

Collaborative ring trial for the detection of cow, sheep and goat milk in dairy samples by real-time PCR targeting mitochondrial DNA

Journal of Consumer Protection and Food Safety, Journal für Verbraucherschutz und Lebensmittelsicherheit

Kristina Kappel, Maik Döring, Stefan Nöbel, Katrin Neumann, Claudia Brünen-Nieweler, Ingo Krujatz, Bert Pöpping, Kathrin Szabo, Ilka Haase

Corresponding author: Maik Döring, National Reference Centre for Authentic Food (NRZ-Authent), Max Rubner-Institut (MRI), E.-C.-Baumann-Straße 20, 95326 Kulmbach, Germany, maik.doering@mri.bund.de

Online Resource 3, Supplementary Information

Table of contents

1	Real-time PCR efficiencies and R^2	2
2	Calculation methods of DNA proportions	2
3	Statistical analysis of the ring trial	4
4	Visualisation of the determined proportions of cattle, sheep and goat DNA	9
5	Tables of the statistical parameters	13
6	Comparison of the calculation methods	25
7	Influence of the sample type	26

1 Real-time PCR efficiencies and R²

All laboratories except one achieved real-time PCR efficiencies and coefficients of determination in the accepted limits from 90 % to 110 % and > 0.98 , respectively (see Table S4). The DNA standard dilution series of laboratory C showed efficiencies < 90 % for all four real-time PCR systemassays, thus, the results were either excluded from the evaluation, or, if not, labelled accordingly.

Table S4 Real-time PCR efficiencies and R². The data of laboratory C is highlighted as the efficiencies were outside the excepted limits

Lab	cattle		16S cattle		sheep		16S sheep		Goat		16S goat	
	E(%)	R ²	E(%)	R ²	E(%)	R ²	E(%)	R ²	E(%)	R ²	E(%)	R ²
A	100.6	0.999	93.3	0.999	99.8	0.995	101.3	0.998	96.9	0.998	98.5	0.997
B	99.1	≥ 0.9995	98.2	0.996	96.7	0.999	97.5	≥ 0.9995	98.9	0.998	97.9	0.999
C	80.5	0.997	79.2	0.998	75.9	0.997	77.2	0.999	81.1	0.997	88.9	≥ 0.9995
D	96.5	0.999	98.5	0.998	93.0	0.998	97.1	0.998	97.9	0.999	106.2	0.999
E	98.8	0.999	97.6	0.999	99.4	0.999	97.8	0.999	96.9	≥ 0.9995	96.9	0.999
F	96.9	0.999	98.0	≥ 0.9995	97.4	0.997	97.3	≥ 0.9995	99.2	0.998	99.6	≥ 0.9995
G	93.6	0.998	95.4	0.999	99.6	0.999	95.3	0.998	98.8	0.999	99.7	0.999
H	98.3	0.997	94.9	0.998	98.2	0.998	98.2	≥ 0.9995	99.1	0.998	104.2	0.999
I	94.8	0.999	99.8	0.998	97.9	0.999	102.2	0.999	100.4	0.998	101.2	≥ 0.9995
J	100.8	0.999	99.1	0.997	99.7	0.998	102.8	0.999	100.9	0.997	100.7	0.999
K	101.1	0.997	100.0	0.998	101.8	0.997	100.2	0.999	106.4	0.994	102.4	0.999
L	96.0	0.999	98.1	0.999	98.9	0.998	95.3	0.999	97.5	≥ 0.9995	97.0	0.999
M	96.3	0.998	98.4	0.999	95.1	0.999	93.1	0.998	92.5	0.998	93.9	0.998
N	97.2	≥ 0.9995	94.2	≥ 0.9995	99.4	0.999	94.8	≥ 0.9995	98.1	0.999	97.5	≥ 0.9995
O	98.5	0.999	102.0	0.998	90.1	0.993	101.3	0.996	90.1	0.995	99.2	0.995

2 Calculation methods of DNA proportions

Calculation of the relative DNA proportion for cattle, sheep and goat based on the DNA standard dilution series and without normalisation based on the mammalian DNA real-time PCR assay (mpC.won)

To determine the DNA quantities for cattle, sheep and goat, a linear regression analysis of the Cq values for the respective DNA standard dilution series against the logarithmised amounts of DNA (in ng/rxn) of cattle, sheep and goat was performed. The Cq values measured for the samples were assigned to the respective DNA quantities from the regression lines. The proportions (%) of cattle, sheep, and goat DNA in the sample were calculated by dividing each species' DNA quantity (ng/rxn) by the total DNA quantity (sum of cattle, sheep and goat) and expressing the result as a percentage of the total.

Calculation of the relative DNA proportion for cattle, sheep and goat based on only one DNA standard and without normalisation based on the mammalian DNA real-time PCR assay (spC.woN)

To determine the DNA quantities for cattle, sheep or goat (ng/rxn) using only one DNA standard (here with the concentration of 10 ng/rxn) instead of a standard series, a straight line fit with slope $-\log_2(10)$ (corresponds to an efficiency of 100 %) was performed through the Cq value at the point of the decadic logarithmised quantity of DNA of the cattle, sheep or goat DNA standard (ng/rxn). The proportions (%) of cattle, sheep, and goat DNA in the sample were calculated by dividing each species' DNA quantity (ng/rxn) by the total DNA quantity and expressing the result as a percentage of the total.

Calculation of the relative DNA proportions for cattle, sheep and goat based on the DNA standard dilution series with prior normalisation based on the mammalian DNA real-time PCR assay (mpC.wN)

DNA quantities for cattle, sheep and goat were calculated based on the respective DNA standard dilution series. In addition the amount of mammalian DNA (in ng/rxn) was calculated for the normalisation of the cattle DNA using the cattle DNA standards, for the normalisation of the sheep DNA using the sheep DNA standards and for the normalisation of the goat DNA using the goat DNA standards, respectively. The relative DNA proportions (%) of cattle, sheep and goat DNA contained in the sample were calculated by dividing the animal species DNA quantity (ng/rxn) by the respective 16S rDNA quantity (ng/rxn) and by dividing these ratio by the sum of the determined ratios.

Calculation of the relative DNA proportions for cattle, sheep and goat based on only one DNA standard with prior normalisation with the mammalian DNA real-time PCR assay (spC.wN)

To determine the DNA quantities for cattle, sheep or goat (%) using only one standard, a straight line was fitted with a slope of $-\log_2(10)$ (corresponds to an efficiency of 100%) through the Cq value at the point of the decadic logarithmised amount of DNA of the standard from cattle, sheep, goat or the mammalian animal DNA amount (ng/rxn). The Cq values measured in the samples were assigned to the respective DNA quantities from the regression lines. The relative DNA proportions (%) of cattle, sheep and goat DNA contained in the sample were calculated by dividing the animal species DNA quantity (ng/rxn) by the respective 16S rDNA quantity (ng/rxn) and by dividing these ratio by the sum of the determined ratios.

Calculation of the relative DNA proportions for cattle, sheep and goat based on a calibration sample (spC, calibration sample)

If a reference sample with a known distribution of animal species proportions is used instead of a DNA standard series, the absolute DNA quantities of the individual animal species in the reference sample (in ng/rxn) are generally unknown. However, the DNA quantities of the individual animal species of the laboratory samples can be determined relative to the DNA quantities of the reference sample. This involves a straight line fitting Cq value against decadic logarithmised quantities of DNA in the unit as a multiple of the unknown DNA quantity of the reference sample. The straight line is defined by the known reference point (relative DNA quantity and Cq value of the reference sample) and the slope $-\log_2(10)$ (corresponds to an efficiency of 100 %).

From the regression lines, the Cq values measured for the DNA samples are assigned to the respective DNA quantities as multiples of the DNA quantity of the reference sample. The relative DNA proportions (%) of the amount of cattle, sheep and goat DNA contained in the sample are calculated by normalising the determined animal species DNA amounts (ng/rxn) to 100 %, whereby the animal species proportions of the reference sample are also included.

3 Statistical analysis of the ring trial

The result of a laboratory was excluded from the sample analysis if the calculated DNA proportion for at least one of the three animal species was significant with respect to the Mandel's h-test at a 1 % level. For comparison, laboratories were marked as near outlier for a sample if the proportion for at least one of the three animal species was significant at the 5 % level for at least one of the three tests: Mandel's h, Grubbs I, or Grubbs II. Following the outlier analysis, the assumption of normal distribution was examined for each sample-species combination. This was done visually by comparing a nonparametric kernel density estimate (limit of the histogram as class width approaches zero) with the density of an adjusted normal distribution, as well as by comparing empirical sample quantiles with the theoretical quantiles of a normal distribution (Normal Q-Q plot). Additionally, the Shapiro-Wilk test was applied.

Since there were no false-positive results, the quantitative evaluation only applies to combinations of animal-sample combinations with positive mass fractions. Consequently, the ring trial samples P03 and P05 with 100% goat content regarding the species composition values are unsuitable for a meaningful quantitative evaluation. The outlier proportion of the laboratory-sample combinations was 5/140 (3.57 %) for mpC.wON, 4/140 (2.86 %) for spC.wN, 4/140 (2.86 %) for mpC.wN and 3/140 (2.14 %) for spC.wN (Tables S5-S12). Furthermore, there were no noticeable deviations from the normal distribution with regard to the outlier-adjusted samples.

