Evaluation Report
proficiency test

DLA 45/2019

Animal Species-Screening III:

Buffalo milk, cow's milk, sheep's milk and goat's milk in dairy product (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.

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 45/2019
EP-Koordinator *PT-Coordinator*	Alexandra Scharf MSc.
Status des EP-Bericht *Status of PT-Report*	Abschlussbericht / Final report (30. Januar 2020) 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: 30. Januar 2020
Unteraufträge *Subcontractors*	Im Rahmen dieser Eignungsprüfung nachstehende Leistungen im Unterauftrag vergeben: Keine As part of the present proficency test the following services were subcontracted: none
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.

Inhalt

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 buffalo milk, cow's milk, sheep's milk and goat's milk were provided for qualitative determination. The parameters added to the matrix dairy product (herder cheese of one animal species) were present in contents of 8 - 12%.

The raw materials for the animal species used were commercial herder cheese 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: Content (in %) of the respective animal species in the herder cheese samples 1-4.

Ingredients*	Sample 1	Sample 2	Sample 3	Sample 4
Cow's milk herder cheese	positive (92%)	positive (10%)	positive (89%)	negative
Buffalo milk herder cheese	positive (8%)	positive (81%)	negative	negative
Goat's milk herder cheese	negative	negative	positive (11%)	positive (90%)
Sheep's milk herder cheese	negative	positive (9%)	negative	positive (10%)

**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.*

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. 0,25 - 0,41 (22-25°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 35th week of 2019. The testing method was optional. The tests should be finished at October 11th 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 Buffalo, Cow's, Sheep's and Goat's Milk for qualitative determination. The parameters are contained in the matrix of a Milk product (cheese) with amounts of 5 - 20%.

Analytical methods for determination are optional.
The evaluation is carried out strictly qualitatively (positive/negative) with indication of the obtained agreements with the consensus values of the participants and the spiking of samples 1-4.

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. 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 26 participants submitted their results in time.

3. Evaluation

Different protein-based methods (e.g. Isoelectric Focusing, ELISA) or PCR 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. In Addition, matrix and/or processing as well as storage and maturation 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 PCR results and results of other methods 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 from 4 or more results if ≥ 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 PCR results and results of other methods 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 PCR methods and other 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 Herder Cheese

4.1.1 PCR-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		
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP	
21	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP	
23	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP	
25	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP	
3	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	MS	Low DNA traces in sample 4; generally low DNA yield
19	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	RF	
14	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	SFA-3P	
24	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-ID	
2	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
9	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
10	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	div	
12	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
13	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
17	positive	positive		negative	3/3 (100%)	3/3 (100%)	div	Sample 3 traces
18	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	
26	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	14	16	0	0
Number negative	2	0	15	16
Percent positive	88	100	0	0
Percent negative	13	0	100	100
Consensus value	positive	positive	negative	negative
Spiking	positive	positive	negative	negative

Methods:

CP = Chipron LCD Array Kit MEAT 5.0

MS = Microsynth

RF= RapidFinder™ ID Kit, ThermoFisher

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

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

div = not indicated / other method

Comments:

The results are in qualitative agreement with the spiking of samples 1 and 2.

Two participants obtained a negative result for the lower spiked sample 1 (8% buffalo milk herder cheese). Sample 2 contained 81% buffalo milk herder cheese.

4.1.2 Results other methods: 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		
5	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU/IEF	Currently no differentiation is made between buffalo and cow's milk
6	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU/IEF	
7	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU/IEF	ASU method modified see documentation
1	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	IEF	
10	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	IEF	
22	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	LC-MS	
11	positive	positive	negative	negative	4/4 (100%)	4/4 (100%)	NGS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	6	7	0	0
Number negative	1	0	7	7
Percent positive	86	100	0	0
Percent negative	14	0	100	100
Consensus value	positive	positive	negative	negative
Spiking	positive	positive	negative	negative

Methods:

ASU/IEF = Isoelectric Focusing according ASU §64 method

IEF= Isoelectric Focusing

LC-MS= Liquid Chromatography-Mass Spectrometry

NGS = Next Generation Sequencing/Amplicon Sequencing

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 1 and sample 2.

One participant obtained a negative result with the method used (Isoelectric Focusing) for the lower-spiked sample 1 (8% buffalo milk herder cheese).
Participant 5 indicated that a differentiation between buffalo and cow's milk was currently not possible with the ASU/IEF method used.
Nevertheless, together with the other participants who used this method, he was able to evaluate sample 3 in accordance with the spiking of the samples (as negative), even though it contains, besides other ingredients, 89% cow's milk herder cheese.

4.2 Proficiency Test Cow's Milk Herder Cheese

4.2.1 PCR-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		
7	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	CP	
21	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	CP	
23	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	CP	
25	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	CP	
8	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	GI	
14	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	GI	Sample 4: traces > 0,01 %.
15	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	GI	
3	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	MS	Sample 2: traces
20	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	MS	
19	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RF	
24	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
2	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
9	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
10	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
12a	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
12b	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
13	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
17	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
18	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	div	
26	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	20	16	20	0
Number negative	0	4	0	20
Percent positive	100	80	100	0
Percent negative	0	20	0	100
Consensus value	positive	positive	positive	negative
Spiking	positive	positive	positive	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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples 1-3.

