Evaluation Report

proficiency test

DLA ptAUS3 (2020)

Animal Species-Screening III:

Buffalo, Cow's, Sheep's and Goat's Milk in Dairy Product (Mozzarella and Herder Cheese)

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

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

Coordinator of this PT:
Alexandra Scharf MSc.

1st Correction 09/12/2020:
A transfer error has occurred in the table "DNA-based results sheep" (p. 13): For participant 4 and participant 6 there were positive results for the evaluation of sample 1, which are missing in the table. The table was accordingly corrected.

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

EP-Anbieter *PT-Provider*	**DLA - Proficiency Tests GmbH** Kalte Weide 21, 24641 Sievershütten, Germany Geschäftsführer/CEO: Dr. Matthias Besler-Scharf Stellv. Leitung/Deputy Lead: Alexandra Scharf MSc. Tel. ++49-(0)4532-9183358 Mob. ++49(0)171-1954375 Fax. ++49(0)4102-9944976 eMail. proficiency-testing@dla-lvu.de
EP-Nummer *PT-Number*	DLA ptAUS3 (2020)
EP-Koordinator *PT-Coordinator*	Alexandra Scharf MSc.
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (9. Dezember 2020) 1. Korrektur / 1st Correction Gültig ist die jeweils letzte Version/Korrektur des Berichts. Sie ersetzt alle vorangegangenen Versionen. Only the latest version/correction of the report is valid. It replaces all preceding versions.
EP-Bericht Freigabe *PT-Report Authorization*	Dr. Matthias Besler-Scharf (Technischer Leiter / Technical Manager) *- gezeichnet / signed M. Besler-Scharf* Alexandra Scharf MSc. (QM-Beauftragte / Quality Manager) *- gezeichnet / signed A. Scharf* Datum / Date: 9. Dezember 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung 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.

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 PT samples with possible contents of buffalo milk and cow's milk in the matrix mozzarella and cow's milk, sheep's milk and goat's milk in the matrix herder cheese were provided for qualitative determination. The parameters were present in the respective milk product matrix with contents of 9 - 13%.

The raw materials for the animal species used were commercial herder cheese and mozzarella preparations, each made exclusively from the milk of one animal species. The corresponding quantitative amounts of raw materials for each sample (see Table 1) were minced using a cutter, mixed thoroughly and stirred until a creamy, homogeneous mixture was obtained. The samples were lyophilized and then again minced and homogenized. The samples were filled into plastic containers in portions of about 25 g.

Table 1: Contents (in %) of the respective animal species in the herder cheese samples (1-2) and mozzarella samples (3-4).

Ingredients*	Sample 1	Sample 2	Sample 3.1	Sample 4
Cow's milk herder cheese	positive (91%)	positive (87%)	negative	negative
Goat's milk herder cheese	negative	positive (13%)	negative	negative
Sheep's milk herder cheese	positive (9%)	negative	negative	negative
Cow's milk mozzarella	negative	negative	positive (89%)	positive (11%)
Buffalo milk mozzarella	negative	negative	positive (11%)	positive (89%)

**Animal species contents of „food item" as indicated in the column of ingredients according gravimetric mixing*
Note: *The metrological traceability of temperature, mass and volume during production of the PT samples is ensured by DAkkS calibrated reference materials.*

The identification of the respective animal species in the samples was carried out using the DNA-based LCD array kit MEAT 5.0 (Chipron GmbH) and corresponds to the spiking of the LVU samples 1-4 (see Tab. 2).

