Bioside

CP = Chipron LCD Array Kit MEAT 5.0

MS = Microsynth

RF= RapidFinder™ ID Kit, ThermoFisher

SFA-4P= SureFood® ANIMAL ID 4plex, R-Biopharm / Congen

SFA-ID= SureFood Animal ID, R-Biopharm / Congen

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 3.

Participant 10 obtained a negative result for sample 3 and a positive result for sample 4.

4.2 Proficiency Test Horse meat

4.2.1 ELISA-Results: Horse meat

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		
5	positive	negative	negative	negative	-	4/4 (100%)	ETM	
9	positive	negative	negative	negative	-	4/4 (100%)	ETM	

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

Methods:

ETM = ELISA-TEK Cooked Meat species ELISA Kit

Comments:

The results are in qualitative agreement with the spiking of sample 1.

A consensus value for consistent results independent of the spiking of the samples is not specified until at least 4 results are available.

4.2.2 PCR-Results: Horse meat

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	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	ASU	
5	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	ASU	Cross-reactions to donkey cannot be excluded (differentiation not reliably possible); detection more equide-specific
7	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	ASU	Low traces (< 1% in L2) possible cross-reaction to donkey
3	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	BS	
6	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	CP	Horse and donkey are not distinguishable
2	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	MS	
1	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	RF	
10	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	SFA-4P	
9	positive	negative	negative	negative	3/3 (100%)	4/4 (100%)	SFA-ID	
8	positive	positive	negative	negative	3/3 (100%)	3/4 (75%)	div	Sample 2 is only w eakly positive, < 1%, could also be donkey

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	10	7	0	0
Number negative	0	3	10	10
Percent positive	100	70	0	0
Percent negative	0	30	100	100
Consensus value	positive	keiner	negative	negative
Spiking	positive	negative	negative	negative

Methods:

ASU = ASU §64 Methode/method

BS= Qualyfast® MEAT ID, Bioside

CP = Chipron LCD Array Kit MEAT 5.0

MS = Microsynth

RF= RapidFinder™ ID Kit, ThermoFisher

SFA-4P= SureFood® ANIMAL ID 4plex, R-Biopharm / Congen

SFA-ID= SureFood Animal ID, R-Biopharm / Congen

div = not indicated / other method

Comments:

The consensus values of the results for samples 1, 3 and 4 are in qualitative agreement with the spiking of sample 1.

For sample 2 (without addition of horse meat, but spiking with donkey meat) inconsistent results were obtained so that no consensus value ≥75% could be determined. Seven positive results were reported for sample 2, probably resulting from a cross-reaction with donkey.
Two participants indicated that a differentiation between horse and donkey is not possible with the applied method ASU or CP.
Two further participants have indicated a weak cross-reactivity. Possible cross reactivities should be documented in the test kit information of the manufacturers.

4.3 Proficiency Test Beef meat

4.3.1 ELISA-Results: Beef meat

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		
5	negative	negative	positive	negative	-	4/4 (100%)	ETM	
9	negative	negative	positive	negative	-	4/4 (100%)	ETM3	

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

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

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

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 3

A consensus value for consistent results independent of the spiking of the samples is not specified until at least 4 results are available.

4.3.2 PCR-Results: Beef meat

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	negative	4/4 (100%)	4/4 (100%)	ASU	
7	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	ASU	
3	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	BS	
6	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	CP	
2	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	MS	
1	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	RF	
10	negative	negative	negative	positive	2/4 (50%)	2/4 (50%)	SFA-4P	
9	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
5	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	
8	negative	negative	positive	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	0	9	1
Number negative	10	10	1	9
Percent positive	0	0	90	10
Percent negative	100	100	10	90
Consensus value	negative	negative	positive	negative
Spiking	negative	negative	positive	negative

Methods:

ASU = ASU §64 Methode/method

BS= Qualyfast® MEAT ID, Bioside

CP = Chipron LCD Array Kit MEAT 5.0

MS = Microsynth

RF= RapidFinder™ ID Kit, ThermoFisher

SFA-4P= SureFood® ANIMAL ID 4plex, R-Biopharm / Congen

SFA-ID= SureFood Animal ID, R-Biopharm / Congen

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 3.