Four participants obtained a negative result with the methods used (CP, GI, MS and an unspecified method (div)) for the lower spiked sample 2 (10% cow's milk herder cheese). Participant 3 indicates that traces of bovine DNA were detected in sample 2.

4.2.2 Results other methods: 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		
5	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU/IEF	Currently no differentiation is made between buffalo and cow's milk
6	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	ASU/IEF	
7	positive	negative	positive	negative	3/4 (75%)	3/4 (75%)	ASU/IEF	ASU method modified (see documentation)
1	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	IEF	
4	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	IEF	No differentiation between buffalo and cow's milk
10	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	IEF	
22	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	LC-MS	
20	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	MALDI-TOF	
11	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	NGS	
2	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	
16	positive	positive	positive	negative	4/4 (100%)	4/4 (100%)	RS	

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

Methods:

ASU/IEF = Isoelectric Focusing according ASU §64 method

IEF= Isoelectric Focusing

LC-MS= Liquid Chromatography-Mass Spectrometry

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

NGS = Next Generation Sequencing/Amplicon Sequencing

RS = Ridascreen® CIS, R-Biopharm ELISA

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples 1-3.

One participant obtained a negative result with a modified ASU/IEF method for the lower spiked sample 2 (10% cow's milk herder cheese).
Participants 4 and 5 indicated that a differentiation between buffalo and cow's milk was currently not possible with the ASU and IEF methods used.

4.3 Proficiency Test Sheep's Milk Herder Cheese

4.3.1 PCR-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		
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
21	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
23	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
25	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI	
14	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	GI	
15	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	GI	
3	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	MS	
20	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	MS	
19	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	RF	
24	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	SFA-4P	Cross-reactivity to springbok (Antidorcas marsupialis) 100 %
2	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
9	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
10	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	div	
12	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
17	negative	positive	negative		3/3 (100%)	3/3 (100%)	div	Sample 4: traces
18	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
26	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	18	1	17
Number negative	18	1	18	1
Percent positive	5	95	5	94
Percent negative	95	5	95	6
Consensus value	negative	positive	negative	positive
Spiking	negative	positive	negative	positive

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

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 2 and 4.

One participant obtained a negative result with the method GI for sample 2 (9% sheep's milk herder cheese) and one participant obtained a negative result with the method SFA-4P for sample 4 (10% sheep's milk herder cheese). Participant 17 indicates that he detected traces of sheep's milk in sample 4.
One participant has obtained positive results for all 4 samples using a method not further specified.

4.3.2 Results other methods: 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	negative	negative	negative	positive	2/4 (50%)	2/4 (50%)	ASU/IEF	No differentiation is currently made between sheep's and goat's milk
6	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	ASU/IEF	Sheep's milk protein/goat's milk protein cannot be differentiated by using this method.
7	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	ASU/IEF	ASU method modified (see documentation)
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IEF	visual evaluation
4	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	IEF	
10	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	IEF	
22	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	LC-MS	
20	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	MALDI-TOF-MS	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	NGS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	8	2	9
Number negative	8	1	7	0
Percent positive	11	89	22	100
Percent negative	89	11	78	0
Consensus value	negative	positive	negative	positive
Spiking	negative	positive	negative	positive

Methods:

ASU/IEF = Isoelectric Focusing according ASU §64 method

IEF= Isoelectric Focusing

LC-MS= Liquid Chromatography-Mass Spectrometry

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

NGS = Next Generation Sequencing/Amplicon Sequencing

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 2 and 4.

One participant obtained with the method ASU/IEF for sample 2 (9% sheep-'s milk herder cheese) a negative result. Participants 5 and 6 indicated that a differentiation between sheep's and goat's milk is currently not possible with the ASU/IEF method used. Accordingly, participant 6 reported a positive result for all samples containing sheep's milk or goat's milk herder cheese (samples 2-4). Another participant was able to evaluate all samples in accordance with the spiking of the samples with a modification (see documentation) of the ASU/IEF method. One participant obtained a positive result for all samples using the IEF method, although sample 1 was not spiked with either sheep's milk or goat's milk herder cheese.