Table 2: Verification of detectability of the present animal species by LCD Array Kit MEAT 5.0 (Chipron GmbH)

chipron	LCD-Array Kit MEAT 5.0*			
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
Rind / Cattle	positive	positive	positive	positive
Ziege / Goat	negative	positive	negative	negative
Schaf / Sheep	positive	negative	negative	negative
Wasserbüffel / Water Buffalo	negative	negative	positive	positive

*LCD-Array Kit MEAT 5.0 Limit of Detection: 0.5% (w/w)

2.1.2 Stability

A water activity (a_W) of < 0,5 is an important factor to ensure the stability of dry or dried products during storage. Optimum conditions for storage is the a_W value range of 0,15 - 0,3. In this range the lowest possible degradation rate is to be expected [16].
The experience with various DLA test materials showed good storage stability with respect to the durability of the sample (spoilage) and the content of the PT parameters for comparable food matrices and water activity (a_W value <0,5).
The a_W value of the PT samples was approx. 0,31 - 0,35 (21-22°C). The stability of the sample material was thus ensured during the investigation period under the specified storage conditions.

2.2 Sample shipment and information to the test

The portions of the test materials (sample 1 to 4) were sent to every participating laboratory in the 29th week of 2020. The testing method was optional. The tests should be finished at September 25th 2020 the latest.

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

There are 4 different samples possibly containing Buffalo and Cow's Milk (in the matrix of Mozzarella) and Cow's, Sheep's and Goat's Milk (in the matrix of Herder Cheese).The parameters are contained in the related matrix with amounts of 5 – 20%.

Analytical methods for determination are optional. The evaluation of results is ***strictly qualitative (positive / negative).***

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

Before analysis, the entire sample quantity should be homogenized, since components such as fat can separate during the production/processing of the samples.

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

17 of 18 participants submitted at least one result in time. One participant did not submit any results.

3. Evaluation

Different protein-based methods (e.g. isoelectric focusing, ELISA) and DNA-based methods for the determination of animal species in foods are eventually using different pH-gradients, antibodies and target-DNA, are usually calibrated with different reference materials and may utilize differing extraction methods. Among others this can induce different valuation of the presence and/or content of the analyte. Furthermore, matrix and/or processing as well as storage and maturing time (for cheese) can strongly influence the detectability of animal species [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 Buffalo Milk Cheese

4.1.1 DNA-based Results: Buffalo

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	
8	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP	
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RF	
15	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RF	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-ID	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SGS	
17	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SGS	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
14			positive	positive	2/2 (100%)	2/2 (100%)	div	

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

Methods:

CP = Chipron LCD Array Kit MEAT 5.0

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 3 (11% buffalo milk mozzarella) and sample 4 (89% buffalo milk mozzarella).

All participants obtained positive results for samples 3 and 4.

4.1.2 Protein-based Results: Buffalo

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		
9				positive	1/1 (100%)	1/1 (100%)	MALDI-TOF-MS	
12	negative	negative	negative	positive	1/1 (100%)	3/4 (75%)	MALDI-TOF-MS	

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

Methods:

MALDI-TOF-MS= Matrix Assisted Laser Desorption Ionization — Time of Flight Mass Spectrometry

Comments:

The results of the two participants are in qualitative agreement with the spiking of sample 4 (89% buffalo milk mozzarella).
For the lower spiked sample 3 (11% buffalo milk mozzarella) participant 12 obtained a negative result, while participant 9 received no result for sample 3.

No consensus values could be determined for samples 1-3, as only one result was available.

4.2 Proficiency Test Cow's Milk Cheese

4.2.1 DNA-based Results: Cow

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP	
2	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP	
8b	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP	
13a	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	EF-ID	
12	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	GI-2	
8a	positive	positive			2/2 (100%)	2/2 (100%)	MS	
11	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	RF	
15	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	RF	
3	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
4	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
16	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
13b	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	SGS	
17	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	SGS	
6	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	div	
7	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
9	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
14	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	Result Sample 4 is not secured

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	17	17	16	15
Number negative	0	0	0	1
Percent positive	100	100	100	94
Percent negative	0	0	0	6
Consensus value	positive	positive	positive	positive
Spiking	positive	positive	positive	positive

Methods:

CP = Chipron LCD Array Kit MEAT 5.0

EF-ID= DNAnimal Ident IPC, Eurofins

GI-2= GEN-IAL® First-duplex PCR kit

MS= Microsynth

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of the samples 1-2 (cow's milk herder cheese) and samples 3-4 (cow's milk mozzarella).

