Evaluation Report
proficiency test

DLA ptAUS3 (2021)

Animal Species-Screening III:

Donkey Milk, Mare's Milk, Camel Milk, Cow's Milk, Sheep's and Goat's Milk in Milk Powder

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

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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: 18 January 2022
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 Donkey Milk, Mare's Milk, Camel Milk, Cow's Milk, Sheep's and Goat's Milk in the matrix milk powder were provided for qualitative determination. The parameters were present in the milk powders at levels of 2.5 - 92%.

The raw materials for the animal species used were commercial milk powders, each made exclusively from the milk of one animal species.
The milk powders were homogenised and subsequently tested for the presence of the declared animal species and a total of 23 further, non-declared animal species using PCR analysis (donkey milk) or the LCD array kit MEAT 5.0 from Chipron (milk from equines, camel milk, cow's milk, sheep's milk and goat's milk) (for tested animal species see product information LCD array MEAT 5.0, Chipron GmbH). All milk powders contained the declared animal species. No further adulterations or contaminations with the respective 23 other animal species (detection limit: 0.5% (w/w)) could be detected.

The corresponding quantitative amounts of raw materials for each sample (see Table 1) were mixed and, after homogenisation, filled into portions of approx. 25 g in metallised PET film bags.

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

Ingredients*	Sample 1	Sample 2	Sample 3	Sample 4
Cow's milk powder	positive (92%)	positive (88%)	positive (42%)	Positive (2,5%)
Sheep's milk powder	negative	positive (5%)	positive (41%)	negative
Goat's milk powder	negative	negative	positive (17%)	positive (91%)
Donkey milk powder	negative	negative	negative	positive (7%)
Mare's milk powder	negative	positive (7%)	negative	negative
Camel milk powder	positive (8%)	negative	negative	negative

**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.*

2.1.1 Homogeneity

The **mixture homogeneity before bottling** was examined 8-fold by **micro-tracer analysis.** It is a standardized method that is part of the international GMP certification system for feed [14].
Before mixing dye coated iron particles of µm size are added to the sample and the number of particles is determined after homogenization in taken aliquots. The evaluation of the mixture homogeneity is based on the Poisson distribution using the chi-square test. A probability of ≥ 5 % is equivalent to a good homogeneous mixture and of ≥ 25% to an excellent mixture [14, 15].
The microtracer analysis of the present PT samples 1-4 showed probabilities of 100%, 58%, 89% and 71%, respectively. Additionally particle number results were converted into concentrations, statistically evaluated according to normal distribution and compared to the standard deviation according to Horwitz. For the assessment HorRat values between 0,3 and 1,3 are to be accepted under repeat conditions (measurements within the laboratory) [17]. This gave HorRat values of 0,4, 1,3, 0,8 and 1,0, respectively. The results of microtracer analysis are given in the documentation.

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. 0,43 - 0,47 (19-21°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 39th week of 2021. The testing method was optional. The tests should be finished at November 26th 2021 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 the animal products **(Donkey Milk, Mare's Milk, Camel Milk, Cow's Milk, Sheep's and Goat's Milk)**. The parameters are contained in the matrix of **Milk Powder** with amounts of 2 – 98%. The evaluation of results is strictly qualitative (positive / negative).*

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

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

Please note the attached information on the proficiency test.
(see documentation, section 5.3 Information on the PT)

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website. The results given as positive/negative were evaluated.
Queried and documented were the indicated results and details of the test methods like specificities, test kit manufacturer and hints about the procedure.
In case participants submitted several results for the same parameter obtained by different methods these results were evaluated with the same evaluation number with a letter as a suffix and indication of the related method.

14 of 15 participants submitted at least one result.
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 Cow's Milk Powder

4.1.1 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		
7	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	ASU	
2	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP ID1.0	
6	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
8	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
13	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
3	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	NGS	
9	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	RF	
12	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	RF	
5	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
1	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
4	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
10	positive	positive	positive	negative	3/4 (75%)	3/4 (75%)	div	
11	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	
14	positive	positive	positive	positive	4/4 (100%)	4/4 (100%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	14	14	14	11
Number negative	0	0	0	3
Percent positive	100	100	100	79
Percent negative	0	0	0	21
Consensus value	positive	positive	positive	positive
Spiking	positive	positive	positive	positive

Methods:

ASU = ASU §64 Methode/method

CP ID1.0 = Chipron LCD Array Kit MILK 1.0

CP ID5.0 = Chipron LCD Array Kit MEAT 5.0

NGS = Next-Generation Sequencing

RF= RapidFinder™ ID Kit, ThermoFisher

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of samples 1-4.

Three participants obtained negative results for the low spiked sample 4 (2.5% cow's milk powder) with the methods RF or an unspecified method (div).