4.4 Proficiency Test Goat's Milk Herder Cheese

4.4.1 PCR-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		
7	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	CP	
21	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	CP	
23	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	CP	
25	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	CP	
8	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	GI	
14	positive	positive	positive	positive	2/3 (67%)	2/4 (50%)	GI	
15	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	GI	
3	negative	negative	positive	negative	2/3 (67%)	3/4 (75%)	MS	Low DNA traces in sample 4; generally low DNA yield
20	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	MS	
19	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	RF	
24	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	SFA-4P	
2	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	
9	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	div	
10	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	
12a	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	div	
12b	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	div	
13	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	Sample 2: traces goat < 0,5%
17	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	div	
18	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	
26	negative	negative	positive	positive	3/3 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	8	20	19
Number negative	19	12	0	1
Percent positive	5	40	100	95
Percent negative	95	60	0	5
Consensus value	negative	none	positive	positive
Spiking	negative	negative	positive	positive

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

div = not indicated / other method

Comments:

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

For sample 2 (without addition of goat's milk herder cheese, but spiked with 9% sheep's milk herder cheese) inconsistent results were obtained, so that no consensus value ≥75% could be observed.
One participant obtained a negative result for sample 4 (90% goat's milk herder cheese, 10% sheep's milk herder cheese) with the method MS used. For sample 2, positive results were obtained using the GI, CP and other (div) methods, possibly due to cross-reactivity to sheep or low cross-contamination of sheep's cheese with goat's milk. One participant obtained a positive result for sample 1 using the GI method (92% cow's milk herder cheese, 8% buffalo milk herder cheese).

4.4.2 Results other methods: 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	positive	positive	3/4 (75%)	3/4 (75%)	ASU/IEF	No differentiation is currently made between sheep's and goat's milk
6	negative	positive	positive	positive	3/4 (75%)	3/4 (75%)	ASU/IEF	Sheep's milk protein/goat's milk protein cannot be differentiated by using this method
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	ASU/IEF	ASU method modified (see documentation)
1	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IEF	visual evaluation
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IEF	
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	IEF	
22	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	LC-MS	
20	negative	-	-	positive	2/2 (100%)	2/2 (100%)	MALDI-TOF-MS	
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	NGS	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	2	8	9
Number negative	9	6	0	0
Percent positive	0	25	100	100
Percent negative	100	75	0	0
Consensus value	negative	negative	positive	positive
Spiking	negative	negative	positive	positive

Methods:

ASU/IEF = Isoelectric Focusing according ASU §64 method

IEF= Isoelectric Focusing

LC-MS= Liquid Chromatography-Mass Spectrometry

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

NGS = Next Generation Sequencing/Amplicon Sequencing

Comments:

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

Two participants obtained a positive result for sample 2 (81% buffalo milk-, 10% cow's milk- and 9% sheep's milk herder cheese) using the ASU/IEF method. Both participants indicated that no differentiation between sheep and goat is possible with this method. Another participant successfully evaluated all samples in accordance with the spiking of the samples using a modified ASU/IEF method.

4.5 Proficiency Test Mammalian Identification

4.5.1 PCR-Results: Mammal

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg		
13	positive	positive	positive	positive	div	

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 PCR: Buffalo

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	%	e.g. food / food protein	Test-Kit + Provider
CP	7	positive	positive	negative	negative	0.5	DNA	Chipron LCD-Array
CP	21	positive	positive	negative	negative		DNA	Chipron Micro-Array Milk Chip
CP	23	positive	positive	negative	negative	n.d.		MEAT 5.0, Chipron
CP	25	positive	positive	negative	negative			
MS	3	positive	positive	negative	negative		DNA	All Milk, Microsynth
RF	19	positive	positive	negative	negative	2		ThermoFisher Rapidfinder PCR Kit
SFA-3P	14	negative	positive	negative	negative	0.01	DNA	SureFood® ANIMAL ID 3plex Water Buffalo/Beef+IAAC
SFA-ID	24	positive	positive	negative	negative	0.1	Meat	SureFood Animal ID Water Buffalo IAAC, R-Biopharm
div	2	positive	positive	negative	negative	0.1		
div	9	positive	positive	negative	negative	0.01	Buffalo-DNA	
div	10	negative	positive	negative	negative	0.5		
div	12	positive	positive	negative	negative	1	Total of amplifiable DNA in 100 ng DNA	biomers
div	13	positive	positive	negative	negative	< 0.01	DNA	house method
div	17	positive	positive	traces	negative			
div	18	positive	positive	negative	negative			house method
div	26	positive	positive	negative	negative	0,001	DNA	literature 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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
CP	7	Meat 5.0	Bubalus bubalis	According to testkit instructions	
CP	21				
CP	23	A-500-12	16S-rRNA-Gen	Extraction: Promega Maxwell 16 FFS Nucleic CID Extraction System, Custom	
CP	25			PCR + cheap DNA (chipron)	Low DNA traces in sample 4; generally low DNA yield
MS	3		EF597572, Bubalis bubalus	DNA Extraction with Proteinase K + RNase, Clean Up with Chloroform and Column /Amplif with RealTime PCR 45 Cycles	LOD 2% milk/cheese
RF	19	IMG-188	According to testkit instructions	ThermoFisher RapidFinder GMO Extraction Kit	
SFA-3P	14	Art. No.: S6130		Real Time PCR	
SFA-ID	24	S6117	Bubalus arnee	SureFood Prep Basic (S1052)	DNA-Extraction with DNeasy® mericon ™ Food Kit
div	2		Cytochrome b Sequence	Multiplex qPCR system "AllMilk" according to Rentsch, J.; Weibel, S.; Ruf, J.; Eugster, A.; Beck, K.; Köppel R. (2013): Interlaboratory validation of two multiplex quantitative real-time PCR methods to dertermine species DNA of cow, sheep and goat as a measure of milk proportions in cheese. Eur. Food Res. Technol. 336:217-227	
div	9		Cytochrome b		
div	10			PCR end point	
div	12	Rüggeberg H. (2013), Huber I. (2016)	Lactoferrin-Gen	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	13		mitochondrial	Real Time PCR, 45 Cycles	
div	17		cyt B	Real-Time PCR	
div	18			Proteinase/ Silika-Columns/Real-Time PCR	
div	26	literature method	mt D-loop control region	CTAB.lysis+Prot. K+ Phenol:Chloroform+ Chloroform+ Isopropanol precipitation+ FFS-Kit (Promega; Maxwell)	