Participant 10 reported a negative result for sample 3 and a positive result for sample 4.

4.4 Proficiency Test Donkey meat

4.4.1 ELISA-Results: Donkey meat

Qualitative valuation of results

No results were submitted for the parameter donkey by ELISA methods.

4.4.2 PCR-Results: Donkey meat

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	positive	positive	negative	negative	3/4 (75%)	3/4 (75%)	ASU	Specificity: donkey/horse
6	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	CP	
9	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-3P	
10	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
2	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
7	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	Low traces (approx. 0.1%) in L1 possibly cross reaction to horse

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	5	0	0
Number negative	5	1	6	6
Percent positive	17	83	0	0
Percent negative	83	17	100	100
Consensus value	negative	positive	negative	negative
Spiking	negative	positive	negative	negative

Methods:

ASU = ASU §64 Methode/method

CP = Chipron LCD Array Kit MEAT 5.0

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

SFA-4P= SureFood® ANIMAL ID 4plex, R-Biopharm / Congen

div = not indicated / other method

Comments:

The results of the participants are in qualitative agreement with the spiking of sample 2.

For sample 1 (no donkey meat added, but spiking with horse meat) a positive result was obtained. The participant points out that a differentiation between donkey and horse is not possible with the ASU method used.

4.5 Proficiency Test Pork meat

4.5.1 ELISA-Results: Pork meat

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		
5	positive	positive	positive	positive	-	4/4 (100%)	ETM	
9	positive	positive	positive	positive	-	4/4 (100%)	ETM3	

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

Methods:

ETM = ELISA-TEK™ Cooked Meat Species Kits

ETM3= ELISA-TEK™ Cooked Meat 3 Species Kit

Comments:

The results are in qualitative agreement with the basis "pork meat" of the samples.

A consensus value for consistent results independent of the spiking of the samples is not specified until at least 4 results are available.

4.5.2 PCR-Results: Pork meat

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	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
7	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
3	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	BS	
6	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP	
2	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	MS	
9	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
5	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
8	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

BS= Qualyfast® MEAT ID, Bioside

CP = Chipron LCD Array Kit MEAT 5.0

MS = Microsynth

SFA-ID= SureFood Animal ID, R-Biopharm / Congen

div = not indicated / other method

Comments:

The consensus values of 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 ELISA: Poultry meat

Primary data

Meth. Abr.	Evaluation-number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of Detection	Limit of detection given as	Method
		qualitative	qualitative	qualitative	qualitative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ETM	5	negative	negative	positive	positive	2	meat	r-biopharm/ Elisa Technologies
ETM3	9	negative	negative	positive*	positive*	0,01	protein	Cooked Meat ELISA Kit Beef, Pork,Poultry from ELISA Technologies/Distribution via R-biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	5			according to instructions	sample 4 (poultry) w eak positive
ETM3	9	510603		according to instructions	*The ELISA-TEK does not differentiate betw een chicken and turkey, it recognizes both as "poultry".

5.1.2 ELISA: Horse meat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ETM	5	positive	negative	negative	negative	2	meat	r-biopharm/ Elisa Technologies
ETM	9	positive	negative	negative	negative	0,01	protein	Cooked Meat Horse Kit from ELISA Technologies/Distribution via R-biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	5			according to instructions	w eak cross-reactivity to donkey
ETM	9	510651		according to instructions	

5.1.3 ELISA: Beef meat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ETM	5	negative	negative	positive	negative	2	meat	r-biopharm/ Elisa Technologies
ETM3	9	negative	negative	positive	negative	0,01	protein	Cooked Meat ELISA Kit Beef, Pork,Poultry von ELISA Technologies/Distribution via R-biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	5			according to instructions	
ETM3	9	510603		according to instructions	

5.1.4 ELISA: Donkey

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ETM	9	-	-	-	-			Cooked Meat Horse Kit von ELISA Technologies/Distribution via R-biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	9	510651		according to instructions	The ELISA TEK is suitable for horses, but can also have cross reactions with donkey. In sample 2 there was an extinction close to the cut off, suggesting that sample 2 might contain donkey. But officially no statement about donkey in all 4 samples is made by ELISA.