For the lower spiked sample 4 (11% cow's milk mozzarella) one participant obtained a negative result with an unspecified method (div).

4.2.2 Protein-based Results: Cow

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		
14	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	EP	
5	positive	positive	positive		3/3 (100%)	3/3 (100%)	IEF	No differentiation of buffalo/cow
9	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	MALDI-TOF-MS	
12	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	MALDI-TOF-MS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	4	4	4	3
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:

EP = EuroProxima ELISA Bovine Milk

IEF = Isoelektrische Fokussierung

MALDI-TOF-MS= Matrix Assisted Laser Desorption Ionization — Time of Flight Mass Spectrometry

Comments:

The consensus values of results are in qualitative agreement with the spiking of the samples 1-2 (cow's milk herder cheese) and samples 3-4 (cow's milk mozzarella).

Participant 5 points out that a differentiation of buffalo and cow's milk is not yet possible with the IEF method used.

4.3 Proficiency Test Sheep's Milk Cheese

4.3.1 DNA-based Results: Sheep

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
8b	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP	
12	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	GI	
8a	positive				1/1 (100%)	1/1 (100%)	MS	
11	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	RF	
15	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	RF	
3	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	SFA-4P	
4	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	QE to Springbok (Antidorcas marsupialis) 100%
16	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
13	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SGS	
17	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SGS	
6	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
9	positive		negative	negative	3/3 (100%)	3/3 (100%)	div	
14	questionable						div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	14	0	0	0
Number negative	1	13	14	14
Percent positive	93	0	0	0
Percent negative	7	100	100	100
Consensus value	positive	negative	negative	negative
Spiking	positive	negative	negative	negative

Methods:

CP = Chipron LCD Array Kit MEAT 5.0

GI= GEN-IAL® First-Meat PCR kit

MS = Microsynth

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of the sample 1 (9% sheeps's milk herder cheese).

One participant received a negative result for sample 1 using the SFA-4P method. Participant 14 could not get a clear result for the parameter sheep in sample 1.

4.3.2 Protein-based Results: Sheep

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	3/3 (100%)	4/4 (100%)	IEF	
9	positive	negative	positive	negative	3/3 (100%)	3/4 (75%)	MALDI-TOF-MS	traces of sheep's cheese

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	2	0	1	0
Number negative	0	2	1	2
Percent positive	100	0	50	0
Percent negative	0	100	50	100
Consensus value	positive	negative	none	negative
Spiking	positive	negative	negative	negative

Methods:

IEF = Isoelektrische Fokussierung

MALDI-TOF-MS= Matrix Assisted Laser Desorption Ionization — Time of Flight Mass Spectrometry

Comments:

The consensus values of the results for samples 1, 2 and 4 are in qualitative agreement with the spiking of the sample 1 (9% sheeps's milk herder cheese).

For the unspiked sample 3 a postive and a negative result were obtained.

4.4 Proficiency Test Goat's Milk Cheese

4.4.1 DNA-based Results: Goat

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
1	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP	
2	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP	
8b	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP	
12	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	GI	
8a		positive			1/1 (100%)	1/1 (100%)	MS	
11	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	RF	
15	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	RF	
3	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
4	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
16	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
13	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SGS	
17	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SGS	
6a	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
6b	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
7	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
9	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
14		positive			1/1 (100%)	1/1 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	17	0	0
Number negative	15	0	15	15
Percent positive	0	100	0	0
Percent negative	100	0	100	100
Consensus value	negative	positive	negative	negative
Spiking	negative	positive	negative	negative

Methods:

CP = Chipron LCD Array Kit MEAT 5.0

GI= GEN-IAL® First-Meat PCR kit

MS = Microsynth

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of the sample 2 (13% goat's milk herder cheese).

4.4.2 Protein-based Results: Goat

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	positive	negative	negative		4/4 (100%)	IEF	

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

Methods:

IEF = Isoelektrische Fokussierung

Comments:

The results of participant 5 are in qualitative agreement with the spiking of sample 2, as well as with the results of the DNA-based methods.