5.1.2 PCR: Cow

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	%	e.g. food / food protein	Test-Kit + Provider
CP	7	positive	negative	positive	negative	0.5	DNA	Chipron LCD-Array
CP	21	positive	positive	positive	negative		DNA	Chipron Micro-Array Milk Chip
CP	23	positive	positive	positive	negative	5	Lysate mixture	MEAT 5.0, Chipron
CP	25	positive	positive	positive	negative			
GI	8	positive	positive	positive	negative	1	others: Food	GEN-IAL® First-Cattle PCR Kit
GI	14	positive	positive	positive	negative	0.01	DNA	GEN-IAL First-Beef-PCR-Kit
GI	15	positive	negative	positive	negative	0.1		GEN-IAL First-beef Kit
MS	3	positive	negative	positive	negative		DNA	All Milk, Microsynth
MS	20	positive	positive	positive	negative	0,001	DNA	AllMilk-PCR gemäß Rentsch et al. 2013 (European Food Research and Technology)
RF	19	positive	positive	positive	negative	2		ThermoFisher Rapidfinder PCR Kit
SFA-4P	24	positive	positive	positive	negative	0.1	meat	SureFood Animal ID 4plex Beef/Sheep/Goat + IAAC, R-Biopharm
div	2	positive	positive	positive	negative	0.1	relative DNA content	
div	9	positive	positive	positive	negative	0.01	cow-DNA	
div	10	positive	positive	positive	negative	0.5		
div	12a	positive	positive	positive	negative			
div	12b	positive	positive	positive	negative	0.5	Total amplifiable DNA in 100 ng DNA	biomers
div	13	positive	positive	positive	negative	< 0.01	DNA	house method
div	17	positive	positive	positive	negative			
div	18	positive	positive	positive	negative			house method
div	26	positive	negative	positive	negative	0,001	DNA	literture 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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
CP	7	Meat 5.0	Bos spez.	According to testkit instructions	DNA extraction using DNeasy® mericon ™ Food Kit
CP	21				Bovine DNA in sample 2 in traces
CP	23	A-500-12	16S-rRNA-Gen	Extraction: Promega Maxwell 16 FFS Nucleic CID Extraction System, Custom	
CP	25			PCR + cheap DNA (chipron)	Protocol 1, 200mg sample weight
GI	8	5207082		SureFood® PREP Advanced, S1053	
GI	14	10004677 romer labs	beef (bos taurus) specific Beta-Actin-Gene, 96 bp	Real Time PCR	
GI	15			GEN-IAL Simplex Easy Spin Food Kit	
MS	3		AY526085, Bos taurus mitoch.	DNA extraction with Proteinase K + RNase, Clean Up with chloroform and columns /Amplif m RealTime PCR 45 cycles	
MS	20			200 mg sample weigh-in, extraction: Macherey&Nagen NucleoSpin Food Kit, QuantiNoxa multiplex PCR kit (Qiagen), 40 cycles	
RF	19	A24391	According to testkit instructions	ThermoFisher RapidFinder GMO Extraction Kit	in sample 4 traces of cow's milk were detected > 0,01
SFA-4P	24	S6121	Bos taurus	SureFood Prep Basic (S1052)	
div	2		tRNA-Lys sequence	Multiplex qPCR system "AllMilk" according to Rentsch, J.; Weibel, S.; Ruf, J.; Eugster, A.; Beck, K.; Köppel R. (2013): Interlaboratory validation of two multiplex quantitative real-time PCR methods to dertermine species DNA of cow, sheep and goat as a measure of milk proportions in cheese. Eur. Food Res. Technol. 336:217-227	
div	9		Cytochrom b		
div	10				LOD 2% milk/cheese
div	12a	International Journal of Food Science and Technology 2007, 42, 9-17	Cyclic GMP phosphodiesterase gene from cattle		
div	12b	Eur Food Res Technol (2013) 236:217–227	Beta-Actin-Gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	13		mitochondrial	Real Time PCR, 45 Cycles	
div	17		ACC.: EH170825	Real-Time PCR	
div	18			Proteinase / silica columns / Real-Time PCR	
div	26	literature method	beta-Actin	CTAB.lysis+Prot. K+ Phenol:Chloroform+ Chloroform+ Isopropanol precipitation+ FFS-Kit (Promega; Maxwell)	