Table S5 Statistical outlier: mpC.woN (stat sign. 1%, near sign. 5%)

Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P04	cattle	10	near	ok	ok	M
P04	sheep	85	stat	near	ok	M
P04	goat	5	near	ok	ok	M
P10	cattle	2	stat	stat	near	G
P10	sheep	2	ok	ok	ok	G
P10	goat	96	ok	ok	ok	G
P10	cattle	2	ok	ok	ok	O
P10	sheep	2	stat	stat	stat	O
P10	goat	96	stat	stat	near	O
P11	cattle	33.33	ok	ok	ok	B
P11	sheep	33.33	ok	ok	ok	B
P11	goat	33.33	stat	ok	near	B
P12	cattle	5	stat	near	ok	O
P12	goat	95	stat	near	ok	O

Table S6 Near outlier: mpC.woN (stat sign. 1%, near sign. 5%)

Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P01	cattle	2	near	ok	ok	D
P01	goat	98	near	ok	ok	D
P06	cattle	10	ok	ok	ok	M
P06	sheep	85	near	ok	ok	M
P06	goat	5	near	ok	ok	M
P09	cattle	5	near	ok	ok	G
P09	goat	95	near	ok	ok	G
P10	cattle	2	ok	ok	near	D
P10	sheep	2	ok	ok	stat	D
P10	goat	96	ok	ok	near	D
P11	cattle	33.33	ok	ok	ok	M
P11	sheep	33.33	ok	ok	ok	M
P11	goat	33.33	ok	ok	near	M

Table S7 Statistical outlier: spC.woN (stat sign. 1%, near sign. 5%)

Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P04	cattle	10	ok	ok	ok	K
P04	sheep	85	ok	ok	ok	K
P04	goat	5	stat	ok	ok	K
P04	cattle	10	stat	near	ok	M
P04	sheep	85	near	ok	ok	M
P04	goat	5	ok	ok	ok	M
P09	cattle	5	stat	ok	ok	O
P09	goat	95	stat	ok	ok	O
P12	cattle	5	stat	stat	stat	O
P12	goat	95	stat	stat	stat	O

Table S8 Near outlier: spC.woN (stat sign. 1%, near sign. 5%)

Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P01	cattle	2	near	ok	ok	D
P01	goat	98	near	ok	ok	D
P02	cattle	5	near	ok	ok	K
P02	goat	95	near	ok	ok	K
P06	cattle	10	near	ok	ok	M
P06	sheep	85	near	ok	ok	M
P06	goat	5	ok	ok	ok	M
P07	cattle	2	near	ok	ok	N
P07	sheep	98	near	ok	ok	N
P08	cattle	1	near	ok	ok	N
P08	sheep	99	near	ok	ok	N
P10	cattle	2	ok	ok	ok	O
P10	sheep	2	near	ok	ok	O
P10	goat	96	near	ok	ok	O
P11	cattle	33.33	ok	ok	ok	B
P11	sheep	33.33	ok	ok	ok	B
P11	goat	33.33	near	ok	ok	B
P11	cattle	33.33	ok	ok	ok	M
P11	sheep	33.33	near	ok	ok	M
P11	goat	33.33	near	ok	ok	M
P12	cattle	5	ok	ok	stat	B
P12	goat	95	ok	ok	stat	B

Table S9 Statistical outlier: mpC.wN (stat sign. 1%, near sign. 5%)

Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P06	cattle	10	near	ok	ok	K
P06	sheep	85	stat	ok	near	K
P06	goat	5	stat	ok	near	K
P08	cattle	1	stat	ok	ok	K
P08	sheep	99	stat	ok	ok	K
P10	cattle	2	stat	stat	stat	G
P10	sheep	2	ok	ok	ok	G
P10	goat	96	ok	ok	ok	G
P12	cattle	5	stat	ok	ok	O
P12	goat	95	stat	ok	ok	O

Table S10 Near outlier: mpC.wN (stat sign. 1%, near sign. 5%)

Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P02	cattle	5	near	ok	near	F
P02	goat	95	near	ok	near	F
P02	cattle	5	near	ok	near	J
P02	goat	95	near	ok	near	J
P04	cattle	10	ok	ok	ok	K
P04	sheep	85	near	ok	near	K
P04	goat	5	near	ok	ok	K
P04	cattle	10	near	ok	ok	M
P04	sheep	85	near	ok	near	M
P04	goat	5	ok	ok	ok	M
P06	cattle	10	ok	ok	ok	M
P06	sheep	85	ok	ok	near	M
P06	goat	5	ok	ok	near	M
P09	cattle	5	near	ok	ok	G
P09	goat	95	near	ok	ok	G
P10	cattle	2	ok	ok	stat	B
P10	sheep	2	ok	ok	ok	B
P10	goat	96	ok	ok	ok	B
P10	cattle	2	ok	ok	ok	O
P10	sheep	2	near	ok	ok	O
P10	goat	96	near	ok	ok	O
P11	cattle	33.33	near	ok	ok	B
P11	sheep	33.33	ok	ok	near	B
P11	goat	33.33	near	ok	near	B
P11	cattle	33.33	near	ok	ok	I
P11	sheep	33.33	ok	ok	ok	I
P11	goat	33.33	ok	ok	ok	I
P11	cattle	33.33	ok	ok	ok	K
P11	sheep	33.33	near	ok	near	K
P11	goat	33.33	near	ok	near	K

Table S11 Statistical outlier: spC.wN (stat sign. 1%, near sign. 5%)

Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P04	cattle	10	stat	near	ok	M
P04	sheep	85	stat	near	near	M
P04	goat	5	stat	ok	ok	M
P11	cattle	33.33	ok	ok	ok	M
P11	sheep	33.33	stat	near	ok	M
P11	goat	33.33	stat	ok	near	M
P12	cattle	5	stat	stat	stat	O
P12	goat	95	stat	stat	stat	O

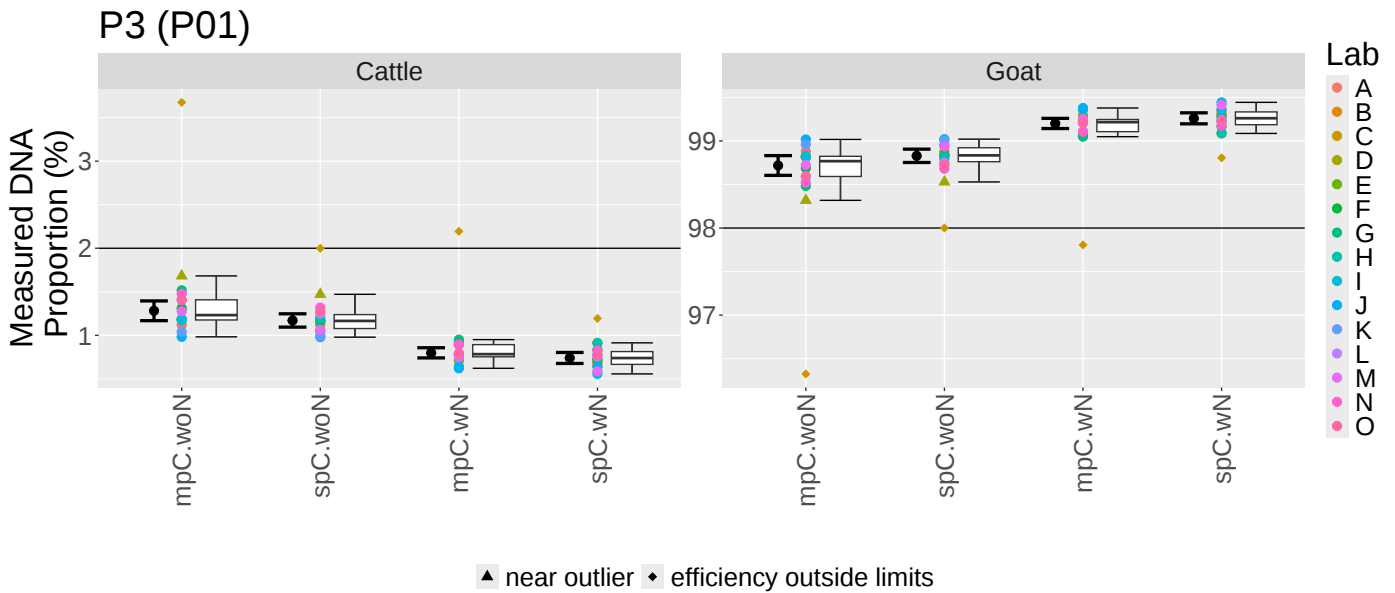
Table S12 Near outlier: spC.wN (stat sign. 1%, near sign. 5%)

