

# **A global assessment of the performance of quantitative PCR assays targeting human- and ruminant-associated *Bacteroidetes* for microbial source tracking**

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**Table S1. Selected qPCR assays analyzed in this study.**

Assay	Target	Primers and probes	Temp.	Ref.
AllBac	<i>Bacteroides</i>	AllBac296f (GAGAGGAAGGTCCCCCAC) AllBac412r (CGCTACTTGGCTGGTTCAG) AllBac375Bhqr (FAM-CCATTGACCAATATTCCTCACTGCTGCCT-NFQ)	60	(9)
BacH	human	BacH_f (CTTGGCCAGCCTTCTGAAAG) BacH_r (CCCCATCGTCTACCGAAAATAC) BacH_pC (FAM-TCATGATCCCATCCTG-NFQ-MGB) BacH_pT (FAM-TCATGATGCCATCTTG-NFQ-MGB)	61	(15)
BacHum	human	BacHum-160f (TGAGTTCACATGTCCGCATGA) BacHum-241r (CGTTACCCCGCCTACTATCTAATG) BacHum-193p (FAM-TCCGGTAGACGATGGGGATGCGTT-NFQ)	60	(10)
BacCow	ruminant	BacCow-CF128f (CCAACYTTCCCGWTACTC) BacCow-305 (GGACCGTGTCTCAGTTCAGTG) BacCow-257p (FAM-TAGGGGTTCTGAGAGGAAGGTCCCC-NFQ)	60	(10)
BacR	ruminant	BacR_f (GCGTATCCAACCTTCCCG) BacR_r (CATCCCCATCCGTTACCG) BacR_p (FAM-CTTCCGAAAGGGAGATT-NFQ-MGB)	60	(16)
BoBac	bovine <sup>a</sup>	BoBac367f (GAAG(G/A)CTGAACCAGCCAAGTA) BoBac467r (GCTTATTCATACGGTACATAACAAG) BoBac402Bhqr (FAM-TGAAGGATGAAGGTTCTATGGATTGTAACTT-NFQ)	57 53	(9)

<sup>a</sup> Although this assay was published as cattle-associated the results of this study lead to the decision to handle it as a ruminant-associated assay.  
Ref., reference; Temp., optimised annealing temperature; FAM, 6-carboxyfluorescein; NFQ, nonfluorescent quencher; MGB, minor groove binder

**Table S2. Sample provenance and host species.**

species		sum of individuals	Argentina	Australia	Austria	Germany	Hungary	Nepal	Nether-lands	Romania	South Korea	Spain	Sweden	Tanzania	Uganda	UK	USA	
<b>human</b>	<b>human</b>	61	5	6	5	5	4	2	3	3	5	3	5	2	3	5	5	
<b>ruminants</b>	<b>ruminants</b>																	
	cattle	47	3	5	1	3	2	4	-	3	5	5	4	-	1	5	6	
	sheep	16	2	-	1	1	2	-	-	2	-	-	2	1	-	5	-	
	deer	8	-	-	1	-	-	-	5	-	-	-	-	-	-	-	2	
	chamois	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
	goat	6	-	-	1	1	2	1	-	-	-	-	-	-	1	-	-	
	llama	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
	<b>group sum</b>	<b>79</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>6</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>6</b>	<b>1</b>	<b>2</b>	<b>10</b>	<b>9</b>	
	<b>non-ruminant herbivores</b>																	
	horse		3	-	3	2	-	-	-	-	3	-	-	2	-	-	-	4
donkey	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	
zebra	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
kangaroo	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hare/rabbit	3	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	1	
groundhog	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>group sum</b>	<b>28</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>2</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>3</b>	<b>-</b>	<b>1</b>	<b>2</b>	<b>-</b>	<b>3</b>	<b>-</b>	<b>5</b>		
<b>omnivores</b>																		
pig	21	-	-	2	2	-	2	5	2	5	-	-	-	2	-	1	-	
wild boar	8	-	-	1	-	-	-	5	-	-	-	-	-	2	-	-	-	
<b>group sum</b>	<b>29</b>	<b>-</b>	<b>-</b>	<b>3</b>	<b>2</b>	<b>-</b>	<b>2</b>	<b>10</b>	<b>2</b>	<b>5</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>4</b>	<b>-</b>	<b>1</b>	<b>-</b>	
<b>carnivores</b>																		
dog	29	3	3	2	2	-	2	-	2	-	5	2	-	1	3	4		
cat	7	-	-	-	1	-	1	-	-	-	-	2	-	-	-	3		
coyote	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1		
opossum	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1		
otter	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1		
<b>group sum</b>	<b>39</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>-</b>	<b>3</b>	<b>-</b>	<b>2</b>	<b>-</b>	<b>5</b>	<b>4</b>	<b>-</b>	<b>1</b>	<b>3</b>	<b>10</b>		
<b>birds</b>																		
chicken	19	1	-	-	1	-	2	-	2	5	-	2	1	1	3	1		
duck	5	-	2	-	-	-	-	-	1	-	-	2	-	-	-	-		
pigeon	3	2	-	-	-	-	-	-	-	-	-	-	-	-	1	-		
goose	3	-	-	-	1	-	-	-	-	-	-	1	-	-	-	1		
turkey	2	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-		
starling	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-		
gull	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1		
parrot	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		
coot	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-		
grebe	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1		
owl	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		
pelican	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
bird	4	-	-	-	-	-	3	-	-	-	-	-	1	-	-	-		
<b>group sum</b>	<b>44</b>	<b>3</b>	<b>2</b>	<b>-</b>	<b>3</b>	<b>-</b>	<b>2</b>	<b>-</b>	<b>3</b>	<b>5</b>	<b>-</b>	<b>5</b>	<b>2</b>	<b>4</b>	<b>6</b>	<b>5</b>		
<b>sums of sums</b>		<b>280</b>	<b>19</b>	<b>20</b>	<b>20</b>	<b>20</b>	<b>10</b>	<b>14</b>	<b>18</b>	<b>18</b>	<b>20</b>	<b>14</b>	<b>22</b>	<b>9</b>	<b>13</b>	<b>25</b>	<b>34</b>	