4.1.2 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		
11	positive	positive	positive	negative	-	3/4 (75%)	MALDI-TOF	

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

Methods:

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

Comments:

The results for samples 1-3 are in qualitative agreement with the spiking of the samples, as well as the consensus values obtained from DNA-based methods.

For the low-spiked sample 4 (2.5% cow's milk powder), participant 11 obtained a negative result.

4.2 Proficiency Test Sheep's Milk Powder

4.2.1 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		
7	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	ASU	
2	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	CP ID1.0	
6	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	CP ID5.0	
8	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	CP ID5.0	
13	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	CP ID5.0	
3	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	NGS	
9	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	RF	
12	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	RF	
5	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	SFA-4P	
1	positive	positive	positive	negative	2/3 (67%)	3/4 (75%)	div	
4	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	div	
10	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	div	
11	negative	positive	positive	negative	3/3 (100%)	4/4 (100%)	div	
14	negative	positive	positive	positive	3/3 (100%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	1	14	14	4
Number negative	13	0	0	10
Percent positive	7	100	100	29
Percent negative	93	0	0	71
Consensus value	negative	positive	positive	none
Spiking	negative	positive	positive	negative

Methods:

ASU = ASU §64 Methode/method

CP ID1.0 = Chipron LCD Array Kit MILK 1.0

CP ID5.0 = Chipron LCD Array Kit MEAT 5.0

NGS = Next-Generation Sequencing

RF= RapidFinder™ ID Kit, ThermoFisher

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of the results for samples 1-3 are in qualitative agreement with the spiking of samples 2 and 3.

For the unspiked sample 4 (91% goat's milk powder) inconsistent results were obtained, so that no consensus value ≥75% could be determined. Possible contamination with sheep milk powder in the range < 0.5% (w/w) cannot be excluded.

4.2.2 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		
11	negative	negative	positive	negative	-	3/4 (75%)	MALDI-TOF	

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

Methods:

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

Comments:

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

For the low-spiked sample 2 (5% sheep's milk powder) participant 11 has obtained a negative result.

4.3 Proficiency Test Goat's Milk Powder

4.3.1 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		
2	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP ID1.0	
6	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
8	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
13	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
3	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	NGS	
9	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	RF	
12	negative	negative	positive	negative	3/4 (75%)	3/4 (75%)	RF	
5	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
1	positive	positive	positive	positive	2/4 (50%)	2/4 (50%)	div	
4	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
7	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
10	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
11	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	
14	negative	negative	positive	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

CP ID1.0 = Chipron LCD Array Kit MILK 1.0

CP ID5.0 = Chipron LCD Array Kit MEAT 5.0

NGS = Next-Generation Sequencing

RF= RapidFinder™ ID Kit, ThermoFisher

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of the sample 3 (17% goat's milk powder) and 4 (91% goat's milk powder).

One participant received a negative result for sample 4 using the RF method. Participant 1 has obtained positive results for all of the samples.

4.3.2 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		
11	negative	negative	positive	positive	-	4/4 (100%)	MALDI-TOF	

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

Methods:

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

Comments:

The results of participant 11 are in qualitative agreement with the spiking of sample 3 (17% goat's milk powder) and 4 (91% goat's milk powder), as well as with the consensus values obtained from DNA-based methods.

4.4 Proficiency Test Milk Powder from Equidae

4.4.1 DNA-based Results: Equidae

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%)	ASU	
6	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	CP ID5.0	
8	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
13	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	CP ID5.0	
3	negative	positive	negative	negative	3/4 (75%)	3/4 (75%)	NGS	
1	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
11	negative	positive	negative	positive	4/4 (100%)	4/4 (100%)	div	
14	negative	negative	negative	positive	3/4 (75%)	3/4 (75%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	6	0	7
Number negative	8	2	8	1
Percent positive	0	75	0	88
Percent negative	100	25	100	13
Consensus value	negative	positive	negative	positive
Spiking	negative	positive	negative	positive

Methods:

ASU = ASU §64 Methode/method

CP ID5.0 = Chipron LCD Array Kit MEAT 5.0

NGS = Next-Generation Sequencing

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of the sample 2 (7% mare's milk powder) and sample 4 (7% donkey milk powder).

Two participants obtained a negative result for sample 2.
One participant obtained a negative result for sample 4 using the NGS method.

4.5 Proficiency Test Mare's Milk Powder

4.5.1 DNA-based Results: Mare

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	negative	4/4 (100%)	4/4 (100%)	ASU	
10	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	ASU	
2	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	CP ID1.0	
3	negative	negative	negative	negative	3/4 (75%)	3/4 (75%)	NGS	
5	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
12	negative	positive	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
13	-	positive	-	negative	2/2 (100%)	2/2 (100%)	div	
14	negative	negative	negative	positive	2/4 (50%)	2/4 (50%)	div	

	Sample 1	Sample 2	Sample 3	Sample 4
Number positive	0	6	0	1
Number negative	7	2	7	7
Percent positive	0	75	0	13
Percent negative	100	25	100	88
Consensus value	negative	positive	negative	negative
Spiking	negative	positive	negative	negative

Methods:

ASU = ASU §64 Methode/method

CP ID1.0 = Chipron LCD Array Kit MILK 1.0

NGS = Next-Generation Sequencing

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of the sample 2 (7% mare's milk powder).