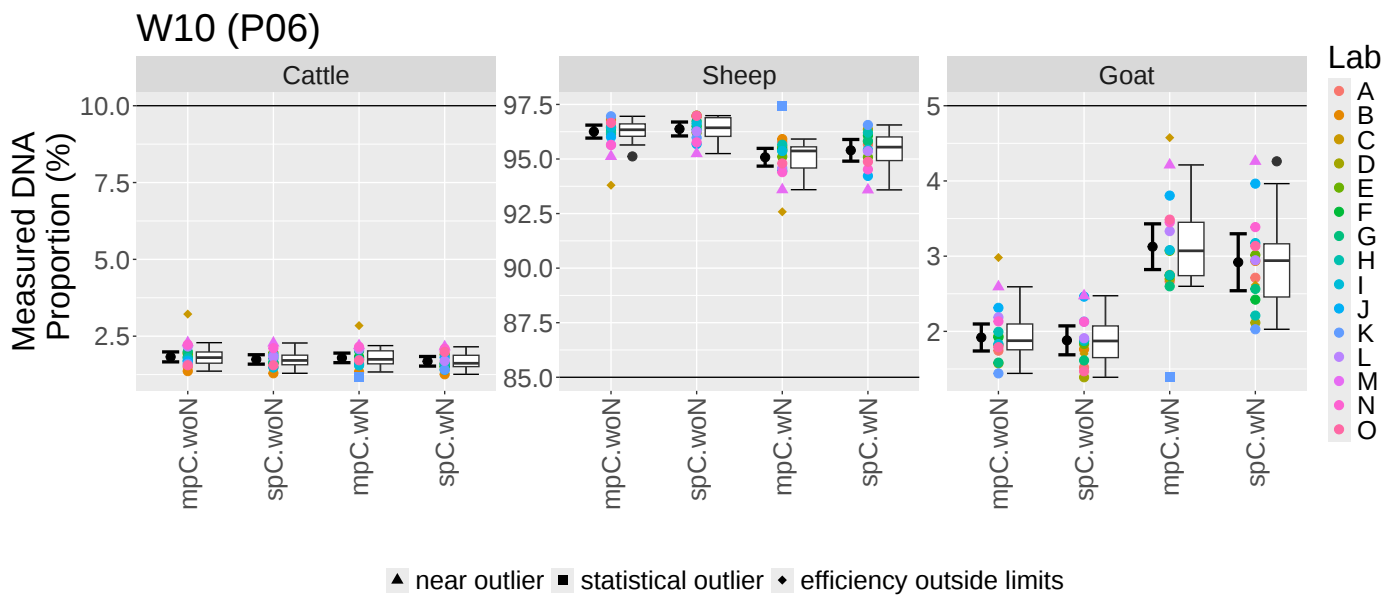
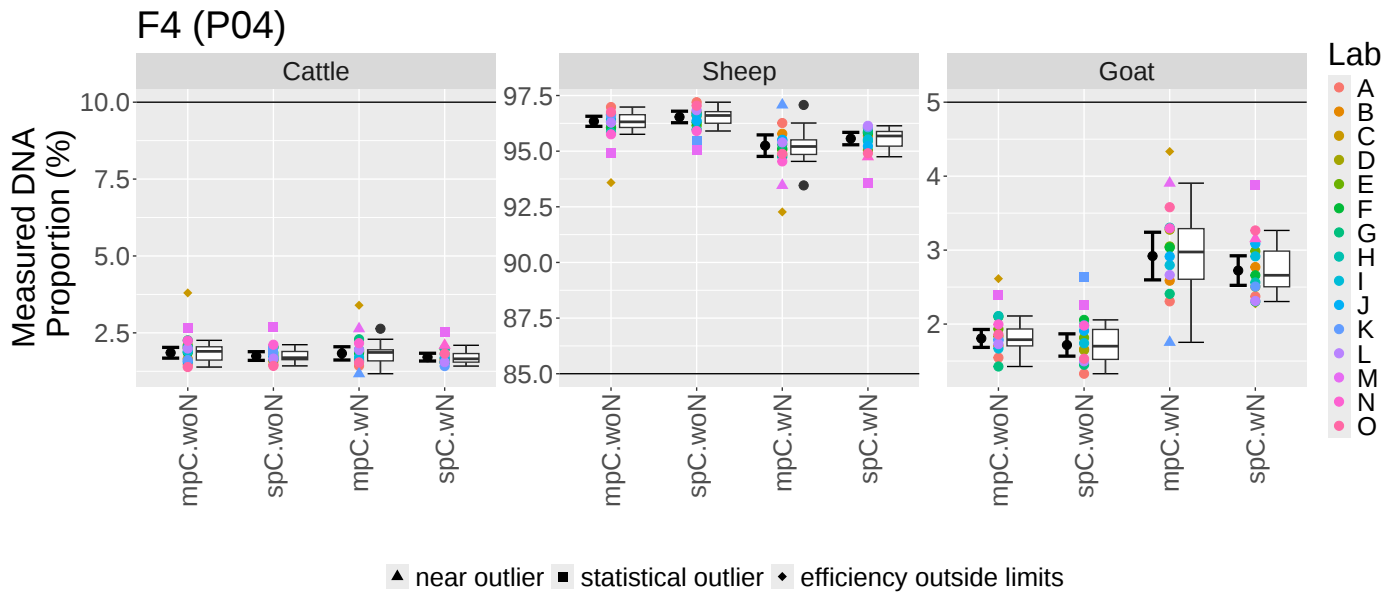
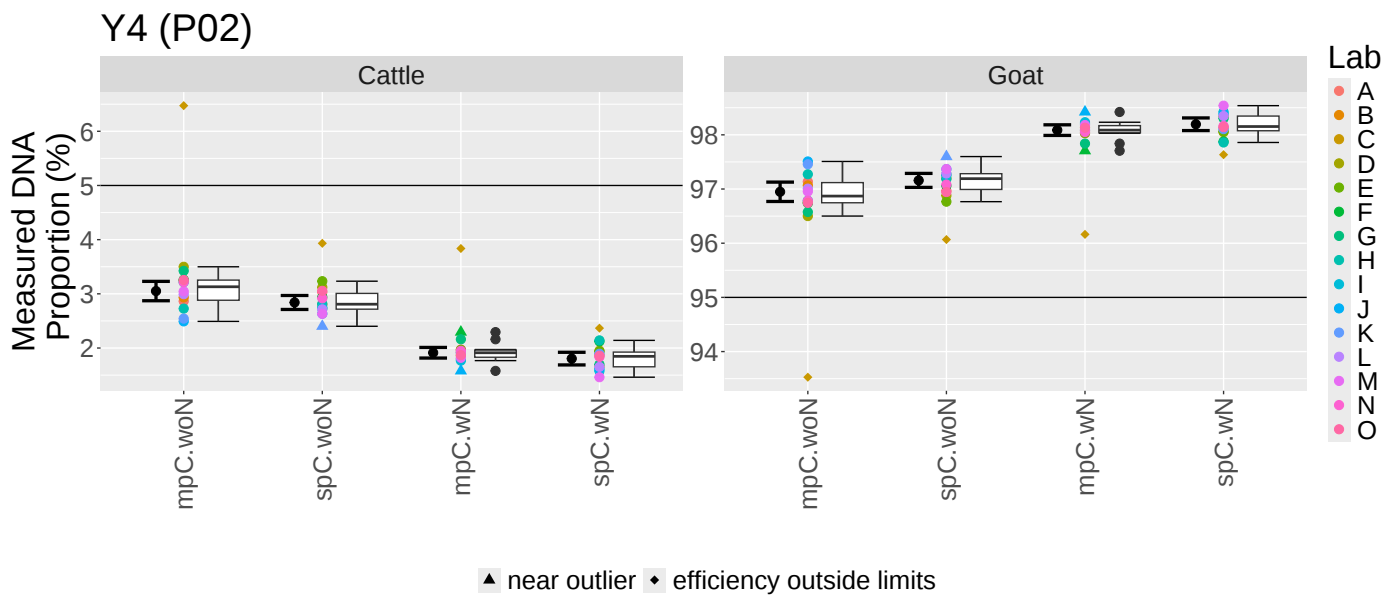
Ring trial sample ID	Animal	Milk mass fraction (%)	Mandel's h	Grubbs I	Grubbs II	Lab
P04	cattle	10	ok	ok	ok	N
P04	sheep	85	ok	ok	near	N
P04	goat	5	ok	ok	ok	N
P06	cattle	10	ok	ok	ok	M
P06	sheep	85	near	ok	ok	M
P06	goat	5	near	ok	ok	M
P07	cattle	2	near	ok	ok	N
P07	sheep	98	near	ok	ok	N
P09	cattle	5	near	ok	ok	M
P09	goat	95	near	ok	ok	M
P09	cattle	5	near	ok	ok	O
P09	goat	95	near	ok	ok	O
P10	cattle	2	ok	ok	ok	H
P10	sheep	2	near	ok	ok	H
P10	goat	96	near	ok	ok	H
P10	cattle	2	near	ok	ok	M
P10	sheep	2	ok	ok	ok	M
P10	goat	96	ok	ok	ok	M
P11	cattle	33.33	ok	ok	ok	B
P11	sheep	33.33	ok	ok	ok	B
P11	goat	33.33	ok	ok	near	B
P11	cattle	33.33	near	ok	ok	I
P11	sheep	33.33	ok	ok	ok	I
P11	goat	33.33	ok	ok	ok	I
P12	cattle	5	ok	ok	stat	H
P12	goat	95	ok	ok	stat	H
P12	cattle	5	ok	ok	near	I
P12	goat	95	ok	ok	near	I