4.5 Proficiency Test Cattle Detection

4.5.1 DNA-based Results: Cattle (Buffalo/ Cow)

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
10	positive	positive	positive	positive		4/4 (100%)	div	

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

Methods:

div = not indicated / other method

4.5.2 Protein-based Results: Cattle (Buffalo/ Cow)

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	positive	positive	positive	positive		4/4 (100%)	EP	Test does not discriminate between milk from cattle and milk from buffalo

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

Methods:

EP = EuroProxima ELISA Bovine Cheese

4.6 Proficiency Test Ruminant Detection

4.6.1 DNA-based Results: Ruminant

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
10	positive	positive	positive	positive		4/4 (100%)	div	

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

Methods:

div = not indicated / other method

5. Documentation

5.1 Details by the participants

Note: Information given in German was translated by DLA to the best of our knowledge (without guarantee of correctness).

5.1.1 DNA-based Methods: Buffalo

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
CP	1	28./29.07.	negative	negative	positive	positive	1%	DNA	Chipron
CP	2		negative	negative	positive	positive			DNA Chip (Chipron)
CP	8		negative	negative	positive	positive	100-250 fg	DNA	Chipron MEAT 5.0 LCD-Array Kit
RF	11	4.9.20	negative	negative	positive	positive	0,1	DNA	RapidFinder Water Buffalo ID Kit, Thermofisher
RF	15		negative	negative	positive	positive	2	DNA	Imegen Rapid Finder
SFA-ID	4	30.7.20	negative	negative	positive	positive	0,1	Food/ Meat	SureFood® Animal ID Water Buffalo IAAC
SGS	13		negative	negative	positive	positive	0,05	Number of reads	All Species ID Meat DNA Anlyser Kit; SGS Molecular
SGS	17		negative	negative	positive	positive	0,3	DNA	All Species ID, SGS MOLECULAR
div	6	13.8.20	negative	negative	positive	positive			house method (conv. PCR-RFLP using consensus primer primer according to Meyer et al.,1995)
div	7	30.7.20	negative	negative	positive	positive	1	DNA	
div	14	18.9.20	-	-	positive	positive	0,05	ng DNA/ PCR	Jürg Rentsch et al.; Eur Food Res Technol (2013) 236:217–227

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
CP	1	A-300-12	mitochondrial 16S rRNA	Implementation according to kit instruction. but with only 30 PCR cycles	
CP	2				
CP	8	A-500-04/-12		CTAB-Extraction	
RF	11	N/A	N/A	GMO Extraction Kit, Real-time PCR	
RF	15		DNA	as per kit instructions	
SFA-ID	4	S6117	Bubalus arnee	SureFood® Prep Basic	K01
SGS	13			Extraction with Macherey Nagel NucleoSpin Food Kit, quantification with Qubit Assay, PCR with All Species ID Kit, gel electrophoresis), purification with AMPure xp magnetic beads, next generation sequencing on Ion Torrent platform (Ion Chef + Ion S5)	
SGS	17			Marchery-Nagel NucleoMag	LOD 130 in 43320 pg
div	6		cytb (359 bp)	Extractions according to ASU §64LFGB L15.05-1 1. SDS / guanidinium chloride buffer with ProtK, purification using the Wizard kit from Promega; 2. CTAB based with ProtK and glycogen; Convention. PCR with 35 cycles and subsequent restriction analysis	LOD for matrices dairy products/ cheeses not conclusively validated (missing control / reference materials)
div	7				
div	14			Wizard; real-time PCR	not examined