5.1.5 ELISA: Pork meat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detec-tion given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ETM	5	positive	positive	positive	positive	2	meat	r-biopharm/ Elisa Technologies
ETM3	9	positive	positive	positive	positive	0,01	protein	Cooked Meat ELISA Kit Beef, Pork,Poultry von ELISA Technologies /Distribution via R-biopharm

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
ETM	5			according instructions	
ETM3	9			according instructions	

5.1.8 PCR: Poultry meat

Primary data ***Chicken***

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	negative	negative	negative	positive	0,1	DNA	
ASU	7	negative	negative	negative	positive	0,001	DNA	RT-PCR, in-house method
BS	3	negative	negative	negative	positive	0,001	w/w	Nucleo Spin Food - MN Qualyfast® MEAT ID- Bioside
CP	6	negative	negative	negative	positive		DNA	LCD Array Kit Meat 5.0; Fa. Chipron
MS	2	negative	negative	negative	positive	0,005	DNA	Microsynth
RF	1	negative	negative	negative	positive	0,1	meat	Thermo Fisher Rapidfinder Kit
SFA-4P	10	negative	negative	positive	negative	0,1	DNA	SureFood Animal 4plex Pork/Chicken/Turkey+IAAC
SFA-ID	9	negative	negative	negative	positive	0,1	meat	SureFood Animal ID Chicken IAAC; Congen/R-biopharm
div	5	negative	negative	negative	positive	1	meat	PCR-RFLP according to literature method (Meyer et al., 1995 - modified according to §64 LFGB RV-Script) using optimized cytochrome b gene specific consensus primer for poultry
div	8	negative	negative	negative	positive	0,001	DNA	in-house method

Other details to the Methods ***Chicken***

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	L 08.00-61	Chicken	CTAB-Extraction, Taqman PCR, 45 Cycles	
ASU	7	L08.00-61 / 2016-03	Cytochrome B	CTAB/Proteinase K/Chloroform Extraction/FFS-Kit Promega/RT-PCR/45 Cycles	
BS	3	FC140945L-A3.01017			
CP	6	A-500-12			
MS	2	1204	TF-GB3X6009	DNA Extraction with Proteinase K, Clean Up with Chloroform and Columns /Amplifm Real-Time PCR 45 Cycles	
RF	1	A24393		Thermo Fisher GVO Extraction Kit 4466336	
SFA-4P	10	S6132	Gallus gallus	SureFood Prep Basic	
SFA-ID	9	S6115		200mg sample, DNeasy Mericon Food; Qia-gen; Real Time PCR 35 Cycles	additional detection by using LCD array, Meat 5.0, company Chipron
div	5		cytb (359 bp)	Extraction according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidinium chloride buffer with Proteinase K, purification with Wizard-Kit from Promega); conventional PCR with 30 cycles and subsequent RFLP analysis	
div	8		Cytochrome B	Real Time PCR	