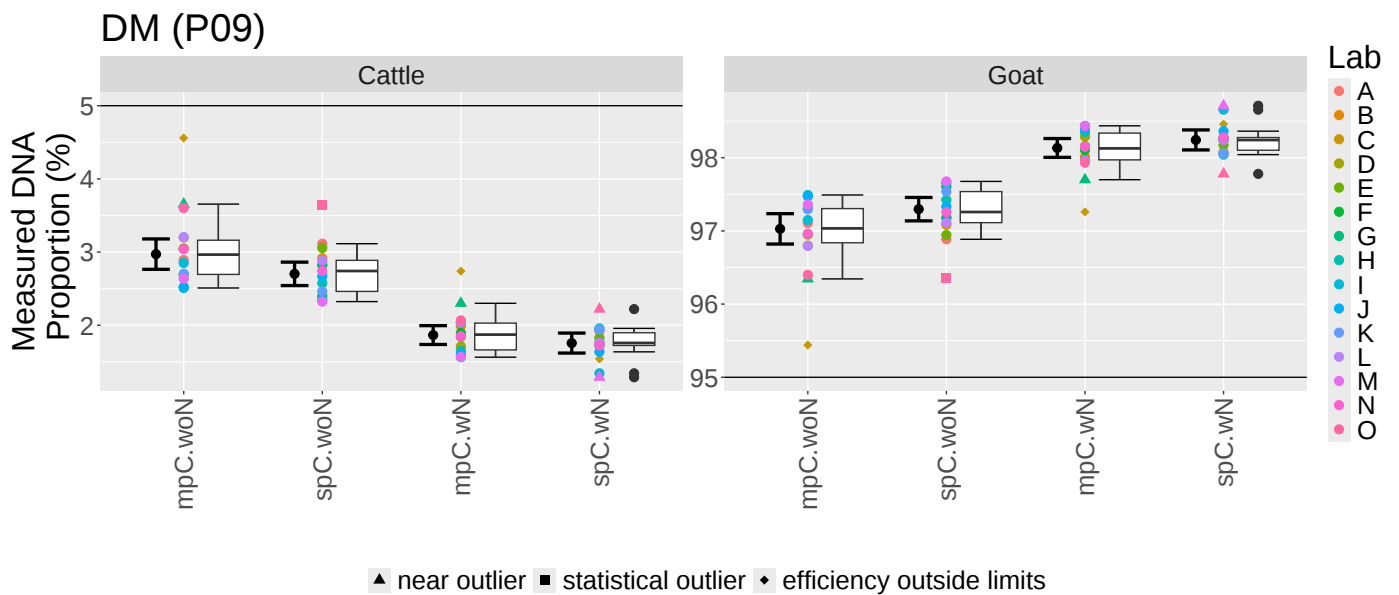
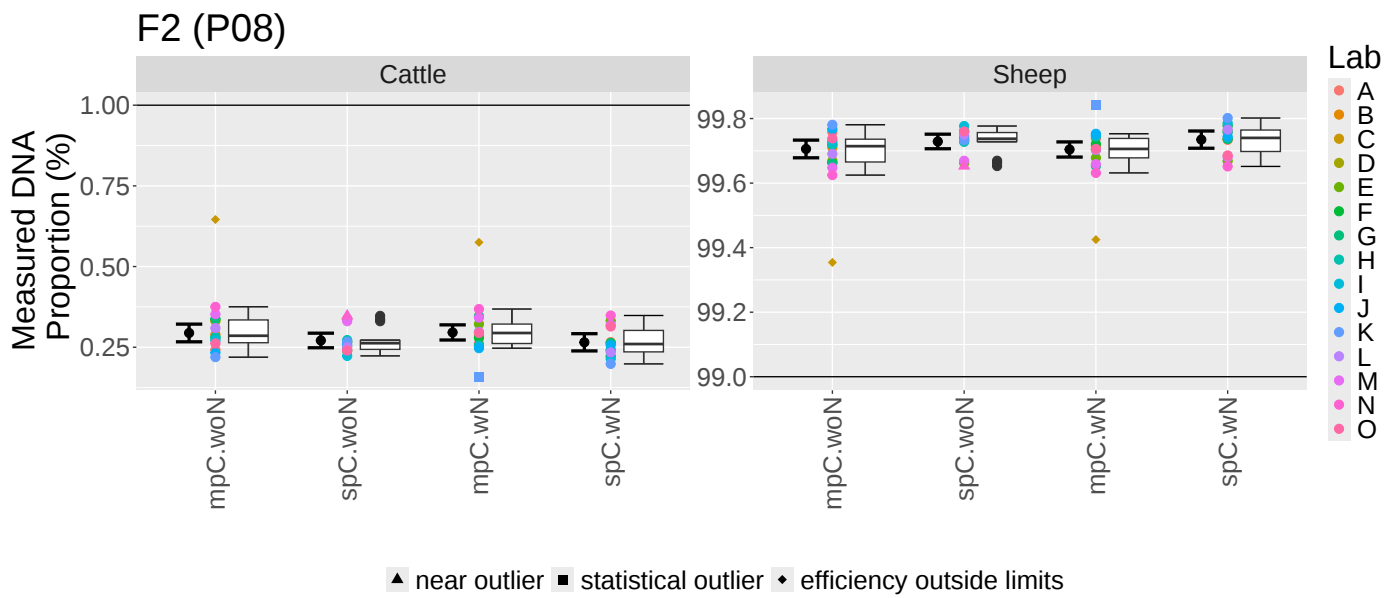
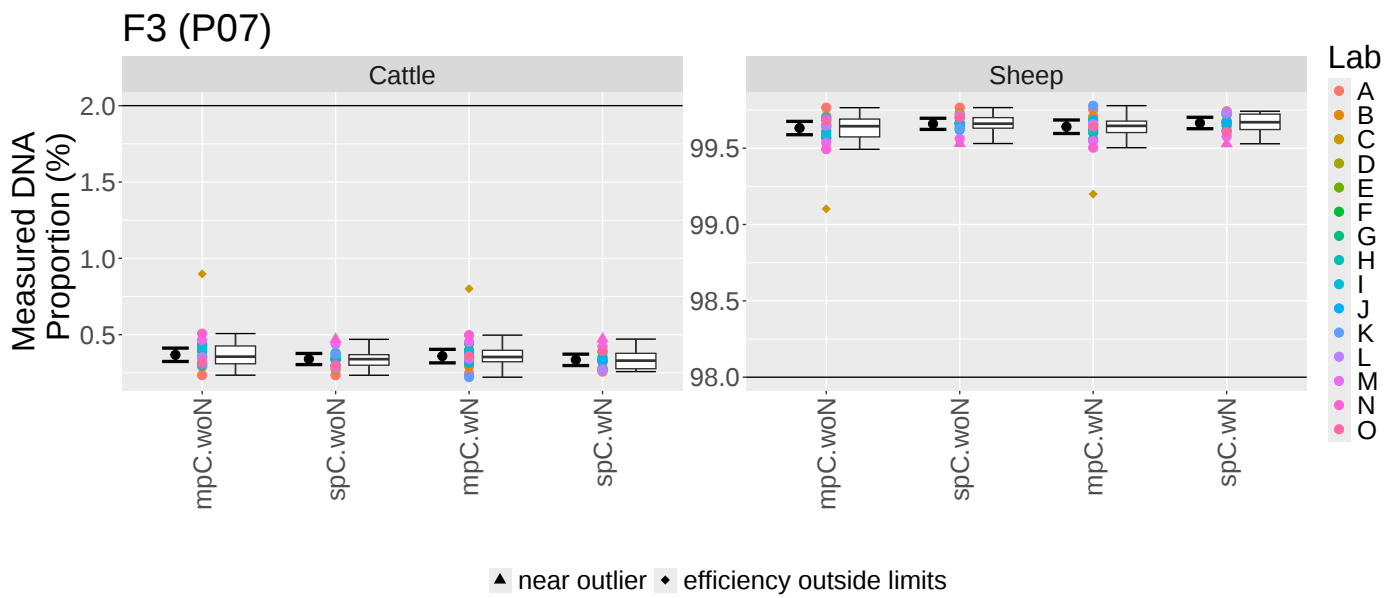
4 Visualisation of the determined proportions of cattle, sheep and goat DNA

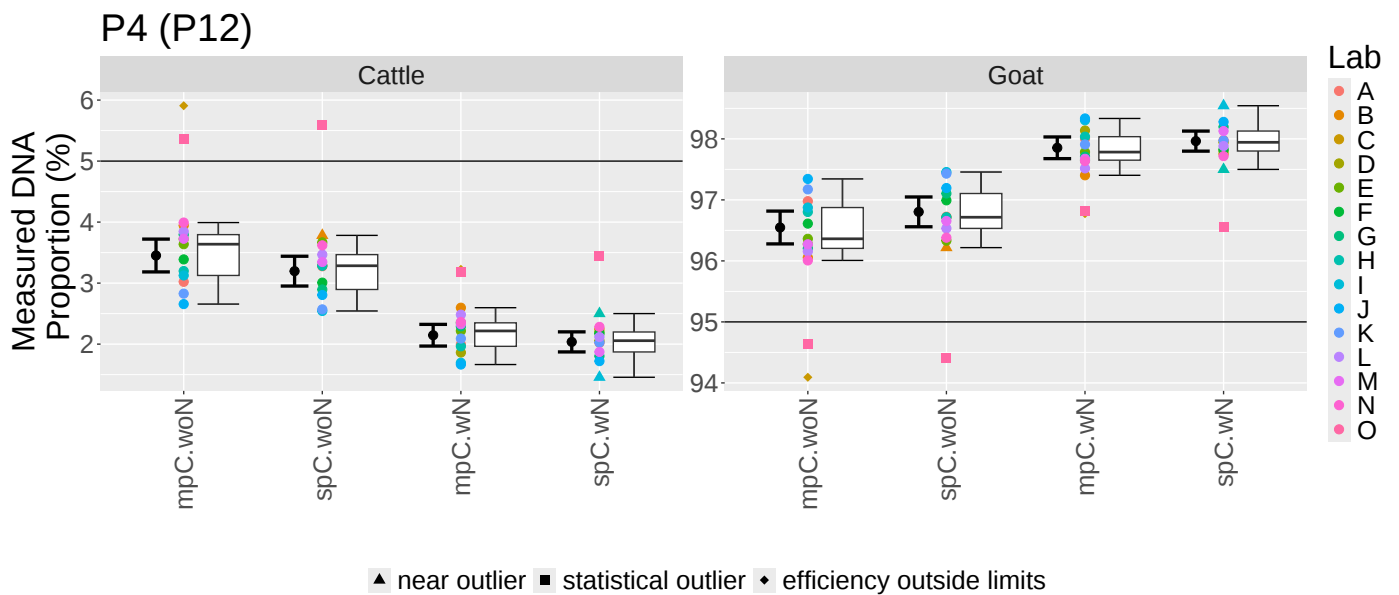
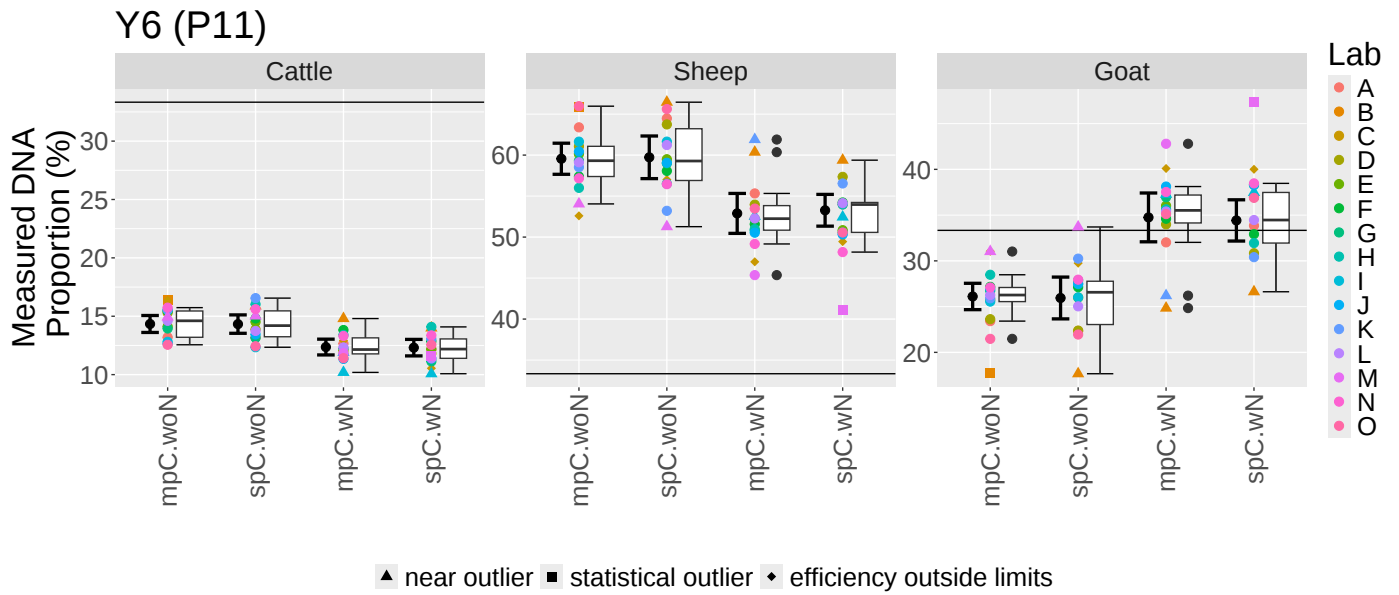
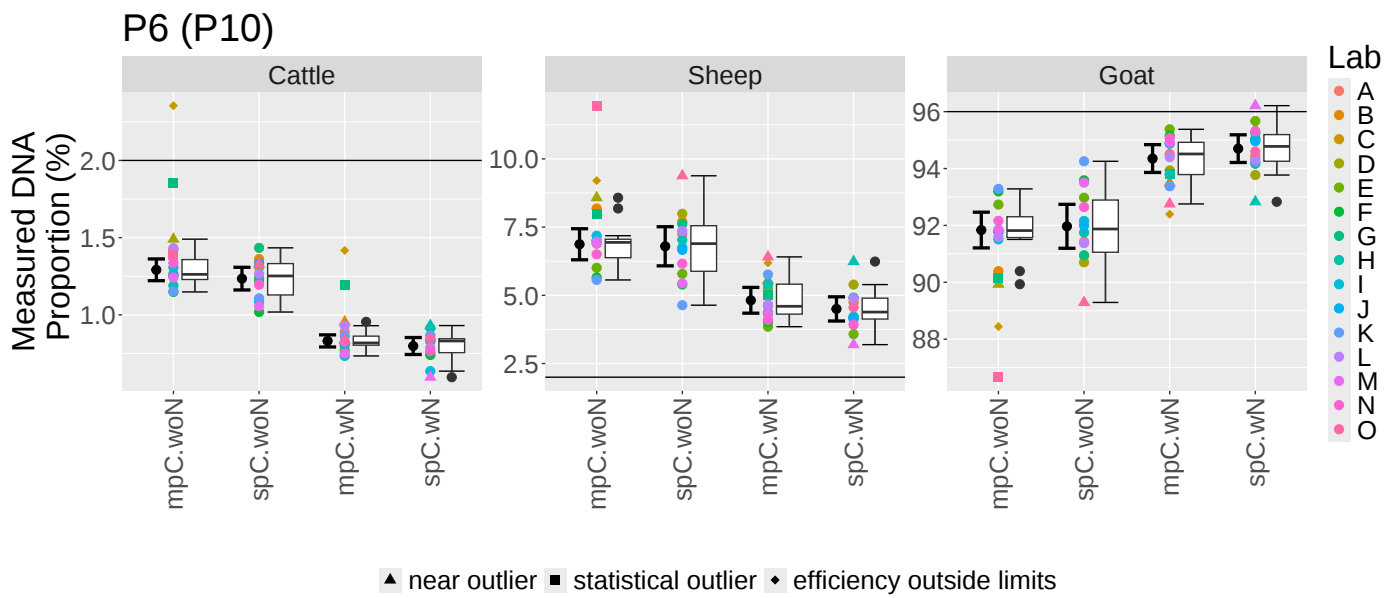
Fig S5