5.1.3 PCR: Sheep

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	%	e.g. food / food protein	Test-Kit + Provider
CP	7	negative	positive	negative	positive	0.5	DNA	Chipron LCD-Array
CP	21	negative	positive	negative	positive		DNA	Chipron Micro-Array Milk Chip
CP	23	negative	positive	negative	positive	n.d.		MEAT 5.0, Chipron
CP	25	negative	positive	negative	positive			
GI	8	negative	positive	negative	positive	1	other: food	GEN-IAL® First-Sheep PCR Kit
GI	14	negative	positive	negative	positive	0.01	DNA	GEN-IAL First-Sheep-PCR-Kit
GI	15	negative	negative	negative	positive	0.1		GEN-IAL First-sheep Kit
MS	3	negative	positive	negative	positive		DNA	All Milk, Microsynth
MS	20	negative	positive	negative	positive	0,005	haploid genome copies	AllMilk-PCR gemäß Rentsch et al. 2013 (European Food Research and Technology)
RF	19	negative	positive	negative	positive	2		ThermoFisher Rapidfinder PCR Kit
SFA-4P	24	negative	positive	negative	negative	0.1	meat	SureFood Animal ID 4plex Beef/Sheep/Goat + IAAC, R-Biopharm
div	2	negative	positive	negative	positive	0.1	relative DNA content	
div	9	negative	positive	negative	positive	0.01	cow-DNA	
div	10	positive	positive	positive	positive	0.5		
div	12	negative	positive	negative	positive	1	Total amplifiable DNA in 100 ng DNA	biomers
div	13	negative	positive	negative	positive	< 0.01	DNA	house method
div	17	negative	positive	negative	Spuren			
div	18	negative	positive	negative	positive			house method
div	26	negative	positive	negative	positive	0,001	DNA	literature 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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
CP	7	Meat 5.0	Ovis aries	According to kit instructions	
CP	21				
CP	23	A-500-12	16S-rRNA-Gen	Extraction: Promega Maxwell 16 FFS Nucleic CID Extraction System, Custom	
CP	25			PCR + cheap DNA (chipron)	
GI	8	5207086		SureFood® PREP Advanced, S1053	Protocol 1, 200mg sample weigh-in
GI	14	10001248 romer labs	schaf (ovis aries) spezifisches zyklisches GMP-Phosphodiesteras e-Gen, 97bp	Real Time PCR	
GI	15			GEN-IAL Simplex Easy Spin Food Kit	
MS	3		CytB DQ459341	DNA extraction with Proteinase K + RNase, Clean Up with chloroform and columns /Amplif m RealTime PCR 45 cycles	
MS	20			200 mg sample weigh-in, extraction: Macherey&Nagen NucleoSpin Food Kit, QuantiNoxa Multiplex PCR-Kit (Qiagen), 40 cycles	
RF	19	A24395	According to kit instructions	ThermoFisher RapidFinder GMO Extraction Kit	LOD 2% milk/cheese
SFA-4P	24	S6121	Ovis aries	SureFood Prep Basic (S1052)	Cross-reactivity to springbok (Antidorcas marsupialis) 100%
div	2		Cytochrome b Sequence	Multiplex qPCR system "AllMilk" according to Rentsch, J.; Weibel, S.; Ruf, J.; Eugster, A.; Beck, K.; Köppel R. (2013): Interlaboratory validation of two multiplex quantitative real-time PCR methods to dertermine species DNA of cow, sheep and goat as a measure of milk proportions in cheese. Eur. Food Res. Technol. 336:217-227	DNA extraction using DNeasy® mericon ™ Food Kit
div	9		Cytochrome b		
div	10				
div	12	International Journal of Food Science and Technology 2007, 42, 9-17	cyclic GMP phosphodiesterase gene from lamb	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	13		mitochondrial	Real Time PCR, 45 cycles	
div	17		prolactin receptor	Real-Time PCR	
div	18			Proteinase / silica columns / Real-Time PCR	
div	26	literature method	Cytochrome b	CTAB.lysis+Prot. K+ Phenol:Chloroform+ Chloroform+ Isopropanol precipitation+ FFS-Kit (Promega; Maxwell)	