Primary data ***Turkey***

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	negative	negative	positive	negative	0,1	DNA	
ASU	7	negative	negative	positive	negative	0,001	DNA	RT-PCR, in-house method
CP	6	negative	negative	positive	negative		DNA	LCD Array Kit Meat 5.0; Fa. Chipron
MS	2	negative	negative	positive	negative	0,005	DNA	Microsynth
RF	1	negative	negative	positive	negative	0,1	meat	Thermo Fisher Rapidfinder Kit
SFA-4P	10	negative	negative	negative	positiv	0,1	meat	SureFood Animal 4plex Pork/Chicken/Turkey+IAAC
SFA-ID	9	negative	negative	positive	negative	0,1	DNA	SureFood Animal ID Turkey IAAC; Congen/R-biopharm
div	5	negative	negative	positive	negative	1	meat	PCR-RFLP according to literature method (Meyer et al., 1995 - modified according to §64 LFGB RV-Script) using optimized cytochrome b gene specific consensus primer for poultry
div	8	negative	negative	positive	negative	0,001	DNA	in- house method

Other details to the Methods ***Turkey***

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	L 08.00-61	turkey	CTAB-Extraction, Taqman PCR, 45 Cycles	
ASU	7	L08.00-61 / 2016-03	prolactin receptor	CTAB/Proteinase K/Chloroform Extraction/FFS-Kit Promega/RT-PCR/45 Cycles	
CP	6	A-500-12			
MS	2	1204	prolactin receptor, L76587	DNA Extraction with Proteinase K, Clean Up with Chloroform and Columns /Amplif m RealTIme PCR 45 Cycles	
RF	1	A24394		Thermo Fisher GVO Extraction Kit 4466336	
SFA-4P	10	S6132	Meleagris gallopavo	SureFood Prep Basic	
SFA-ID	9	S6116		200mg sample, DNeasy Mericon Food; Qiagen; Real Time PCR 35 Cycles	additional detection by using LCD array, Meat 5.0, company Chipron
div	5		cytb (359 bp)	Extraction according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidinium chloride buffer with Proteinase K, purification with Wizard Kit from Promega); conventional PCR with 30 cycles and subsequent RFLP analysis	
div	8		Cytochrome B	Real Time PCR	

5.1.9 PCR: Horse meat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	positive	positive	negative	negative	0,1	DNA	
ASU	5	positive	positive	negative	negative	0,1	meat	PCR-RFLP according to § 64 LFGB Method L 06.26/27-2 (December 2007)
ASU	7	positive	negative	negative	negative	0,001	DNA	RT-PCR, in-house method
BS	3	positive	positive	negative	negative	0,001	w/w	Nucleo Spin Food - MN Qualyfast® MEAT ID- Bioside
CP	6	positive	positive	negative	negative		DNA	LCD Array Kit Meat 5.0; Fa. Chipron
MS	2	positive	negative	negative	negative	0,005	DNA	Microsynth
RF	1	positive	positive	negative	negative	0,1	meat	Thermo Fisher Rapidfinder Kit
SFA-4P	10	positive	positive	negative	negative	0,1	meat	SureFood Animal 4plex Camel/Horse/Donkey+IAAC
SFA-ID	9	positive	negative	negative	negative	0,1	DNA	SureFood Animal ID 3plex Horse/Donkey + IAAC; Congen/R-biopharm
div	8	positive	positive	negative	negative	0,001	DNA	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	L 08.00-62	donkey/ horse	CTAB-Extraction, Taqman PCR, 45 Cycles	
ASU	5	L 06.26/27-2 (dezember 2007)	cytb (146 bp)	Extraction according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidinium chloride buffer with Proteinase K, purification with Wizard-Kit from Promega); conventional PCR with 40 cycles and subsequent RFLP analysis	Cross-reactions to donkey cannot be excluded (differentiation not certain possible); detection more equide-specific
ASU	7	L08.00-62 / 2016-03	growth hormone receptor	CTAB/Proteinase K/Chloroformextraktion/FFS-Kit Promega/RT-PCR/45 Cycles	low traces (<1% in L2) possibly cross reaction to donkey
BS	3	FC140945L - A3.01017			
CP	6	A-500-12			Horse and donkey not distinguishable
MS	2	1206	growth hormone receptor	DNA Extraction with Proteinase K, Clean Up with Chloroform and Columns /Amplif m RealTIme PCR 45 Cycles	
RF	1	A15570		Thermo Fisher GVO Extraction Kit 4466336	
SFA-4P	10	S6131	Equus caballus	SureFood Prep Basic	
SFA-ID	9	S6119		200 mg sample, DNeasy Mericon Food; Qiagen; Real Time PCR 35 Cycles	additional detection by using LCD array, Meat 5.0, company Chipron
div	8		Cytochrome B	Real Time PCR	Sample 2 is only weakly positive, <1%, could also be donkey