The determined proportions of cattle, sheep and goat DNA for the ring trial samples containing milk from at least two species and for animal-sample combinations with positive milk mass fraction. Displayed are the calculated individual proportions of each laboratory (middle with shape denoting: triangle for near outlier, square for statistical outlier, diamond for efficiency exclusion outside expected limits). Additionally, boxplots (right) and mean values with 95 % confidence intervals assuming normality (left, the length of the interval is proportional to the standard deviation of reproducibility) are shown following outlier adjustment. The horizontal lines depict the mass fractions used for production.









5 Tables of the statistical parameters

Statistical parameters according to the specification of BVL (2006) based on DIN ISO 5725-2 for determining the relative DNA proportion for cattle, sheep and goat based on four calculation methods (mpC.woN, spC.woN, mpC.wN, spC.wN) for ring trial samples containing milk from at least two species and for animal-sample combinations with positive milk mass fraction (Tables S13-S24).

Table S13 Statistical parameters: mpC.woN, Cattle

Sample ID	P01	P02	P04	P06	P07	P08	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	Feta	Feta	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	13	14	14	14	14	12	13	13
Number of outlier laboratories	0	0	1	0	0	0	0	2	1	1
Milk mass fraction (%)	2	5	10	10	2	1	5	2	33.33	5
Mean (%)	1.28	3.05	1.85	1.83	0.37	0.29	2.97	1.29	14.34	3.45
Standard deviation of reproducibility (%)	0.2	0.31	0.29	0.28	0.08	0.05	0.36	0.11	1.2	0.44
Reproducibility limit (%)	0.55	0.87	0.81	0.79	0.21	0.13	1.01	0.31	3.37	1.25
Mean / mass fraction (%)	64.09	61.02	18.52	18.26	18.42	29.42	59.44	64.59	43.01	69.05
Lower limit 95%-confidence interval* mean / mass fraction (%)	58.42	57.45	16.78	16.63	16.23	26.68	55.29	61.06	40.83	63.67
Upper limit 95%-confidence interval* mean / mass fraction (%)	69.76	64.6	20.27	19.88	20.61	32.16	63.59	68.11	45.19	74.43
Mean - mass fraction (%)	-0.72	-1.95	-8.15	-8.17	-1.63	-0.71	-2.03	-0.71	-19	-1.55
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	-35.91	-38.98	-81.48	-81.74	-81.58	-70.58	-40.56	-35.41	-56.99	-30.95
Standard deviation of reproducibility mass fraction (%)	9.82	6.19	2.89	2.81	3.79	4.74	7.19	5.54	3.61	8.9
Standard deviation of reproducibility mean (%)	15.32	10.15	15.61	15.4	20.56	16.13	12.1	8.58	8.39	12.88
Uncertainty** (%)	0.74	1.97	8.15	8.18	1.63	0.71	2.06	0.72	19.03	1.61
Relative uncertainty*** (%)	37.23	39.47	81.53	81.79	81.67	70.74	41.19	35.85	57.1	32.2
Logit**** mass fraction	-3.89	-2.94	-2.2	-2.2	-3.89	-4.6	-2.94	-3.89	-0.69	-2.94
Mean of Logits	-4.35	-3.46	-3.98	-4	-5.62	-5.84	-3.49	-4.34	-1.79	-3.34
Standard deviation of reproducibility of Logits	0.15	0.11	0.16	0.16	0.21	0.16	0.12	0.09	0.1	0.14

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S14 Statistical parameters: mpC.woN, Sheep

Sample ID	P04	P06	P07	P08	P10	P11
Sample type	Feta	Feta whey	Feta	Feta	Picodon	yoghurt
Number of participating laboratories	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14
Number of laboratories after outlier elimination	13	14	14	14	12	13
Number of outlier laboratories	1	0	0	0	2	1
Milk mass fraction (%)	85	85	98	99	2	33.33
Mean (%)	96.34	96.26	99.63	99.71	6.87	59.55
Standard deviation of reproducibility (%)	0.37	0.52	0.08	0.05	0.9	3.14
Reproducibility limit (%)	1.05	1.44	0.21	0.13	2.51	8.79
Mean / mass fraction (%)	113.34	113.24	101.66	100.71	343.57	178.66
Lower limit 95%-confidence interval* mean / mass fraction (%)	113.08	112.89	101.62	100.69	315.1	172.97
Upper limit 95%-confidence interval* mean / mass fraction (%)	113.61	113.59	101.71	100.74	372.03	184.35
Mean - mass fraction (%)	11.34	11.26	1.63	0.71	4.87	26.22
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	13.34	13.24	1.66	0.71	243.57	78.66
$\frac{\text{Standard deviation of reproducibility}}{\text{mass fraction}}$ (%)	0.44	0.61	0.08	0.05	44.8	9.42
$\frac{\text{Standard deviation of reproducibility}}{\text{mean}}$ (%)	0.39	0.54	0.08	0.05	13.04	5.27
Uncertainty** (%)	11.35	11.27	1.63	0.71	4.95	26.41
Relative uncertainty*** (%)	13.35	13.26	1.67	0.71	247.65	79.23
Logit**** mass fraction	1.73	1.73	3.89	4.6	-3.89	-0.69
Mean of Logits	3.28	3.26	5.62	5.84	-2.61	0.39
Standard deviation of reproducibility of Logits	0.11	0.14	0.21	0.16	0.14	0.13

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S15 Statistical parameters: mpC.woN, Goat