Two participants obtained a negative result for sample 2.
Another participant received a positive result for sample 4 spiked with donkey milk (7% donkey milk powder).

4.6 Proficiency Test Donkey Milk Powder

4.6.1 DNA-based Results: Donkey

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
7	negative	-	negative	positive	3/3 (100%)	3/3 (100%)	ASU	
2	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	CP ID1.0	
3	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	NGS	
5	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
12	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	SFA-4P	
14	negative	negative	negative	positive	4/4 (100%)	4/4 (100%)	div	

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

Methods:

ASU = ASU §64 Methode/method

CP ID1.0 = Chipron LCD Array Kit MILK 1.0

NGS = Next-Generation Sequencing

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of the sample 4 (7% donkey milk powder).

4.7 Proficiency Test Camel Milk Powder

4.7.1 DNA-based Results: Camel

Qualitative valuation of results

Evaluation number	Sample 1	Sample 2	Sample 3	Sample 4	Qualitative Valuation	Qualitative Valuation	Method	Remarks
	pos/neg	pos/neg	pos/neg	pos/neg	Agreement with consensus value	Agreement with spiking of samples		
2	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP ID1.0	
6	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP ID5.0	
8	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP ID5.0	
13	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	CP ID5.0	
3	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	NGS	
5	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
12	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	SFA-4P	
7	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	
14	positive	negative	negative	negative	4/4 (100%)	4/4 (100%)	div	

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

Methods:

CP ID1.0 = Chipron LCD Array Kit MILK 1.0

CP ID5.0 = Chipron LCD Array Kit MEAT 5.0

NGS = Next-Generation Sequencing

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

div = keine genaue Angabe / andere Methode

div = not indicated / other method

Comments:

The consensus values of results are in qualitative agreement with the spiking of sample 1 (8% camel milk powder).

4.8 Further results of the Proficiency Test

4.8.1 DNA-based Results: Lama

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		
13	negative	negative	negative	negative	-	4/4 (100%)	CP ID3.0	

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

Methods:

CP ID3.0 = Chipron LCD Array Kit MEAT 3.0

Comments:

The results are in agreement with the spiking of the samples: No milk from Lama was spiked in any of the samples.

5. Documentation

5.1 Details by the participants

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

5.1.1 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
ASU	7	05-11.10.2021	positive	positive	positive	positive	1	food	QuantiTect Multipleks PCR NoROX kit (Qiagen)/primers and probes (TibMolBiol)/ Supreme NzyTaq II DNA Polymerase (NZYTech)
CP ID1.0	2	10.11.21 11.11.21	positive	positive	positive	positive	1	DNA	Chipron
CP ID5.0	6	05.10.21	positive	positive	positive	positive	2	DNA	LCD-Array kit MEAT 5.0 Chipron
CP ID5.0	8		positive	positive	positive	positive	0.1	DNA	Chipron LCD-Array Meat 5.0
CP ID5.0	13	19.10.21	positive	positive	positive	positive	0.1 ng/PCR	DNA	LCD Array Kit MEAT 5.0, Chipron
NGS	3	11.11.2021	positive	positive	positive	positive	1	% number of reads	NGS - Ion Torrent
RF	9	08.10.21	positive	positive	positive	negative	0.1	DNA	RapidFinder ID Kit, Thermofisher
RF	12	05.10.21	positive	positive	positive	negative	2		A24391 Thermo Fisher RapidFinder
SFA-4P	5	05.10.21	positive	positive	positive	positive	0.1	DNA	SureFood® Animal ID 4plex Beef/Sheep/Goat+IAAC
div	1	15/11/2021	positive	positive	positive	positive	1	DNA	In-House Test Kit
div	4		positive	positive	positive	positive	1%	DNA	Internal method
div	10		positive	positive	positive	negative	0.1%	Animal Species	J. Rentsch et al 2013; Interlaboratory validation of two multiplex quantitative real-time PCR methods to determine species DNA of cow, sheep and goat as a measure of milk proportions in cheese
div	11		positive	positive	positive	positive	0.1	DNA	
div	14		positive	positive	positive	positive		DNA	AllMilch in house