5.1.2 DNA-based Methods: Cow

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
CP	1	28./29.07.	positive	positive	positive	positive	1%	DNA	Chipron
CP	2		positive	positive	positive	positive			DNA Chip (Chipron)
CP	8b		positive	positive	positive	positive	100-250 fg	DNA	Chipron MEAT 5.0 LCD-Array Kit
EF-ID	13a		positive	positive	positive	positive	0,01	DNA	DNAnimal Ident Beef IPC; Eurofins
GI-2	12	14.8.20	positive	positive	positive	positive	0,01	DNA	GEN-IAL® First-Cattle PCR Kit
MS	8a		positive	positive					AllMilch, Microsynth
RF	11	4.9.20	positive	positive	positive	positive	0,1	DNA	RapidFinder Beef ID Kit, Thermofisher
RF	15		positive	positive	positive	positive	2	DNA	Imegen Rapid Finder
SFA-4P	3	22.7.20	positive	positive	positive	positive	0,05	Food	Surefood ANIMAL ID Beef/Sheep/Goat (r-biopharm)
SFA-4P	4	30.7.20	positive	positive	positive	positive	0,1	Food/ Meat	SureFood® Animal ID 4plex Beef/Sheep/ Goat+IAAC
SFA-4P	16	23.9.20	positive	positive	positive	positive	0,1	DNA	SureFood Animal ID 4plex Beef/Sheep/Goat
SGS	13b		positive	positive	positive	positive	0,05	Number of reads	All Species ID Meat DNA Anlyser Kit; SGS Molecular
SGS	17		positive	positive	positive	positive	0,3	DNA	All Species ID, SGS MOLECULAR
div	6	18.8.20	positive	positive	positive	negative			house method (conv. PCR-RFLP using con-sensus primer-primer according to Wolf et al.,1999)
div	7	30.7.20	positive	positive	positive	positive	1	DNA	
div	9		positive	positive	positive	positive	0,1	DNA	Rentsch et al; European Food Research and Technology 2013
div	14	18.9.20	positive	positive	positive	positive	0,05	ng DNA/ PCR	Jürg Rentsch et al.; Eur Food Res Technol (2013) 236:217–228

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
CP	1	A-300-13	mitochondrial 16S rRNA	Implementation according to kit instructions, but with only 30 PCR cycles	
CP	2				
CP	8b	A-500-04/-12		CTAB-Extraction	
EF-ID	13a	5422220610			
GI-2	12	Art. No.: PHC	the cattle (bos taurus) specific region of the cyclic GMP phosphodiesterase-gene (102bp)	Real time PCR,	
MS	8a	1217		CTAB	
RF	11	N/A	N/A	GMO Extraction Kit, Real-time PCR	
RF	15		DNA	as per kit instructions	
SFA-4P	3	S6121	DNA	Extraction, clean up, enzymes, real time PCR	
SFA-4P	4	S6121	Bos taurus	SureFood® Prep Basic	K01
SFA-4P	16	S6121, according to manual		Dneay s Mericon Food, 35 cycles	
SGS	13b			Extraction with Macherey Nagel NucleoSpin Food Kit, quantification with Qubit Assay, PCR with All Species ID Kit, gel electrophoresis), purification with AMPure xp magnetic beads, next generation sequencing on Ion Torrent platform (Ion Chef + Ion S5)	
SGS	17			Marchery-Nagel NucleoMag	Result Sample 4 is not secured
div	6		cytb (464 bp)	Extractions according to ASU § 64 LFGB L 15.05-1 1. SDS / guanidinium chloride buffer with ProtK, purification using the Wizard kit from Promega; 2. CTAB based with ProtK and glycogen; Convention. PCR with 40 cycles and subsequent restriction analysis	LOD for matrices dairy products/ cheeses not conclusively validated (missing control / reference materials)
div	7				
div	9			M&N Food Kit. Quantinova Mastermix	
div	14			Wizard; Real-time PCR	