Sample ID	P01	P02	P04	P06	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	13	14	14	12	13	13
Number of outlier laboratories	0	0	1	0	0	2	1	1
Milk mass fraction (%)	98	95	5	5	95	96	33.33	95
Mean (%)	98.72	96.95	1.81	1.92	97.03	91.84	26.11	96.55
Standard deviation of reproducibility (%)	0.2	0.31	0.2	0.31	0.36	0.99	2.38	0.44
Reproducibility limit (%)	0.55	0.87	0.56	0.87	1.01	2.77	6.68	1.25
Mean / mass fraction (%)	100.73	102.05	36.11	38.38	102.13	95.66	78.32	101.63
Lower limit 95%-confidence interval* mean / mass fraction (%)	100.62	101.86	33.69	34.79	101.92	95.01	74	101.35
Upper limit 95%-confidence interval* mean / mass fraction (%)	100.85	102.24	38.54	41.97	102.35	96.32	82.65	101.91
Mean - mass fraction (%)	0.72	1.95	-3.19	-3.08	2.03	-4.16	-7.23	1.55
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	0.73	2.05	-63.89	-61.62	2.13	-4.34	-21.68	1.63
$\frac{\text{Standard deviation of reproducibility}}{\text{mass fraction}}$ (%)	0.2	0.33	4.01	6.22	0.38	1.03	7.15	0.47
$\frac{\text{Standard deviation of reproducibility}}{\text{mean}}$ (%)	0.2	0.32	11.09	16.2	0.37	1.08	9.13	0.46
Uncertainty** (%)	0.74	1.97	3.2	3.1	2.06	4.28	7.61	1.61
Relative uncertainty*** (%)	0.76	2.08	64.01	61.94	2.17	4.46	22.83	1.69
Logit**** mass fraction	3.89	2.94	-2.94	-2.94	2.94	3.18	-0.69	2.94
Mean of Logits	4.35	3.46	-4	-3.95	3.49	2.43	-1.04	3.34
Standard deviation of reproducibility of Logits	0.15	0.11	0.12	0.16	0.12	0.13	0.12	0.14

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S16 Statistical parameters: spC.woN, Cattle

Sample ID	P01	P02	P04	P06	P07	P08	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	Feta	Feta	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	12	14	14	14	13	14	14	13
Number of outlier laboratories	0	0	2	0	0	0	1	0	0	1
Milk mass fraction (%)	2	5	10	10	2	1	5	2	33.33	5
Mean (%)	1.17	2.84	1.75	1.74	0.34	0.27	2.7	1.23	14.33	3.2
Standard deviation of reproducibility (%)	0.13	0.22	0.22	0.27	0.06	0.04	0.26	0.13	1.36	0.4
Reproducibility limit (%)	0.37	0.63	0.62	0.74	0.18	0.11	0.74	0.36	3.82	1.13
Mean / mass fraction (%)	58.54	56.82	17.46	17.43	17.03	27.1	54.05	61.73	42.98	63.92
Lower limit 95%-confidence interval* mean / mass fraction (%)	54.71	54.23	16.05	15.9	15.21	24.84	50.85	58.06	40.62	59.04
Upper limit 95%-confidence interval* mean / mass fraction (%)	62.36	59.4	18.87	18.96	18.86	29.36	57.25	65.4	45.34	68.81
Mean - mass fraction (%)	-0.83	-2.16	-8.25	-8.26	-1.66	-0.73	-2.3	-0.77	-19.01	-1.8
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	-41.46	-43.18	-82.54	-82.57	-82.97	-72.9	-45.95	-38.27	-57.02	-36.08
Standard deviation of reproducibility mass fraction (%)	6.62	4.47	2.22	2.66	3.16	3.91	5.3	6.35	4.09	8.09
Standard deviation of reproducibility mean (%)	11.32	7.87	12.72	15.23	18.55	14.43	9.8	10.29	9.53	12.65
Uncertainty** (%)	0.84	2.17	8.26	8.26	1.66	0.73	2.31	0.78	19.06	1.85
Relative uncertainty*** (%)	41.99	43.41	82.57	82.61	83.03	73.01	46.25	38.79	57.17	36.97
Logit**** mass fraction	-3.89	-2.94	-2.2	-2.2	-3.89	-4.6	-2.94	-3.89	-0.69	-2.94
Mean of Logits	-4.44	-3.54	-4.04	-4.04	-5.69	-5.92	-3.59	-4.39	-1.79	-3.42
Standard deviation of reproducibility of Logits	0.11	0.08	0.13	0.15	0.19	0.14	0.1	0.11	0.11	0.13

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S17 Statistical parameters: spC.woN, Sheep

Sample ID	P04	P06	P07	P08	P10	P11
Sample type	Feta	Feta whey	Feta	Feta	Picodon	yoghurt
Number of participating laboratories	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14
Number of laboratories after outlier elimination	12	14	14	14	14	14
Number of outlier laboratories	2	0	0	0	0	0
Milk mass fraction (%)	85	85	98	99	2	33.33
Mean (%)	96.54	96.38	99.66	99.73	6.8	59.73
Standard deviation of reproducibility (%)	0.41	0.55	0.06	0.04	1.24	4.5
Reproducibility limit (%)	1.14	1.55	0.18	0.11	3.48	12.6
Mean / mass fraction (%)	113.57	113.38	101.69	100.74	339.86	179.19
Lower limit 95%-confidence interval* mean / mass fraction (%)	113.27	113.01	101.66	100.71	304.01	171.39
Upper limit 95%-confidence interval* mean / mass fraction (%)	113.88	113.76	101.73	100.76	375.71	186.99
Mean - mass fraction (%)	11.54	11.38	1.66	0.73	4.8	26.4
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	13.57	13.38	1.69	0.74	239.86	79.19
$\frac{\text{Standard deviation of reproducibility}}{\text{mass fraction}}$ (%)	0.48	0.65	0.06	0.04	62.09	13.5
$\frac{\text{Standard deviation of reproducibility}}{\text{mean}}$ (%)	0.42	0.57	0.06	0.04	18.27	7.54
Uncertainty** (%)	11.54	11.39	1.66	0.73	4.96	26.78
Relative uncertainty*** (%)	13.58	13.4	1.69	0.74	247.77	80.33
Logit**** mass fraction	1.73	1.73	3.89	4.6	-3.89	-0.69
Mean of Logits	3.33	3.29	5.69	5.92	-2.63	0.4
Standard deviation of reproducibility of Logits	0.12	0.15	0.19	0.14	0.2	0.19

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S18 Statistical parameters: spC.woN, Goat

Sample ID	P01	P02	P04	P06	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	12	14	13	14	14	13
Number of outlier laboratories	0	0	2	0	1	0	0	1
Milk mass fraction (%)	98	95	5	5	95	96	33.33	95
Mean (%)	98.83	97.16	1.72	1.88	97.3	91.97	25.94	96.8
Standard deviation of reproducibility (%)	0.13	0.22	0.24	0.33	0.26	1.34	3.96	0.4
Reproducibility limit (%)	0.37	0.63	0.66	0.93	0.74	3.75	11.08	1.13
Mean / mass fraction (%)	100.85	102.27	34.34	37.62	102.42	95.8	77.83	101.9
Lower limit 95%-confidence interval* mean / mass fraction (%)	100.77	102.14	31.33	33.77	102.25	95	70.98	101.64
Upper limit 95%-confidence interval* mean / mass fraction (%)	100.92	102.41	37.36	41.46	102.59	96.6	84.68	102.16
Mean - mass fraction (%)	0.83	2.16	-3.28	-3.12	2.3	-4.03	-7.39	1.8
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	0.85	2.27	-65.66	-62.38	2.42	-4.2	-22.17	1.9
$\frac{\text{Standard deviation of reproducibility}}{\text{mass fraction}}$ (%)	0.14	0.24	4.74	6.66	0.28	1.39	11.87	0.43
$\frac{\text{Standard deviation of reproducibility}}{\text{mean}}$ (%)	0.13	0.23	13.81	17.7	0.27	1.45	15.25	0.42
Uncertainty** (%)	0.84	2.17	3.29	3.14	2.31	4.25	8.38	1.85
Relative uncertainty*** (%)	0.86	2.28	65.83	62.74	2.43	4.42	25.15	1.95
Logit**** mass fraction	3.89	2.94	-2.94	-2.94	2.94	3.18	-0.69	2.94
Mean of Logits	4.44	3.54	-4.06	-3.97	3.59	2.45	-1.06	3.42
Standard deviation of reproducibility of Logits	0.11	0.08	0.14	0.18	0.1	0.18	0.21	0.13

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S19 Statistical parameters: mpC.wN, Cattle

Sample ID	P01	P02	P04	P06	P07	P08	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	Feta	Feta	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	14	13	14	13	14	13	14	13
Number of outlier laboratories	0	0	0	1	0	1	0	1	0	1
Milk mass fraction (%)	2	5	10	10	2	1	5	2	33.33	5
Mean (%)	0.8	1.91	1.83	1.79	0.36	0.3	1.87	0.83	12.36	2.15
Standard deviation of reproducibility (%)	0.1	0.17	0.37	0.26	0.08	0.04	0.22	0.06	1.17	0.29
Reproducibility limit (%)	0.28	0.48	1.04	0.72	0.21	0.11	0.62	0.18	3.27	0.82
Mean / mass fraction (%)	39.96	38.26	18.32	17.94	17.99	29.59	37.32	41.57	37.08	42.92
Lower limit 95%-confidence interval* mean / mass fraction (%)	37.04	36.3	16.17	16.38	15.78	27.24	34.75	39.61	35.06	39.37
Upper limit 95%-confidence interval* mean / mass fraction (%)	42.88	40.22	20.47	19.49	20.2	31.93	39.89	43.53	39.11	46.47
Mean - mass fraction (%)	-1.2	-3.09	-8.17	-8.21	-1.64	-0.7	-3.13	-1.17	-20.97	-2.85
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	-60.04	-61.74	-81.68	-82.06	-82.01	-70.41	-62.68	-58.43	-62.92	-57.08
Standard deviation of reproducibility mass fraction (%)	5.06	3.39	3.73	2.58	3.83	3.89	4.46	3.25	3.51	5.88
Standard deviation of reproducibility mean (%)	12.66	8.87	20.34	14.37	21.28	13.13	11.94	7.81	9.46	13.71
Uncertainty** (%)	1.21	3.09	8.18	8.21	1.64	0.71	3.14	1.17	21	2.87
Relative uncertainty*** (%)	60.26	61.83	81.76	82.11	82.1	70.52	62.84	58.52	63.01	57.38
Logit**** mass fraction	-3.89	-2.94	-2.2	-2.2	-3.89	-4.6	-2.94	-3.89	-0.69	-2.94
Mean of Logits	-4.83	-3.94	-4	-4.01	-5.65	-5.83	-3.97	-4.78	-1.96	-3.83
Standard deviation of reproducibility of Logits	0.13	0.09	0.21	0.15	0.23	0.13	0.12	0.08	0.11	0.14

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S20 Statistical parameters: mpC.wN, Sheep