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	7	In house multiplex real-time PCR; ASU L 08.00-62:2016-03; In house PCR-RFLP	beta-actine; Cytochrome b	magnetic beads extraction in MagNa Pure LC 2.0 (Roche)/ multiplex real-time PCR/ 40 cycles and PCR/40 cycles/RFLP with Fast Digest enzymes (Thermofisher)/microchip electrophoresis in MultiNA (Shimadzu)	
CP ID1.0	2	A-300-12	mitochondrial 16S rRNA	Carried out according to kit instructions, except that only 30 PCR cycles are performed.	
CP ID5.0	6	MEAT 5.0 A-500-12	16 S rRNA genes	Extraction/ PCR/ LCD-array	
CP ID5.0	8				
CP ID5.0	13	A-500-12	16S rDNA	Extraction using DNeasy® mericon ™ Food Kit	
NGS	3				
RF	9	N/A	N/A	Real-time PCR	
RF	12				Cow Milk
SFA-4P	5	S6121	Bos taurus	SureFood® Prep Basic	LOD in muscle meat, K01
div	1	-	mitochondrial DNA	In-House CTAB Extraction Method, PCR & Gel Electrophoresis	
div	4				
div	10		F: 5'- AGT TAG AGA TTG AGA GCC ATA TAC TCT CC -3' S: 5'- FAM TGG TGA CAT GCC GCA ACT AGA CAC G BHQ1 -3' R: 5'- TTG ATA AGA TCA TTG TCA GTC ATG TTG -3'	200 mg, M&N Nucleospin Food, Mastermix: 4x QuantiNova PCR-Kit (Fa. Qiagen)	
div	11				
div	14			Wizard +Rotorgene 6000	

5.1.2 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
ASU	7	05-11.10.2021	negative	positive	positive	negative	2	food	QuantiTect Multipleks PCR NoROX kit (Qiagen)/primers and probes (TibMolBiol)/ Supreme NzyTaq II DNA Polymerase (NZYTech)
CP ID1.0	2	10.11.21 11.11.21	negative	positive	positive	negative	1	DNA	Chipron
CP ID5.0	6	05.10.21	negative	positive	positive	negative	2	DNA	LCD-Array kit MEAT 5.0 Chipron
CP ID5.0	8		negative	positive	positive	negative	0,1	DNA	Chipron LCD-Array Meat 5.0
CP ID5.0	13	19.10.21	negative	positive	positive	negative	0,1 ng/PCR	DNA	LCD Array Kit MEAT 5.0, Chipron
NGS	3	11.11.2021	negative	positive	positive	positive	1	% number of reads	NGS - Ion Torrent
RF	9	08.10.21	negative	positive	positive	negative	0,1	DNA	RapidFinder ID Kit, Thermofisher
RF	12	05.10.21	negative	positive	positive	negative	2		A24395 Thermo Fisher RapidFinder
SFA-4P	5	05.10.21	negative	positive	positive	positive	0,1	DNA	SureFood® Animal ID 4plex Beef/Sheep/Goat+IAAC
div	1	15/11/2021	positive	positive	positive	negative	0,001	DNA	In-House Test Kit
div	4		negative	positive	positive	negative	1%	DNA	Internal method
div	10		negative	positive	positive	positive	0,01 ng/PCR	DNA	J. Rentsch et al 2013; Interlaboratory validation of two multiplex quantitative real-time PCR methods to determine species DNA of cow, sheep and goat as a measure of milk proportions in cheese
div	11		negative	positive	positive	negative	0,1	DNA	
div	14		negative	positive	positive	positive		DNA	AllMilch in house

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	7	In house multiplex real-time PCR; ASU L 08.00-62:2016-03; In house PCR-RFLP	prolactin receptor; Cytochrome b	magnetic beads extraction in MagNa Pure LC 2.0 (Roche)/ multiplex real-time PCR/ 40 cycles and PCR/40 cycles/RFLP with Fast Digest enzymes (Thermofisher)/microchip electrophoresis in MultiNA (Shimadzu)	
CP ID1.0	2	A-300-12	mitochondrial 16S rRNA	Carried out according to kit instructions, except that only 30 PCR cycles are performed.	
CP ID5.0	6	MEAT 5.0 A-500-12	16 S rRNA genes	Extraction/ PCR/ LCD-array	
CP ID5.0	8				
CP ID5.0	13	A-500-12	16S rDNA	Extraction using DNeasy® mericon ™ Food Kit	
NGS	3				
RF	9	N/A	N/A	Real-time PCR	
RF	12				Sheep Milk
SFA-4P	5	S6121	Ovis aries	SureFood® Prep Basic	LOD in muscle meat, K01, QE to springbok (Antidorcas marsupialis) 100 %,
div	1	-	mitochondrial DNA	In-House CTAB Extraction Method, PCR & Gel Electrophoresis	
div	4				
div	10		F: 5'- TTT CGC CTT TCA CTT TAT TTT CCC -3' R: 5'- GAA TTC CTG TGG GGT TGT TGG -3' S: 5'- GAA TTC CTG TGG GGT TGT TGG -3'	200 mg, M&N Nucleospin Food, Mastermix: 4x QuantiNova PCR-Kit (Fa. Qiagen)	
div	11				
div	14			Wizard +Rotorgene 6000	