5.1.3 DNA-based Methods: Sheep

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
CP	1	28./29.07.	positive	negative	negative	negative	1%	DNA	Chipron
CP	2		positive	negative	negative	negative			DNA Chip (Chipron)
CP	8b		positive	negative	negative	negative	100-250 fg	DNA	Chipron MEAT 5.0 LCD-Array Kit
GI	12	14.8.20	positive	negative	negative	negative	0,01	DNA	GEN-IAL® First-Sheep PCR Kit
MS	8a		positive						AllMilch, Microsynth
RF	11	4.9.20	positive	negative	negative	negative	0,1	DNA	RapidFinder Sheep ID Kit, Thermofisher
RF	15		positive	negative	negative	negative	2	DNA	Imegen Rapid Finder
SFA-4P	3	22.7.20	negative	negative	negative	negative	0,05	Food	Surefood ANIMAL ID Beef/Sheep/Goat (r-biopharm)
SFA-4P	4	11.8.20	positive	negative	negative	negative	0,1	Food/ Meat	SureFood® Animal ID 4plex Beef/Sheep/Goat+IAAC
SFA-4P	16	23.9.20	positive	negative	negative	negative	0,1	DNA	SureFood Animal ID 4plex Beef/Sheep/Goat
SGS	13		positive	negative	negative	negative	0,05	Number of reads	All Species ID Meat DNA Anlyser Kit; SGS Molecular
SGS	17		positive	negative	negative	negative	0,3	DNA	All Species ID, SGS MOLECULAR
div	6	18.8.20	positive	negative	negative	negative			house method (conv. PCR-RFLP using consensus primer-primer according to Wolf et al.,1999)
div	7	30.7.20	positive	negative	negative	negative	1	DNA	
div	9		positive	-	negative	negative	0,000002	DNA	Rentsch et al; European Food Research and Technology 2013
div	14	18.9.20	questio-nable	-	-	-	0,05	ng DNA/ PCR	Jürg Rentsch et al.; Eur Food Res Technol (2013) 236:217–229

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
CP	1	A-300-14	mitochondrial 16S rRNA	Implementation according to kit instructions, but with only 30 PCR cycles	
CP	2				
CP	8b	A-500-04/-12		CTAB-Extraction	
GI	12	Art. No.: PHSP	the sheep (ovis aries) specific cyclic GMP phosphodiesterase-gene (97bp)	Real time PCR,	
MS	8a	1217		CTAB	
RF	11	N/A	N/A	GMO Extraction Kit, Real-time PCR	
RF	15		DNA	as per kit instructions	
SFA-4P	3	S6121	DNA	Extraction, clean up, enzymes, real time PCR	
SFA-4P	4	S6121	Ovis aries	SureFood® Prep Basic	QE for springbok (Antidorcas marsupialis) 100%, K01
SFA-4P	16	S6121, according to manual		Dneay s Mericon Food, 35 cycles	
SGS	13			Extraction with Macherey Nagel NucleoSpin Food Kit, quantification with Qubit Assay, PCR with All Species ID Kit, gel electrophoresis), purification with AMPure xp magnetic beads, next generation sequencing on Ion Torrent platform (Ion Chef + Ion S5)	
SGS	17			Marchery-Nagel NucleoMag	
div	6		cytb (464 bp)	Extractions according to ASU § 64 LFGB L 15.05-1 1. SDS / guanidinium chloride buffer with ProtK, purification using the Wizard kit from Promega; 2. CTAB based with ProtK and glycogen; Convention. PCR with 40 cycles and subsequent restriction analysis	LOD for matrices dairy products/ cheeses not conclusively validated (missing control / reference materials)
div	7				
div	9			M&N Food Kit. Quantinova Mastermix	
div	14			Wizard; Real-time PCR	