Sample ID	P04	P06	P07	P08	P10	P11
Sample type	Feta	Feta whey	Feta	Feta	Picodon	yoghurt
Number of participating laboratories	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	13	14	13	13	14
Number of outlier laboratories	0	1	0	1	1	0
Milk mass fraction (%)	85	85	98	99	2	33.33
Mean (%)	95.25	95.08	99.64	99.7	4.82	52.89
Standard deviation of reproducibility (%)	0.84	0.67	0.08	0.04	0.78	4.22
Reproducibility limit (%)	2.34	1.88	0.21	0.11	2.19	11.81
Mean / mass fraction (%)	112.06	111.86	101.67	100.71	240.91	158.67
Lower limit 95%-confidence interval* mean / mass fraction (%)	111.49	111.38	101.63	100.69	217.31	151.36
Upper limit 95%-confidence interval* mean / mass fraction (%)	112.63	112.34	101.72	100.73	264.5	165.97
Mean - mass fraction (%)	10.25	10.08	1.64	0.7	2.82	19.56
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	12.06	11.86	1.67	0.71	140.91	58.67
Standard deviation of reproducibility mass fraction (%)	0.99	0.79	0.08	0.04	39.05	12.65
Standard deviation of reproducibility mean (%)	0.88	0.71	0.08	0.04	16.21	7.97
Uncertainty** (%)	10.28	10.1	1.64	0.71	2.92	20.01
Relative uncertainty*** (%)	12.1	11.89	1.68	0.71	146.22	60.02
Logit**** mass fraction	1.73	1.73	3.89	4.6	-3.89	-0.69
Mean of Logits	3.01	2.97	5.65	5.83	-3	0.12
Standard deviation of reproducibility of Logits	0.2	0.14	0.23	0.13	0.17	0.17

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S21 Statistical parameters: mpC.wN, Goat

Sample ID	P01	P02	P04	P06	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	14	13	14	13	14	13
Number of outlier laboratories	0	0	0	1	0	1	0	1
Milk mass fraction (%)	98	95	5	5	95	96	33.33	95
Mean (%)	99.2	98.09	2.92	3.13	98.13	94.35	34.75	97.85
Standard deviation of reproducibility (%)	0.1	0.17	0.56	0.5	0.22	0.81	4.62	0.29
Reproducibility limit (%)	0.28	0.48	1.56	1.41	0.62	2.27	12.93	0.82
Mean / mass fraction (%)	101.23	103.25	58.39	62.52	103.3	98.28	104.25	103
Lower limit 95%-confidence interval* mean / mass fraction (%)	101.17	103.15	51.95	56.44	103.16	97.77	96.25	102.82
Upper limit 95%-confidence interval* mean / mass fraction (%)	101.28	103.35	64.83	68.59	103.43	98.79	112.25	103.19
Mean - mass fraction (%)	1.2	3.09	-2.08	-1.87	3.13	-1.65	1.42	2.85
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	1.23	3.25	-41.61	-37.48	3.3	-1.72	4.25	3
$\frac{\text{Standard deviation of reproducibility}}{\text{mass fraction}}$ (%)	0.1	0.18	11.15	10.06	0.23	0.84	13.86	0.31
$\frac{\text{Standard deviation of reproducibility}}{\text{mean}}$ (%)	0.1	0.17	19.1	16.09	0.23	0.86	13.29	0.3
Uncertainty** (%)	1.21	3.09	2.15	1.94	3.14	1.84	4.83	2.87
Relative uncertainty*** (%)	1.23	3.25	43.08	38.81	3.31	1.91	14.49	3.02
Logit**** mass fraction	3.89	2.94	-2.94	-2.94	2.94	3.18	-0.69	2.94
Mean of Logits	4.83	3.94	-3.52	-3.44	3.97	2.82	-0.64	3.83
Standard deviation of reproducibility of Logits	0.13	0.09	0.21	0.16	0.12	0.15	0.21	0.14

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S22 Statistical parameters: spC.wN, Cattle

Sample ID	P01	P02	P04	P06	P07	P08	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	Feta	Feta	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	13	14	14	14	14	14	13	13
Number of outlier laboratories	0	0	1	0	0	0	0	0	1	1
Milk mass fraction (%)	2	5	10	10	2	1	5	2	33.33	5
Mean (%)	0.74	1.8	1.71	1.68	0.34	0.27	1.76	0.8	12.31	2.04
Standard deviation of reproducibility (%)	0.11	0.2	0.21	0.27	0.06	0.05	0.24	0.1	1.17	0.27
Reproducibility limit (%)	0.31	0.57	0.58	0.76	0.18	0.13	0.66	0.27	3.28	0.76
Mean / mass fraction (%)	37.01	36.09	17.1	16.83	16.76	26.53	35.13	39.92	36.93	40.73
Lower limit 95%-confidence interval* mean / mass fraction (%)	33.85	33.76	15.84	15.27	14.89	23.86	32.4	37.17	34.81	37.44
Upper limit 95%-confidence interval* mean / mass fraction (%)	40.17	38.42	18.36	18.39	18.63	29.2	37.86	42.67	39.05	44.02
Mean - mass fraction (%)	-1.26	-3.2	-8.29	-8.32	-1.66	-0.73	-3.24	-1.2	-21.02	-2.96
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	-62.99	-63.91	-82.9	-83.17	-83.24	-73.47	-64.87	-60.08	-63.07	-59.27
Standard deviation of reproducibility mass fraction (%)	5.47	4.04	2.09	2.71	3.24	4.62	4.73	4.76	3.51	5.44
Standard deviation of reproducibility mean (%)	14.78	11.19	12.21	16.09	19.31	17.43	13.48	11.92	9.5	13.36
Uncertainty** (%)	1.26	3.2	8.29	8.32	1.67	0.74	3.25	1.21	21.06	2.98
Relative uncertainty*** (%)	63.22	64.04	82.92	83.22	83.3	73.62	65.04	60.27	63.17	59.52
Logit**** mass fraction	-3.89	-2.94	-2.2	-2.2	-3.89	-4.6	-2.94	-3.89	-0.69	-2.94
Mean of Logits	-4.91	-4	-4.06	-4.08	-5.71	-5.94	-4.03	-4.83	-1.97	-3.88
Standard deviation of reproducibility of Logits	0.15	0.11	0.12	0.16	0.19	0.17	0.14	0.13	0.11	0.14

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S23 Statistical parameters: spC.wN, Sheep

Sample ID	P04	P06	P07	P08	P10	P11
Sample type	Feta	Feta whey	Feta	Feta	Picodon	yoghurt
Number of participating laboratories	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14
Number of laboratories after outlier elimination	13	14	14	14	14	13
Number of outlier laboratories	1	0	0	0	0	1
Milk mass fraction (%)	85	85	98	99	2	33.33
Mean (%)	95.57	95.4	99.66	99.73	4.5	53.27
Standard deviation of reproducibility (%)	0.46	0.86	0.06	0.05	0.77	3.2
Reproducibility limit (%)	1.29	2.41	0.18	0.13	2.15	8.97
Mean / mass fraction (%)	112.43	112.23	101.7	100.74	225.11	159.81
Lower limit 95%-confidence interval* mean / mass fraction (%)	112.1	111.65	101.66	100.72	202.94	154
Upper limit 95%-confidence interval* mean / mass fraction (%)	112.76	112.82	101.74	100.77	247.28	165.62
Mean - mass fraction (%)	10.57	10.4	1.66	0.73	2.5	19.94
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	12.43	12.23	1.7	0.74	125.11	59.81
Standard deviation of reproducibility mass fraction (%)	0.54	1.01	0.07	0.05	38.4	9.61
Standard deviation of reproducibility mean (%)	0.48	0.9	0.06	0.05	17.06	6.01
Uncertainty** (%)	10.58	10.43	1.67	0.74	2.62	20.19
Relative uncertainty*** (%)	12.44	12.27	1.7	0.74	130.87	60.58
Logit**** mass fraction	1.73	1.73	3.89	4.6	-3.89	-0.69
Mean of Logits	3.08	3.05	5.71	5.94	-3.07	0.13
Standard deviation of reproducibility of Logits	0.11	0.19	0.19	0.17	0.18	0.13

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

Table S24 Statistical parameters: spC.wN, Goat

Sample ID	P01	P02	P04	P06	P09	P10	P11	P12
Sample type	Picodon	yoghurt	Feta	Feta whey	drinking milk	Picodon	yoghurt	Picodon
Number of participating laboratories	15	15	15	15	15	15	15	15
Number of laboratories with evaluable results	14	14	14	14	14	14	14	14
Number of laboratories after outlier elimination	14	14	13	14	14	14	13	13
Number of outlier laboratories	0	0	1	0	0	0	1	1
Milk mass fraction (%)	98	95	5	5	95	96	33.33	95
Mean (%)	99.26	98.2	2.72	2.92	98.24	94.7	34.42	97.96
Standard deviation of reproducibility (%)	0.11	0.2	0.33	0.66	0.24	0.84	3.73	0.27
Reproducibility limit (%)	0.31	0.57	0.93	1.84	0.66	2.36	10.44	0.76
Mean / mass fraction (%)	101.29	103.36	54.47	58.38	103.41	98.65	103.26	103.12
Lower limit 95%-confidence interval* mean / mass fraction (%)	101.22	103.24	50.47	50.81	103.27	98.14	96.5	102.95
Upper limit 95%-confidence interval* mean / mass fraction (%)	101.35	103.49	58.47	65.96	103.56	99.15	110.02	103.29
Mean - mass fraction (%)	1.26	3.2	-2.28	-2.08	3.24	-1.3	1.09	2.96
$\frac{\text{mean} - \text{mass fraction}}{\text{mass fraction}}$ (%)	1.29	3.36	-45.53	-41.62	3.41	-1.35	3.26	3.12
$\frac{\text{Standard deviation of reproducibility}}{\text{mass fraction}}$ (%)	0.11	0.21	6.61	13.12	0.25	0.88	11.19	0.29
$\frac{\text{Standard deviation of reproducibility}}{\text{mean}}$ (%)	0.11	0.21	12.14	22.47	0.24	0.89	10.84	0.28
Uncertainty** (%)	1.26	3.2	2.3	2.18	3.25	1.55	3.88	2.98
Relative uncertainty*** (%)	1.29	3.37	46.01	43.64	3.42	1.61	11.65	3.13
Logit**** mass fraction	3.89	2.94	-2.94	-2.94	2.94	3.18	-0.69	2.94
Mean of Logits	4.91	4	-3.58	-3.53	4.03	2.89	-0.65	3.88
Standard deviation of reproducibility of Logits	0.15	0.11	0.12	0.23	0.14	0.17	0.17	0.14