5.1.4 PCR: Goat

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	%	e.g. food / food protein	Test-Kit + Provider
CP	7	negative	negative	positive	positive	0.5	DNA	Chipron LCD-Array
CP	21	negative	negative	positive	positive		DNA	Chipron Micro-Array Milk Chip
CP	23	negative	negative	positive	positive	n.d.		MEAT 5.0, Chipron
CP	25	negative	positive	positive	positive			
GI	8	negative	positive	positive	positive	1	other: food	GEN-IAL® First-Goat PCR Kit
GI	14	positive	positive	positive	positive	0.01	DNA	GEN-IAL First-Goat-PCR-Kit
GI	15	negative	positive	positive	positive	0.1		GEN-IAL First-goat Kit
MS	3	negative	negative	positive	positive		DNA	All Milk, Microsynth
MS	20	negative	negative	positive	positive	0,002	haploid genome copies	AllMilk-PCR according Rentsch et al. 2013 (European Food Research and Technology)
RF	19	negative	negative	positive	positive	2		ThermoFisher Rapidfinder PCR Kit
SFA-4P	24	negative	negative	positive	positive	0.1	meat	SureFood Animal ID 4plex Beef/Sheep/Goat + IAAC, R-Biopharm
div	2	negative	negative	positive	positive	0.1	relative DNA content	
div	9	negative	positive	positive	positive	0.01	goat-DNA	
div	10	negative	negative	positive	positive	0.5		
div	12a	negative	positive	positive	positive			
div	12b	negative	positive	positive	positive	1	Total amplifiable DNA in 100 ng DNA	biomers
div	13	negative	negative	positive	positive	< 0.01	DNA	house method
div	17	negative	positive	positive	positive			
div	18	negative	negative	positive	positive			house method
div	26	negative	negative	positive	positive	0,001	DNA	literature 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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
CP	7	Meat 5.0	Capra hircus	According to kit instructions	
CP	21				
CP	23	A-500-12	16S-rRNA-Gene	Extraction: Promega Maxwell 16 FFS Nucleic CID Extraction System, Custom	
CP	25			PCR + cheap DNA (chipron)	
GI	8	5207085		SureFood® PREP Advanced, S1053	Protocol 1, 200mg sample weigh-in
GI	14	10001247 romer labs	Goat (capra) specific GMP phosphodiesterase gene, 96bp	Real Time PCR	
GI	15			GEN-IAL Simplex Easy Spin Food Kit	
MS	3		CytB DG514544	DNA extraction with Proteinase K + RNase, Clean Up with chloroform and columns /Amplif m RealTime PCR 45 cycles	Low DNA traces in sample 4; generally low DNA yield
MS	20			200 mg sample weigh-in, extraction: Macherey&Nagen NucleoSpin Food Kit, QuantiNoxa Multiplex PCR-Kit (Qiagen), 40 cycles	
RF	19	IMG-175	According to kit instructions	ThermoFisher RapidFinder GMO Extraction Kit	LOD 2% milk/cheese
SFA-4P	24	S6121	Capra hircus	SureFood Prep Basic (S1052)	
div	2		Cytochrome b Sequence	Multiplex qPCR system "AllMilk" according to Rentsch, J.; Weibel, S.; Ruf, J.; Eugster, A.; Beck, K.; Köppel R. (2013): Interlaboratory validation of two multiplex quantitative real-time PCR methods to dertermine species DNA of cow, sheep and goat as a measure of milk proportions in cheese. Eur. Food Res. Technol. 336:217-227	DNA extraction using DNeasy® mericon ™ Food Kit
div	9				
div	10				
div	12a	Eur Food Res Technol (2013) 236:217–227 ;	growth hormone receptor-gene		
div	12b	International Journal of Food Science and Technology 2007, 42, 9-17	Cyclic GMP phosphodiesterase gene	Maxwell® RSC PureFood GMO and Authentication Kit, Promega	
div	13		mitochondrial	Real Time PCR, 45 cycles	Sample 2 traces of goat < 0,5
div	17		growth hormone receptor	Real-Time PCR	
div	18			Proteinase / silica columns / Real-Time PCR	
div	26	literature method	Cytochrome b	CTAB.lysis+Prot. K+ Phenol:Chloroform+ Chloroform+ Isopropanol precipitation+ FFS-Kit (Promega; Maxwell)	

5.1.5 PCR: Mammal

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	%	e.g. food / food protein	Test-Kit + Provider
div	13	positive	positive	positive	positive	< 0,01	DNA	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.	Antibody	e.g. Extractionbuffer / Time / Temperature	
div	13		mitochondrial	Real Time PCR, 45 cycles	

5.1.6 Other methods: Buffalo

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	%	e.g. food / food protein	Test-Kit + Provider
ASU/IEF	5	positive	positive	negative	negative	10		ASU L 01.00-39
ASU/IEF	6	positive	positive	negative	negative	1	protein	
ASU/IEF	7	positive	positive	negative	negative	2	food	PAGIF/ASU mod.
IEF	1	positive	positive	negative	negative	approx. 3	Buffalo milk casein	
IEF	10	negative	positive	negative	negative	1	Isoelectric focusing	Isoelectric focusing
LC-MS	22	positive	positive	negative	negative	1	food	target proteomic analysis
NGS	11	positive	positive	negative	negative	0.1	Reads of the respective animal species with respect to total number of reads	NGS amplicon sequencing (Dobrovolny et al., 2019)

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/IEF	5	ASU L 01.00-39			Currently no distinction is made between buffalo and cow's milk; detection limit indicated as milk content
ASU/IEF	6	ASU L 01.00-39		Isoelectric focusing	
ASU/IEF	7	L01.00-39		mod.:500 µl Ampholyte ph 6-7, staining solution 1 and 2 with phosphoric acid and aluminium sulphate hydrate, defatting of Proteins with acetone	
IEF	1			Isoelectric focusing	visual evaluation
IEF	10				
LC-MS	22		kappa-casein	Extraction with urea+thiourea+TRIS, aceton precipitation, trypsin digestion, LC-MS/MS	
NGS	11		16S ribosomal DNA	DNA-Extraction: CTAB-Maxwell 16 FFS	