5.1.3 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 ID1.0	2	10.11.21 11.11.21	negative	negative	positive	positive	1	DNA	Chipron
CP ID5.0	6	05.10.21	negative	negative	positive	positive	2	DNA	LCD-Array kit MEAT 5.0 Chipron
CP ID5.0	8		negative	negative	positive	positive	0,1	DNA	Chipron LCD-Array Meat 5.0
CP ID5.0	13	19.10.21	negative	negative	positive	positive	0,1 ng/PCR	DNA	LCD Array Kit MEAT 5.0, Chipron
NGS	3	11.11.2021	negative	negative	positive	positive	1	% number of reads	NGS - Ion Torrent
RF	9	08.10.21	negative	negative	positive	positive	0,1	DNA	RapidFinder ID Kit, Thermofisher
RF	12	05.10.21	negative	negative	positive	negative	2		A24407 Thermo Fisher RapdFinder
SFA-4P	5	05.10.21	negative	negative	positive	positive	0,1	DNA	SureFood® Animal ID 4plex Beef/Sheep/Goat+IAAC
div	1	24/11/2021	positive	positive	positive	positive	0,01	DNA	In-House Test Kit
div	4		negative	negative	positive	positive	1%	DNA	Internal method
div	7	05- 11.10.2021	negative	negative	positive	positive	1	food	QuantiTect Multipleks PCR NoROX kit (Qiagen)/primers and probes (TibMolBiol)/ Supreme NzyTaq II DNA Polymerase (NZYTech)
div	10		negative	negative	positive	positive	0,025 ng/PCR	DNA	J. Rentsch et al 2013; Interlaboratory validation of two multiplex quantitative real-time PCR methods to determine species DNA of cow, sheep and goat as a measure of milk proportions in cheese
div	11		negative	negative	positive	positive	0,1	DNA	
div	14		negative	negative	positive	positive		DNA	AIIMilch in house

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 ID1.0	2	A-300-12	mitochondrial 16S rRNA	Carried out according to kit instructions, except that only 30 PCR cycles are performed	
CP ID5.0	6	MEAT 5.0 A-500-12	16 S rRNA genes	Extraction/ PCR/ LCD-array	
CP ID5.0	8				
CP ID5.0	13	A-500-12	16S rDNA	Extraction using DNeasy® mericon ™ Food Kit	
NGS	3				
RF	9	N/A	N/A	Real-time PCR	
RF	12				Goat Milk
SFA-4P	5	S6121	Capra hircus	SureFood® Prep Basic	LOD in muscle meat, K01
div	1	-	mitochondrial DNA	In-House CTAB Extraction Method, PCR & Gel Electrophoresis	
div	4				
div	7	In house multiplex real-time PCR; In house PCR-RFLP	insertion of a LINE-1 element in the 5'-non-coding region of the growth factor; cytochrom b	magnetic beads extraction in MagNa Pure LC 2.0 (Roche)/ multiplex real-time PCR/ 40 cycles and PCR/40 cycles/RFLP with Fast Digest enzymes (Thermofisher)/microchip electrophoresis in MultiNA (Shimadzu)	
div	10		F: 5'- CAC TTT ATC CTC CCA TTC ATC ATC AC -3' R: 5'- TCT TTA ATG GTG TAG TAA GGG TGA AAT G -3' R: 5'- HEX CCTCGCCATAGTCCAC CTGCTCTTCC BHQ1 -3'	200 mg, M&N Nucleospin Food, Mastermix: 4x QuantiNova PCR-Kit (Fa. Qiagen)	
div	11				
div	14			Wizard +Rotorgene 6000	

5.1.4 DNA-based Methods: Equidae

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
ASU	7	07.10.21	negative	positive	negative	positive	0.5	food	QuantiTect Multipleks PCR NoROX kit (Qiagen)/primers and probes (TibMolBiol)
CP ID5.0	6	05.10.21	negative	negative	negative	positive	2	DNA	LCD-Array kit MEAT 5.0 Chipron
CP ID5.0	8		negative	positive	negative	positive	0,1	DNA	Chipron LCD-Array Meat 5.0
CP ID5.0	13	19.10.21	negative	positive	negative	positive	0,1 ng/PCR	DNA	LCD Array Kit MEAT 5.0, Chipron
NGS	3	11.11.2021	negative	positive	negative	negative	1	% number of reads	NGS - Ion Torrent
div	1	15/11/2021	negative	positive	negative	positive	0,001	DNA	In-House Test Kit
div	11		negative	positive	negative	positive	0,1	DNA	
div	14		negative	negative	negative	positive		DNA	