* confidence interval under the assumption of normal distribution

** uncertainty = $\sqrt{(\text{mean} - \text{mass fraction})^2 + \text{Standard deviation of reproducibility}^2}$

*** rel. uncertainty = uncertainty / mass fraction

**** logit transformation: $\text{logit}(\text{proportion}) = \ln(\text{proportion}) - \ln(1 - \text{proportion})$

6 Comparison of the calculation methods

For comparing the different calculation methods, the Aitchison distances (Aitchison, 1982) between the calculated DNA proportions and the milk mass fraction of the complete sample are shown for all ring trial samples containing material from at least two species and for animal-sample combinations with positive milk mass fraction (Fig. S6). This distance measure is suitable for comparing compositional data in which the relative proportions are interdependent and are totalled to one. On the one hand, the figure shows that the Aitchison distances are generally lower in samples containing only material from cattle and goat (DM, Y4, P3, P4). On the other hand, it depicts differences between the proportions normalised with the mammalian assay and those calculated without normalisation. Again, these differences only occur in the samples with exclusively cow and goat milk, whereby the normalised proportions show somewhat larger distances. The reasons for both observations are not clear. However, one plausible contributing factor could be the higher somatic cell counts in goat and sheep milk—approximately tenfold and eightfold higher, respectively, than in cow milk (Table 8). This disparity may have led to disproportionate representation of species-specific mitochondrial DNA when standardising DNA concentrations across all three species for the PCR reactions.

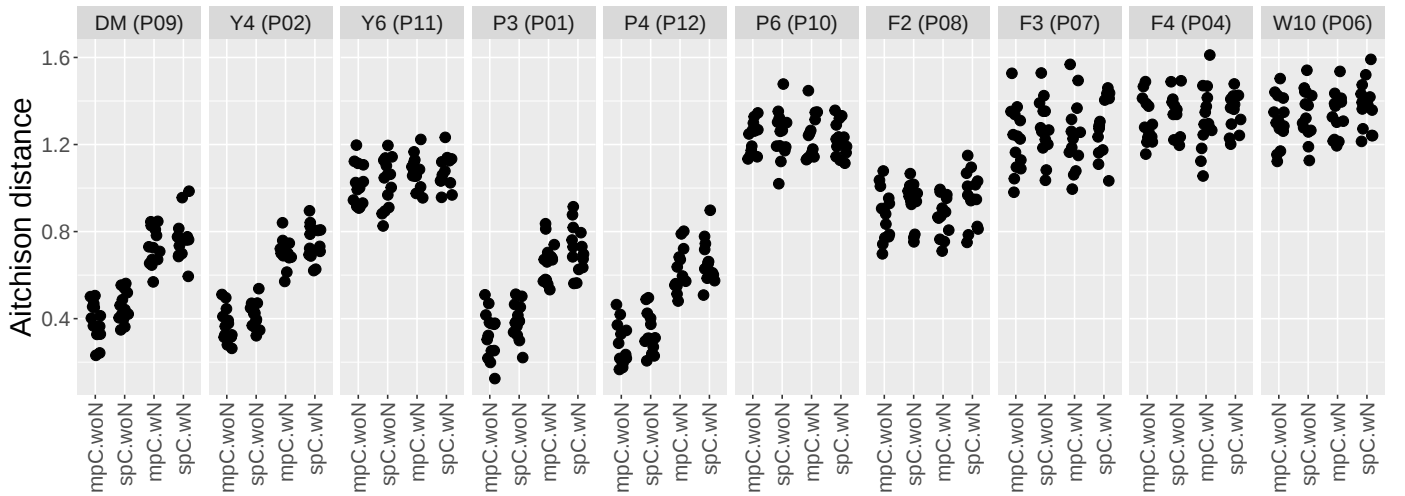


Fig S6 Aitchison distances between the calculated DNA proportions and the milk mass fractions of the complete samples in all ring trial samples containing milk from at least two species and animal-sample combinations with positive milk mass fraction. The DNA proportions were measured by the ring trial participants and were calculated using four different calculation methods: multi-point calibration without normalisation (mpC.woN), single-point calibration without normalisation (spC.woN), multi-point calibration with normalisation (mpC.wN), single-point calibration with normalisation (spC.wN)

7 Influence of the sample type

It was also tested whether the sample type impacted the recovery of the different species. This was of particular interest as the DNA extractions had been performed differently for the fluid and solid samples. In order to obtain sufficient DNA for conducting the ring trial, including performing the preliminary tests, the DNA from the fluid samples was extracted from centrifugation-enriched cells. However, the results indicate that this does not influence the determined recovery of the different species (Fig. S7). The recovery of cattle seems to be influenced more by whether sheep or goat milk was the main component and was better in the samples with high amounts of goat milk. This was also reflected by smaller Aitchison distances between the calculated DNA proportions and the milk mass fractions of the complete samples (Fig. S7). The samples with equal amounts of cow, sheep and goat milk had average recoveries. The reasons for this are unclear and do not appear to lie in the measured somatic cell counts of the raw milks or in the efficiencies of the sheep- and cattle- specific real-time PCR assays.

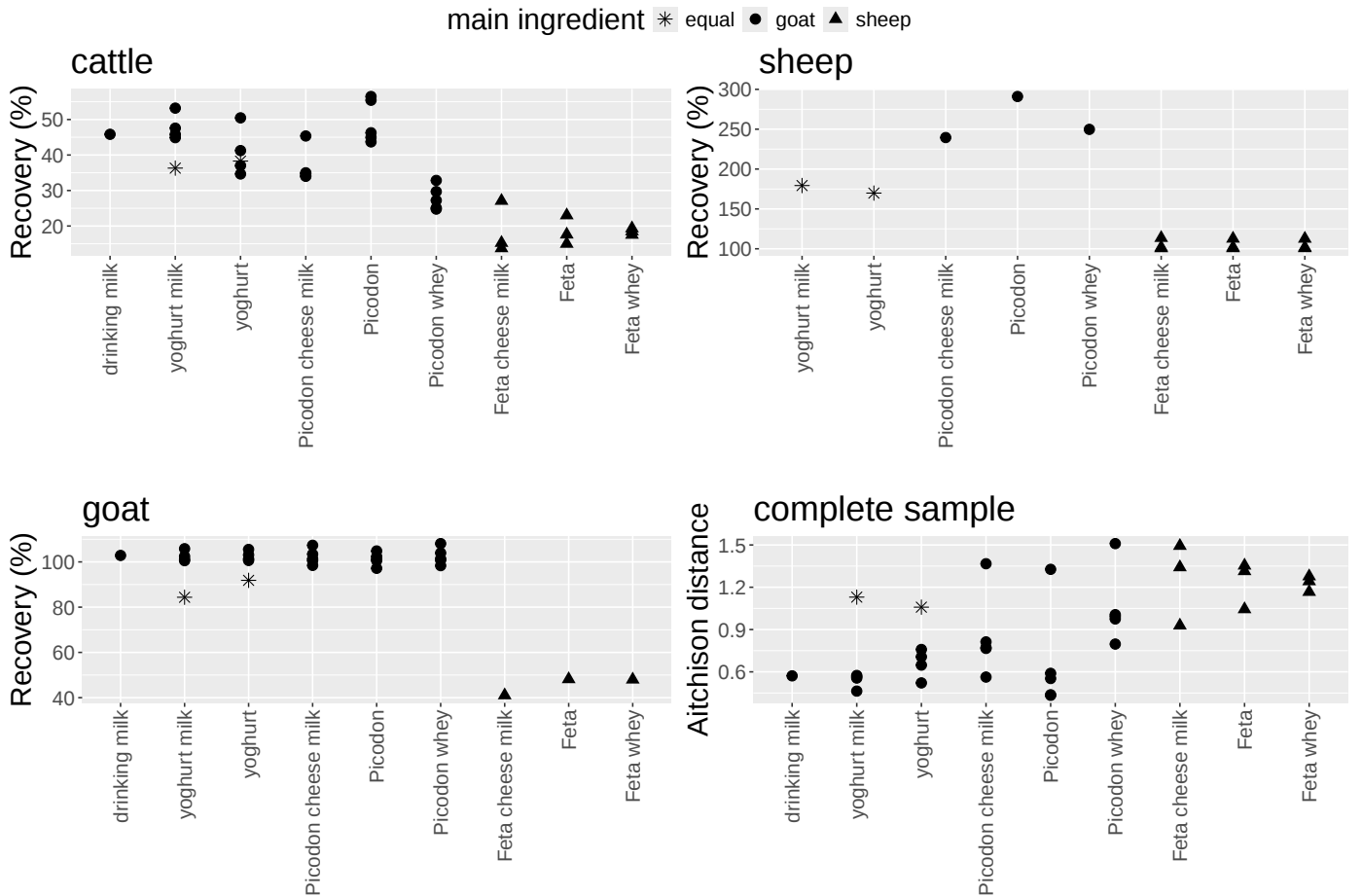


Fig S7 Recovery in % of cattle, sheep and goat and Aitchison distances of the complete samples between the measured proportions and the milk mass fractions for samples containing milk from at least two species and for animal-sample combination with positive milk mass fraction of the single-laboratory analysis. The relative DNA proportion for cattle, sheep and goat was calculated based on the DNA standard dilution series and without normalisation (mpC.woN). The stars represent samples made from equal proportions of cow, sheep and goat milk, the dots represent samples with goat milk as main ingredient and the triangles show samples with sheep milk as main component.