5.1.4 DNA-based Methods: Goat

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
CP	1	28./29.07.	negative	positive	negative	negative	1%	DNA	Chipron
CP	2		negative	positive	negative	negative			DNA Chip (Chipron)
CP	8b		negative	positive	negative	negative	100-250 fg	DNA	Chipron MEAT 5.0 LCD-Array Kit
GI	12	14.8.20	negative	positive	negative	negative	0,01	DNA	GEN-IAL® First-Goat PCR Kit
MS	8a			positive					AllMilch, Microsynth
RF	11	4.9.20	negative	positive	negative	negative	0,1	DNA	RapidFinder Goat ID Kit, Thermofisher
RF	15		negative	positive	negative	negative	2	DNA	Imegen Rapid Finder
SFA-4P	3	22.7.20	negative	positive	negative	negative	0,05	Food	Surefood ANIMAL ID Beef/Sheep/Goat (r-biopharm)
SFA-4P	4	30.7.20	negative	positive	negative	negative	0,1	Food/ Meat	SureFood® Animal ID 4plex Beef/Sheep/Goat+IAAC
SFA-4P	16	23.9.20	negative	positive	negative	negative	0,1	DNA	SureFood Animal ID 4plex Beef/Sheep/Goat
SGS	13		negative	positive	negative	negative	0,05	Number of reads	All Species ID Meat DNA Anlyser Kit; SGS Molecular
SGS	17		negative	positive	negative	negative	0,3	DNA	All Species ID, SGS MOLECULAR
div	6a	18.8.20	negative	positive	negative	negative			house method (conv. PCR-RFLP using consensus primer-primer according to Wolf et al.,1999)
div	6b	11.8.20	negative	positive	negative	negative			house method (conv. PCRusing species-specific primers Altmann et al.,2003)
div	7	30.7.20	negative	positive	negative	negative	1	DNA	
div	9		negative	positive	negative	negative	0,000005	DNA	Rentsch et al; European Food Research and Technology 2013
div	14	18.9.20	-	positive	-	-	0,05	ng DNA/ PCR	Jürg Rentsch et al.; Eur Food Res Technol (2013) 236:217–230

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
CP	1	A-300-15	mitochondrial 16S rRNA	Implementation according to kit instructions, but with only 30 PCR cycles	
CP	2				
CP	8b	A-500-04/-12		CTAB-Extraction	
GI	12	Art. No.: PHG	goat (capra) specific GMP phosphodies-terase -gene (96bp)	Real time PCR,	
MS	8a	1217		CTAB	
RF	11	N/A	N/A	GMO Extraction Kit, Real-time PCR	
RF	15		DNA	as per kit instructions	
SFA-4P	3	S6121	DNA	Extraction, clean up, enzymes, real time PCR	
SFA-4P	4	S6121	Capra hircus	SureFood® Prep Basic	K01
SFA-4P	16	S6121, gemäß Anleitung		Dneays Mericon Food, 35 cycles	
SGS	13			Extraction with Macherey Nagel NucleoSpin Food Kit, quantification with Qubit Assay, PCR with All Species ID Kit, gel electrophoresis), purification with AMPure xp magnetic beads, next generation sequencing on Ion Torrent platform (Ion Chef + Ion S5)	
SGS	17			Marchery-Nagel NucleoMag	
div	6a		cytb (464 bp)	Extractions according to ASU § 64 LFGB L 15.05-1 1. SDS / guanidinium chloride buffer with ProtK, purification using the Wizard kit from Promega; 2. CTAB based with ProtK and glycogen; Convention. PCR with 40 cycles and subsequent restriction analysis	LOD for matrices dairy products/ cheeses not conclusively validated (missing control / reference materials)
div	6b		beta-casein (161 bp)	Extractions according to ASU § 64 LFGB L 15.05-1 1. SDS / guanidinium chloride buffer with ProtK, purification using the Wizard kit from Promega; 2. CTAB based with ProtK and glycogen; Convention. PCR with 33 cycles	LOD for matrices dairy products/ cheeses not conclusively validated (missing control / reference materials)
div	7				
div	9			M&N Food Kit. Quantinova Mastermix	
div	14			Wizard; real-time PCR	

5.1.5 DNA-based Methods: Ruminant Detection

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
div	10	27.7.20	positive	positive	positive	positive			

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	
div	10			in accordance with Regulation (EU) No. 51/2013	

5.1.6 DNA-based Methods: Cattle (Buffalo/ Cow)

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
10	div	27.7.20	positive	positive	positive	positive			

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	
div	10				realtime PCR own method