5.1.7 Other methods: Cow

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	%	e.g. food / food protein	Test-Kit + Provider
ASU/IEF	5	positive	positive	positive	negative	10		ASU L 01.00-39
ASU/IEF	6	positive	positive	positive	negative	1	protein	
ASU/IEF	7	positive	negative	positive	negative	2	food	PAGIF/ASU mod.
IEF	1	positive	positive	positive	negative	approx. 1	Cow's milk casein	
IEF	4	positive	positive	positive	negative	2		IEF, ready-to-use gel plates from Serva (Precotes pH 3-10 and pH 4-6)
IEF	10	positive	positive	positive	negative	1	Isoelectric focusing	Isoelectric focusing
LC-MS	22	positive	positive	positive	negative	1	food	target proteomic analysis
MALDI-TOF-MS	20	positive	positive	positive	negative	<1.8%	protein	OS extraction (Bruker), modified
NGS	11	positive	positive	positive	negative	0.1	Reads of the respective animal species with respect to total number of reads	NGS Amplicon sequencing (Dobrovolny et al., 2019)
RS	2	positive	positive	positive	negative	0.1	Cow's milk in sheep's and goat's cheese or milk	RIDASCREEN CIS of the company r-biopharm
RS	16	positive	positive	positive	negative	0.1	Cow's milk content / milk lower Wdk	r-biopharm Ridascreen CIS

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/IEF	5	ASU L 01.00-39			Currently no differentiation is made between buffalo and cow's milk; detection limit given as milk content
ASU/IEF	6	ASU L 01.00-39		Isoelectric Focusing	
ASU/IEF	7	L01.00-39		mod.:500 µl Ampholyte ph 6-7, staining solution 1 and 2 with phosphoric acid and aluminium sulphate hydrate, defatting of Proteins with acetone	
IEF	1			Isoelectric Focusing	visual evaluation
IEF	4				No differentiation between buffalo and cow
IEF	10				
LC-MS	22		kappa-casein	Extraction with urea+thiourea+TRIS, Acetone Precipitation, Trypsin Digestion, LC-MS/MS	
MALDI-TOF-MS	20			MALDI-TOF house method, qualitativ	
NGS	11		16S ribosomal DNA	DNA-Extraction: CTAB-Maxwell 16 FFS	
RS	2	R4302			
RS	16	R4302	bovine IgG	Charge 15128	

5.1.8 Other methods: Sheep

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	%	e.g. food / food protein	Test-Kit + Provider
ASU/IEF	5	negative	negative	negative	positive	10		ASU L 01.00-39
ASU/IEF	6	negative	positive	positive	positive	1	Protein	
ASU/IEF	7	negative	positive	negative	positive	2	Food	PAGIF/ASU mod.
IEF	1	negative	positive	negative	positive	approx. 3	Sheep milk casein	
IEF	4	negative	positive	negative	positive	5		IEF, ready to use gel plates company Serva (Precotes pH 3-10 and pH 4-6)
IEF	10	positive	positive	positive	positive	1	Isoelectric Focusing	Isoelectric Focusing
LC-MS	22	negative	positive	negative	positive	1	Food	target proteomic analysis
MALDI-TOF-MS	20	negative	positive	negative	positive	0,025	Protein	OS-Extraction (Bruker), modified
NGS	11	negative	positive	negative	positive	0.1	Reads of the respective animal species with respect to the total number of reads	NGS Amplicon Sequencing (Dobrovolny et al., 2019)

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/IEF	5	ASU L 01.00-39			Currently no differentiation is made between sheep's and goat's milk; detection limit given as milk content
ASU/IEF	6	ASU L 01.00-39		Isoelectric Focusing	Sheep milk protein/goat milk protein cannot be differentiated using this method
ASU/IEF	7	L01.00-39		mod.:500 µl Ampholyte ph 6-7, staining solution 1 and 2 with phosphoric acid and aluminium sulphate hydrate, defatting of Proteins with acetone	
IEF	1			Isoelectric Focusing	visual evaluation
IEF	4				
IEF	10				
LC-MS	22		kappa-casein	Extraction with urea+thiourea+TRIS, aceton precipitation, trypsin digestion, LC-MS/MS	
MALDI-TOF-MS	20			MALDI-TOF house method, qualitativ	
NGS	11		16S ribosomal DNA	DNA-Extraction: CTAB-Maxwell 16 FFS	