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	7	ASU L 08.00-62:2016-03	growth hormone receptor	magnetic beads extraction in MagNa Pure LC 2.0 (Roche)/ multiplex real-time PCR/ 40 cycles	
CP ID5.0	6	MEAT 5.0 A-500-12	16 S rRNA genes	Extraction/ PCR/ LCD-array	
CP ID5.0	8				
CP ID5.0	13	A-500-12	16S rDNA	Extraction using DNeasy® mericon ™ Food Kit	
NGS	3				
div	1	-	mitochondrial DNA	In-House CTAB Extraction Method, PCR & Gel Electrophoresis	
div	11				
div	14			Wizard +Rotorgene 6000	

5.1.5 DNA-based Methods: Mare

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detecti-on given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
ASU	7	07-12.10.2021	negative	positivee	negative	negative	0.5	food	QuantiTect Multipleks PCR NoROX kit (Qiagen)/primers and probes (TibMolBiol)/ Supreme NzyTaq II DNA Polymerase (NZYTech)
ASU	10		negative	positive	negative	negative			§64 LFGB ASU L 06.26/27-2 (2007-12)
CP ID1.0	2	10.11.21 11.11.21	negative	positive	negative	negative	1	DNA	Chipron
NGS	3	11.11.2021	negative	negative	negative	negative	1	% number of reads	NGS - Ion Torrent
SFA-4P	5	05.10.21	negative	positive	negative	negative	0,1	DNA	SureFood® Animal ID 4plex Camel/Horse/Donkey+IAAC
SFA-4P	12	08.10.21	negative	positivee	negative	negative	2		S6113 Congen
div	13	18.10.21	-	positive	-	negative	0,10%	DNA	Dobrovolny S., Blaschitz M., Weinmaier T., Pechatschek J., Cichna-Markl M., Indra A., Hufnagl P., Hochegger R. (2019). Development of a DNA metabarcoding method for the identification of fifteen mammalian and six poultry species in food. Food Chemistry, 272, 354-361.
div	14		negative	negative	negative	positive		DNA	

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	7	ASU L 08.00-62:2016-03; ASU L 06.26/27-2:2007-12	growth hormone receptor; cytochrom b	magnetic beads extraction in MagNa Pure LC 2.0 (Roche)/ multiplex real-time PCR/ 40 cycles and PCR/40 cycles/RFLP with Fast Digest enzymes (Thermofisher)/microchip electrophoresis in MultiNA (Shimadzu)	
ASU	10		Primer Primer HO-EX1U : 5'-CAC AgC CCT ggT AgT-3' Primer Primer HO-EX1R: 5'-gCA AgA TCA ggA ggA ggA gT-3'	200 mg, M&N Nucleospin Food, Mastermix: 4x QuantiNova PCR-Kit (Fa. Qiagen); PCR according to ASU, restriction analysis with HpyF3I and MboI	
CP ID1.0	2	A-300-12	mitochondrial 16S rRNA	Carried out according to kit instructions, except that only 30 PCR cycles are performed	
NGS	3				
SFA-4P	5	S6131	Equus caballus	SureFood® Prep Basic	LOD in muscle meat, K01
SFA-4P	12				Horse Milk
div	13		16S rDNA	Extraction using DNeasy® mericon ™ Food Kit, Horse-positive result of sample 2 confirmed by ASU method L 06.26/27-2, 2007-12	
div	14			Wizard +Rotorgene 6000	

5.1.6 DNA-based Methods: Donkey

Primary data

Meth. Abbr.	Evaluation number	Date of analysis	Result Sample 1	Result Sample 2	Result Sample 3	Result Sample 4	Limit of detection	Limit of detecti-on given as	Method
			qualitative	qualitative	qualitative	qualitative	%	e.g. food/ protein	Test-Kit + Manufacturer
ASU	7	07-12.10.2021	negative	-	negative	positive	0.5	food	QuantiTect Multipleks PCR NoROX kit (Qiagen)/primers and probes (TibMolBiol)/ Supreme NzyTaq II DNA Polymerase (NZYTech)
CP ID1.0	2	10.11.21 11.11.21	negative	negative	negative	positive	1	DNA	Chipron
NGS	3	11.11.2021	negative	negative	negative	positive	1	% number of reads	NGS - Ion Torrent
SFA-4P	5	05.10.21	negative	negative	negative	positive	0,1	DNA	SureFood® Animal ID 4plex Camel/Horse/Donkey+IAAC
SFA-4P	12	08.10.21	negative	negative	negative	positive	2		S6113 Congen
div	14		negative	negative	negative	positive		DNA	