5.1.7 Protein-based Methods: Buffalo

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
MALDI-TOF-MS	9		-	-	-	positive	n.a.		
MALDI-TOF-MS	12	13.8.20	negative	negative	negative	positive	5	Protein	

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	
MALDI-TOF-MS	9		Fingerprinting	Processing with organic solvents, recording of the entire spectrum on the MALDI-TOF. Evaluation of the fingerprint using your own database + individual spectrum analysis for evidence of traces	http://maldi-tof-ms-user-platform.ua-bw.de/docs/CVUAS_Stoll_Rau_Tierarten_MALDITOFMS_20150914.pdf
MALDI-TOF-MS	12			Maldi Tof MS; Biotyper microflex LT / SH, Bruker with self-created database entries	

5.1.8 Protein-based Methods: Cow

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
EP	14	7.8.20	positive	positive	positive	positive	10	Protein	EuroProxima Milk Fraud/Bovine ELISA
IEF	5		positive	positive	positive	-	2		IEF, ready-made gel plates from Serva (Precotes pH 3-10 and pH 4-6)
MALDI-TOF-MS	9		positive	positive	positive	positive	0,1	Food	MALDI-TOF-MS with single spectrum analysis
MALDI-TOF-MS	12	13.8.20	positive	positive	positive	positive	5	Protein	

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	
EP	14	5171BKCM	kappa-Casein		
IEF	5				No distinction between buffalo/ cow
MALDI-TOF-MS	9		Fingerprinting	Processing with organic solvents, recording of the entire spectrum on the MALDI-TOF. Evaluation of the fingerprint using your own database + individual spectrum analysis for evidence of traces	http://maldi-tof-ms-user-platform.ua-bw.de/docs/CVUAS_Stoll_Rau_Tierarten_MALDITOFMS_20150914.pdf
MALDI-TOF-MS	12			Maldi Tof MS; Biotyper microflex LT / SH, Bruker with self-created database entries	

5.1.9 Protein-based Methods: Sheep

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
IEF	5		positive	negative	negative	negative	5		IEF, ready-made gel plates from Serva (Precotes pH 3-10 and pH 4-6)
MALDI-TOF-MS	9		positive	negative	positive	negative	n.a.	Food	MALDI-TOF-MS with single spectrum analysis

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	
IEF	5				
MALDI-TOF-MS	9		Fingerprinting	Processing with organic solvents, recording of the entire spectrum on the MALDI-TOF. Evaluation of the fingerprint using your own database + individual spectrum analysis for evidence of evidence	Traces of sheep cheese

5.1.10 Protein-based Methods: Goat

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
IEF	5		negative	positive	negative	negative	5		IEF, ready-made gel plates from Serva (Precotes pH 3-10 and pH 4-6)

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	
IEF	5				

5.1.11 Protein-based Methods: Cattle (Buffalo/ Cow)

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detection given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
EP	16		positive	positive	positive	positive	1	Cow's milk / cheese	EuroProxima R-Biopharm

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
EP	16	5171BKCC, according to manual	bovines para-kappa Casein	Charge VN5962	(Test cannot differentiate between milk from beef and milk from buffalo)

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 ptAUS3 (2020)***
PT name	***Animal Species-Screening III – 4 Samples qualitative: Buffalo, Cow's, Sheep's and Goat's Milk in Dairy Product (Mozzarella and Herder Cheese, freeze-dried Mixtures)***
Sample matrix	*Samples 1-2: Herder Cheese (freeze-dried)* *Samples 3-4: Mozzarella (freeze-dried)*
Number of samples and sample amount	*4 different Samples 1-4: 25 g each*
Storage	*Samples 1-4: cooled 2 - 10°C (long term frozen < -18°C)*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative: Qualitative: Buffalo, Cow's, Sheep's and Goat's Milk* *Samples 1-4: appr. 5-20%*
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 September 25th 2020***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Alexandra Scharf M.Sc.*

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

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country

[Die Adressdaten der Teilnehmer wurden für die allgemeine Veröffentlichung des Auswerte-Berichts nicht angegeben.]

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

7. Index of references

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