5.1.10 PCR: Beef meat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	negative	negative	positive	negative	0,1	DNA	
ASU	7	negative	negative	positive	negative	0,001	DNA	RT-PCR, in-house method
BS	3	negative	negative	positive	negative	0,001	w/w	Nucleo Spin Food - MN Qualyfast® MEAT ID-Bioside
CP	6	negative	negative	positive	negative		DNA	LCD Array Kit Meat 5.0; Fa. Chipron
MS	2	negative	negative	positive	negative	0,005	DNA	Microsynth
RF	1	negative	negative	positive	negative	0,1	meat	Thermo Fisher Rapidfinder Kit
SFA-4P	10	negative	negative	negative	positive	0,1	meat	SureFood Animal 4plex Beef/Sheep/Goat+IAAC
SFA-ID	9	negative	negative	positive	negative	0,1	DNA	SureFood Animal ID Beef IAAC; Congen/R-biopharm
div	5	negative	negative	positive	negative	1	meat	PCR-RFLP according to literature method (Wolf et al., 1999) using cytochrome b gene specific consensus primer
div	8	negative	negative	positive	negative	0,001	DNA	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	L 08.00-61	beef	CTAB extraction, Taqman PCR, 45 cycles	
ASU	7	L08.00-61 / 2016-03	b-Actine	CTAB/Proteinase K/Chloroformextraction/FFS-Kit Promega/RT-PCR/45 cycles	
BS	3	FC140945L-A3.01017			
CP	6	A-500-12			
MS	2	1206	Beta-actine-gene EH170825	DNA Extraction with Proteinase K, Clean Up with Chloroform and Columns /Amplif m RealTime PCR 45 Cycles	
RF	1	A24391		Thermo Fisher GVO Extraction Kit 4466336	
SFA-4P	10	S6121	Bos taurus	SureFood Prep Basic	
SFA-ID	9	S6113		200 mg sample, DNeasy Mericon Food; Qiagen; Real Time PCR 35 Cycles	additional detection by using LCD array, Meat 5.0, company Chipron
div	5		cytb (464 bp)	Extraction according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidinium chloride buffer with Proteinase K, purification with Wizard-Kit from Promega); conventional PCR with 40 cycles and subequent RFLP analysis	
div	8		Satellite IV DNA	Real Time PCR	

5.1.11 PCR: Donkey meat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	positive	positive	negative	negative	0,1	DNA	
CP	6	negative	negative	negative	negative		DNA	LCD Array Kit Meat 5.0; Fa. Chipron
SFA-3P	9	negative	positive	negative	negative	0,1	DNA	SureFood Animal ID 3plex Horse/Donkey + IAAC; Congen/R-biopharm
SFA-4P	10	negative	positive	negative	negative	0,1	meat	SureFood Animal 4plex Camel/Horse/Donkey+IAAC
div	2	negative	positive	negative	negative	0,005	DNA	in-house method
div	7	negative	positive	negative	negative	0,001		RT-PCR, in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	4	L 08.00-62	donkey/horse	CTAB-Extraction, Taqman PCR, 45 Cycles	
CP	6	A-500-12			
SFA-3P	9	S6119		200mg sample, DNeasy Mericon Food; Qiagen; Real Time PCR 35 Cycles	additional detection by means of LCD array, Meat 5.0, company Chipron
SFA-4P	10	S6131	Equus asinus	SureFood Prep Basic	
div	2		X97337, mitochondrial cytochrome b	DNA Extraction with Proteinase K, Clean Up with Chloroform and Columns /Amplif m RealTime PCR 45 Cycles	
div	7	literature method	cytochrome b	CTAB/Proteinase K/Chloroformextraction/FFS-Kit Promega/RT-PCR/45 cycles	Low traces (approx. 0.1%) in L1 possibly cross reaction to horse