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Target-Sequence / -DNA	e.g. Extraction/ Enzymes/ Clean-Up/ Real Time PCR/ Gel electrophoresis/ Cycles	
ASU	7	ASU L 08.00-62:2016-03; ASU L 06.26/27-2:2007-12; In house PCR-RFLP	growth hormone receptor; cytochrom b	magnetic beads extraction in MagNa Pure LC 2.0 (Roche)/ multiplex real-time PCR/ 40 cycles and PCR/ 40 cycles/ RFLP with Fast Digest enzymes (Thermofisher)/ microchip electrophoresis in MultiNA (Shimadzu)	
CP ID1.0	2	A-300-12	mitochondrial 16S rRNA	Carried out according to kit instructions, except that only 30 PCR cycles are performed.	
NGS	3				
SFA-4P	5	S6131	Equus asinus	SureFood® Prep Basic	LOD in muscle meat, K01
SFA-4P	12				Donkey Milk
div	14			Wizard +Rotorgene 6000	

5.1.7 DNA-based Methods: Camel

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 ID1.0	2	10.11.21 11.11.21	positiv	negativ	negativ	negativ	1	DNA	Chipron
CP ID5.0	6	05.10.21	positive	negative	negative	negative	2	DNA	LCD-Array kit MEAT 5.0 Chipron
CP ID5.0	8		positiv	negativ	negativ	negativ	0,1	DNA	Chipron LCD-Array Meat 5.0
CP ID5.0	13	19.10.21	positiv	negativ	negativ	negativ	0,1 ng/PCR	DNA	LCD Array Kit MEAT 5.0, Chipron
NGS	3	11.11.2021	positive	negative	negative	negative	1	% number of reads	NGS - Ion Torrent
SFA-4P	5	05.10.21	positiv	negativ	negativ	negativ	0,1	DNA	SureFood® Animal ID 4plex Camel/Horse/Donkey+IAAC
SFA-4P	12	08.10.21	positive	negative	negative	negative	2		S6113 Congen
div	7	08-11.10.2021	positive	negative	negative	negative	5	food	dNTP (Bioline)/MgCl2 (NZYTech)/ primers (Sigma)/ Supreme NzyTaq II DNA Polymerase (NZYTech)
div	14		positiv	negativ	negativ	negativ		DNA	

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	
CP ID1.0	2	A-300-12	mitochondrial 16S rRNA	Carried out according to kit instructions, except that only 30 PCR cycles are performed	
CP ID5.0	6	MEAT 5.0 A-500-12	16 S rRNA genes	Extraction/ PCR/ LCD-array	
CP ID5.0	8				
CP ID5.0	13	A-500-12	16S rDNA	Extraction using DNeasy® mericon ™ Food Kit	
NGS	3				
SFA-4P	5	S6131	Camelus spp.	SureFood® Prep Basic	LOD in muscle meat, K01
SFA-4P	12				Camel Milk
div	7	In house PCR-RFLP	cytochrom b	magnetic beads extraction in MagNa Pure LC 2.0 (Roche)/ PCR/ 30 cycles/ RFLP with Fast Digest enzymes (Thermofisher)/ microchip electrophoresis in MultiNA (Shimadzu)	
div	14			Wizard +Rotorgene 6000	

5.1.8 Further DNA-based Methods: Lama

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 ID3.0	13	19.10.21	negativ	negativ	negativ	negativ	0,1 ng/PCR	DNA	LCD Array Kit MEAT 3.0, Chipron

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	
CP ID3.0	13	A-925-04	16S rDNA	Extraction using DNeasy® mericon ™ Food Kit	

5.1.9 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
MALDI-TOF	11		positive	positive	positive	negative	0,5	Animal Species	unvalidated MALDI-TOF in-house method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
MALDI-TOF	11			Solvent-based extraction (OS; Bruker), MALDI-TOF Sirius, MBT AutoX	not validated

5.1.10 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
MALDI-TOF	11		negative	negative	positive	negative	n. a.	Animal Species	non-validated MALDI-TOF in-house method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
MALDI-TOF	11			Solvent-based extraction (OS; Bruker), MALDI-TOF Sirius, MBT AutoX	not validated

5.1.11 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
MALDI-TOF	11		negative	negative	positive	positive	n. a.	Animal Species	non-validated MALDI-TOF in-house method

Other details to the Methods

Meth. Abbr.	Evaluation number	Method-No. / Test-Kit No.	Specifity	Remarks to the Method (Extraction and Determination)	Further Remarks
		Article-No./ASU-No.	Antibody	e.g. Extractionbuffer / Time / Temperature	
MALDI-TOF	11			Solvent-based extraction (OS; Bruker), MALDI-TOF Sirius, MBT AutoX	not validated