5.1.8 Other methods: Goat

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	%	e.g. food / food protein	Test-Kit + Provider
ASU/IEF	5	negative	positive	positive	positive	10		ASU L 01.00-39
ASU/IEF	6	negative	positive	positive	positive	1	Protein	
ASU/IEF	7	negative	negative	positive	positive	2	Food	PAGIF/ASU mod.
IEF	1	negative	negative	positive	positive	approx. 3	Goat milk casein	
IEF	4	negative	negative	positive	positive	5		IEF, ready to use gel plates company Serva (Precotes pH 3-10 and pH 4-6)
IEF	10	negative	negative	positive	positive	1	Isoelectric Focusing	Isoelectric Focusing
LC-MS	22	negative	negative	positive	positive	1	Food	target proteomic analysis
MALDI-TOF-MS	20	negative	-	-	positive	keine	Protein	OS-Extraction (Bruker), modified
NGS	11	negative	negative	positive	positive	0.1	Reads of the respective animal species with respect to the total number of reads	NGS Amplicon Sequencing (Dobrovolny et al., 2019)

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/IEF	5	ASU L 01.00-39			Currently no differentiation is made between sheep's and goat's milk; detection limit given as milk content
ASU/IEF	6	ASU L 01.00-39		Isoelectric Focusing	Sheep milk protein/goat milk protein cannot be differentiated using this method
ASU/IEF	7	L01.00-39		mod.:500 µl Ampholyte ph 6-7, staining solution 1 and 2 with phosphoric acid and aluminium sulphate hydrate, defatting of Proteins with acetone	
IEF	1			Isoelectric Focusing	visual evaluation
IEF	4				
IEF	10				
LC-MS	22		kappa-casein	Extraction with Urea+Thiourea+TRIS, Acetone Precipitation, Trypsin Digestion, LC-MS/MS	
MALDI-TOF-MS	20			MALDI-TOF house method, qualitativ	
NGS	11		16S ribosomal DNA	DNA-Extraction: CTAB-Maxwell 16 FFS	

5.2 Information on the Proficiency Test (PT)

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

Information on the Proficiency Test (PT)

PT number	***DLA 45-2019***
PT name	***Animal Species-Screening III – 4 Samples qualitative: Buffalo, Cow's, Sheep's and Goat's Milk in Milk Product (Cheese)***
Sample matrix	*Samples 1-4:* *Milk Product (Feta Cheese, 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/matrix	*Qualitative:* ***Buffalo, Cow's, Sheep's and Goat's Milk*** *Samples 1-4: appr. 5-95%*
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	*positive / negative (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 18th October 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
		AUSTRIA
		GERMANY
		SWITZERLAND
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		CZECHIA
		FRANCE
		GERMANY
		AUSTRIA
		GERMANY
		ITALY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		AUSTRIA
		GREAT BRITAIN
		GERMANY
		GERMANY
		GERMANY
		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.]

7. Index of references

1. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
2. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
3. ISO 13528:2015 & DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche / Statistical methods for use in proficiency testing by inter-laboratory comparisons
4. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methoden-validierung / DIN ISO 5725 series part 1, 2 and 6 Accuracy (trueness and preci-sion) of measurement methods and results
5. Verordnung / Regulation 882/2004/EU; Verordnung über über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz / Regulation on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
6. Evaluation of analytical methods used for regulation of food and drugs; W. Hor-witz; Analytical Chemistry, 54, 67-76 (1982)
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
9. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
10. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Ana-lyst, 125, 385-386 (2000)
11. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories; Pure Appl Chem, 78, 145 – 196 (2006)
12. AMC Kernel Density - Representing data distributions with kernel density estim-ates, amc technical brief, Editor M Thompson, Analytical Methods Committee, AMCTB No 4, Revised March 2006 and Excel Add-in Kernel.xla 1.0e by Royal Society of Chemistry
13. EURACHEM/CITAC Leitfaden, Ermittlung der Messunsicherheit bei analytischen Messun-gen (2003); Quantifying Uncertainty in Analytical Measurement (1999)
14. GMP+ Feed Certification scheme, Module: Feed Safety Assurance, chapter 5.7 Check-ing procedure for the process accuracy of compound feed with micro tracers in GMP+ BA2 Control of residues, Version: 1st of January 2015 GMP+ International B.V.
15. MTSE SOP No. 010.01 (2014): Quantitative measurement of mixing uniformity and carry-over in powder mixtures with the rotary detector technique, MTSE Micro Tracers Services Europe GmbH
16. Homogeneity and stability of reference materials; Linsinger et al.; Accred Qual Assur, 6, 20-25 (2001)
17. AOAC Official Methods of Analysis: Guidelines for Standard Method Performance Re-quirements, Appendix F, p. 2, AOAC Int (2016)
18. Codex Alimentarius Commission (2010) - Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific protiens in foods, CAC/GL 74-2010
19. Lebensmittelchemische Gesellschaft [LChG of the GDCh] "Statement of the AG regarding: Methods for the Differentiation of Animal Species in Food - Status Quo, (2016), Food Chemistry Society of the GDCh]
20. ASU according to § 35 LMBG analysis of food: Detection of animal species in milk, dairy products and cheese using isoelectric focusing (PAGIF). Method L 01.00-39 (1995)
21. Meister, A., Janzen, H., Kauer, T., Schiffer, B., & Schlicht, C. PAGIF method to verify animal species in dairy products: improved separation performance, sensitivity and efficiency. *Journal of Consumer Protection and Food Safety*, *14*(4), 421-428 (2019)