5.1.12 PCR: Pork meat

Primary data

Meth. Abr.	Evaluation number	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
		positive / negative	positive / negative	positive / negative	positive / negative	mg/kg	e.g. food / food protein	Test-Kit + Provider
ASU	4	positive	positive	positive	positive	0,1	DNA	
ASU	7	positive	positive	positive	positive	0,001	DNA	RT-PCR, in-house method
BS	3	positive	positive	positive	positive	0,001	w/w	Nucleo Spin Food - MN Qualyfast® MEAT ID- Bioside
CP	6	positive	positive	positive	positive		DNA	LCD Array Kit Meat 5.0; Fa. Chipron
MS	2	positive	positive	positive	positive	0,01	DNA	Microsynth
SFA-ID	9	positive	positive	positive	positive	0,5	DNA	SureFood Animal ID Pork IAAC; Congen/R-biopharm
div	5	positive	positive	positive	positive	1	meat	PCR-RFLP according to literature method (Meyer et al., 1995) using cytochrome b gene specific consensus primer
div	8	positive	positive	positive	positive	0,001	DNA	in-house method

Other details to the Methods

Meth. Abr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No. / ASU-No.	Target-DNA	e.g. Extraction / Enzymes / Clean-Up / Real Time PCR / Gel electrophoresis / Cycles	
ASU	12	L 08.00-61	pork	CTAB-Extraction, Taqman PCR, 45 Cycles	
ASU	7	L08.00-61 / 2016-03	b-Actin	CTAB/Proteinase K/Chloroform Extraction/FFS-Kit Promega/RT-PCR/45 Cycles	
BS	14	FC140945L-A3.01017			
CP	17	A-500-12			
MS	24	1206	Beta-actin gene DQ452569	DNA Extraction with Proteinase K, Clean Up with Chloroform and Columns /Amplif m RealTime PCR 45 Cycles	
SFA-ID	25	S6114		200 mg sample, DNeasy Mericon Food; Qiagen; Real Time PCR 35 Cycles	additional detection by using LCD array, Meat 5.0, company Chipron
div	28		cytb (359 bp)	Extraction according to ASU § 64 LFGB L 15.05-1 (SDS/Guanidinium chloride buffer with Proteinase K, purification with Wizard-Kit from Promega); conventional PCR with 35 cycles and subsequent RFLP analysis	
div	31	in-house method	Cytochrome B	Real Time PCR	

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	229,0
2	237,5
3	237,5
4	250,9
5	250,8

General average	241,1	
Reapeatability standard deviation	9,52	3,9%

Homogeneity test Sample 2

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%

Homogeneity test Sample 3

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 4

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%

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 44-2019***
PT name	***Allergen-Screening II - 4 Samples qualitative: Donkey, Beef, Horse, Chicken and Turkey in meat product (Pork)***
Sample matrix	*Samples 1-4:* *Sausage meat (heated)/ Ingredients: pork, water, gelatine (pork), salt, sodium citrate and other meat species*
Number of samples and sample amount	*4 different Samples 1-4: 25 g each*
Storage	*Samples A + B: cooled 2 - 10°C (long term frozen < -18°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative:* ***Donkey, Beef, Horse, Chicken and Turkey in meat product (Pork)*** *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***
Deadline	***the latest 23th August 2019***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Alexandra Scharf M.Sc.*

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

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		Germany
		Germany
		Germany
		Germany
		Germany
		Italy
		Germany
		AUSTRIA
		GREAT BRITAIN
		Germany

[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.]

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