5.2 Homogeneity

5.2.1 Mixture homogeneity before bottling

Microtracer Homogeneity Test
DLA -ptAUS3 Sample 1

Weight whole sample	1,30	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	26,1	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	61	24,5
3	5,01	60	24,0
4	5,01	61	24,4
5	5,02	57	22,7
7	5,04	63	25,0
8	5,00	66	26,4
9	4,98	62	24,9
10	5,01	62	24,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	61,5	Particles
Standard deviation	2,60	Particles
χ^2 (CHI-Quadrat)	0,77	
Probability	**100**	%
Recovery rate	94	%

Normal distribution		
Number of samples	8	
Mean	24,6	mg/kg
Standard deviation	1,04	mg/kg
rel. Standard deviaton	4,2	%
Horwitz standard deviation	9,9	%
HorRat-value	**0,4**	
Recovery rate	94	%

Microtracer Homogeneity Test
DLA -ptAUS3 Sample 2

Weight whole sample	1,20	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	24,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	4,98	39	15,7
2	5,02	48	19,1
3	5,02	53	21,1
4	5,01	41	16,4
5	5,01	57	22,8
6	4,98	51	20,5
7	5,05	43	17,0
8	4,95	49	19,8

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	47,6	Particles
Standard deviation	6,22	Particles
χ^2 (CHI-Quadrat)	5,70	
Probability	**58**	%
Recovery rate	77	%

Normal distribution		
Number of samples	8	
Mean	19,0	mg/kg
Standard deviation	2,49	mg/kg
rel. Standard deviaton	13,1	%
Horwitz standard deviation	9,8	%
HorRat-value	**1,3**	
Recovery rate	77	%

Microtracer Homogeneity Test
DLA -ptAUS3 Sample 3

Weight whole sample	1,20	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	29,8	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1	5,02	77	30,7
2	4,99	77	30,9
3	4,98	62	24,9
4	5,01	75	29,9
5	5,05	65	25,7
6	4,98	73	29,3
7	5,01	73	29,1
8	4,99	71	28,5

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	71,6	Particles
Standard deviation	5,51	Particles
χ^2 (CHI-Quadrat)	2,96	
Probability	**89**	%
Recovery rate	96	%

Normal distribution		
Number of samples	8	
Mean	28,6	mg/kg
Standard deviation	2,20	mg/kg
rel. Standard deviaton	7,7	%
Horwitz standard deviation	9,8	%
HorRat-value	**0,8**	
Recovery rate	96	%

Microtracer Homogeneity Test
DLA -ptAUS3 Sample 4

Weight whole sample	1,20	kg
Microtracer	FSS-rot lake	
Particle size	75 – 300	µm
Weight per particle	2,0	µg
Addition of tracer	31,0	mg/kg

Result of analysis

Sample	Weight [g]	Particle number	Particles [mg/kg]
1*	4,99	82	32,9
2*	4,98	75	30,1
3	4,98	76	30,5
4	5,01	83	33,1
5	4,99	67	26,9
6	5,03	78	31,0
7	4,96	62	25,0
8*	5,05	73	28,9

Poisson distribution		
Number of samples	8	
Degree of freedom	7	
Mean	74,5	Particles
Standard deviation	7,01	Particles
χ^2 (CHI-Quadrat)	4,62	
Probability	**71**	%
Recovery rate	96	%

Normal distribution		
Number of samples	8	
Mean	29,8	mg/kg
Standard deviation	2,81	mg/kg
rel. Standard deviaton	9,4	%
Horwitz standard deviation	9,6	%
HorRat-value	**1,0**	
Recovery rate	96	%

5.3 Information on the Proficiency Test (PT)

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

PT number	***DLA ptAUS3 (2021)***
PT name	***Animal Species-Screening III – 4 Samples qualitative: Donkey Milk, Mare's Milk, Camel Milk, Cow's Milk, Sheep's and/or Goat's Milk in Milk Powder***
Sample matrix	*Samples 1-4:* *Milk Powder/ ingredients: Donkey Milk, Mare's Milk, Camel Milk, Cow's Milk, Sheep's and/or Goat's Milk*
Number of samples and sample amount	*4 different Samples 1-4: 25 g each*
Storage	*Samples 1-4: cooled 2 - 10°C*
Intentional use	*Laboratory use only (quality control samples)*
Parameter	*Qualitative: Donkey Milk, Mare's Milk, Camel Milk, Cow's Milk, Sheep's and/or Goat's Milk* *Samples 1-4: appr. 2-98%*
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 November 26th 2021.***
Evaluation report	*The evaluation report is expected to be completed 6 weeks after deadline of result submission and sent as PDF file by e-mail.*
Coordinator and contact person of PT	*Alexandra Scharf M.Sc.*

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

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		ITALIEN
		Deutschland
		Deutschland
		Deutschland
		FRANKREICH
		MALAYSIA
		SCHWEIZ
		SCHWEIZ
		POLEN
		Deutschland
		GROSSBRITANNIEN
		Deutschland
		GROSSBRITANIEN
		SCHWEIZ
		PORTUGAL

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