Figure S1

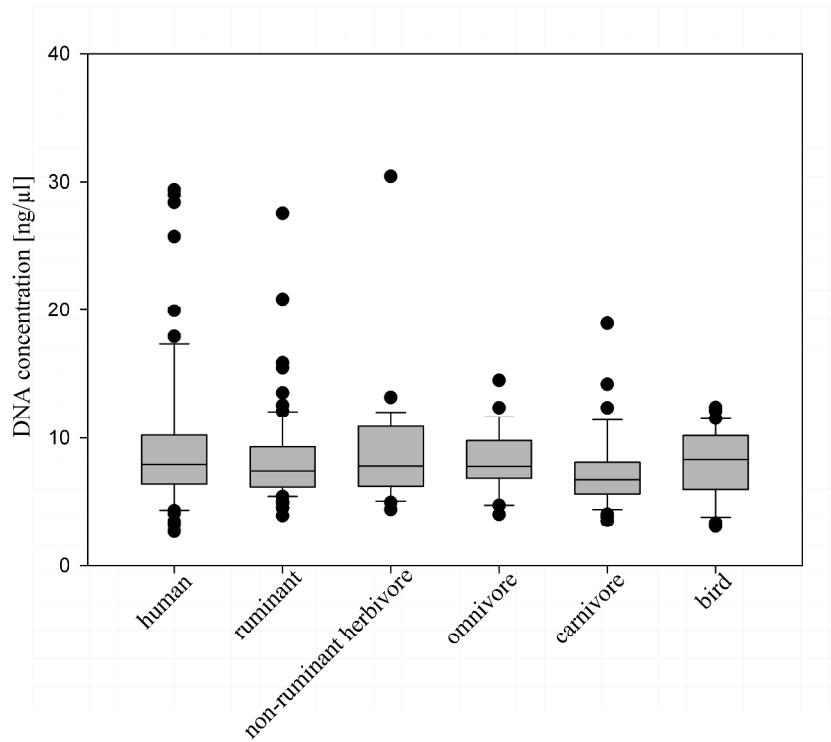


Figure S1 – Distributions of the spectro-photometrically determined DNA concentrations in fecal DNA extracts from the source groups humans (n=61), ruminating mammals (n=79), non-ruminating herbivorous mammals (n=28), omnivorous mammals (n=28), carnivorous mammals (n=29) and birds (n=39). Boxes, 25th and 75th percentile; lines within the boxes, median; whiskers, 10th and 90th percentile, respectively.

Figure S2

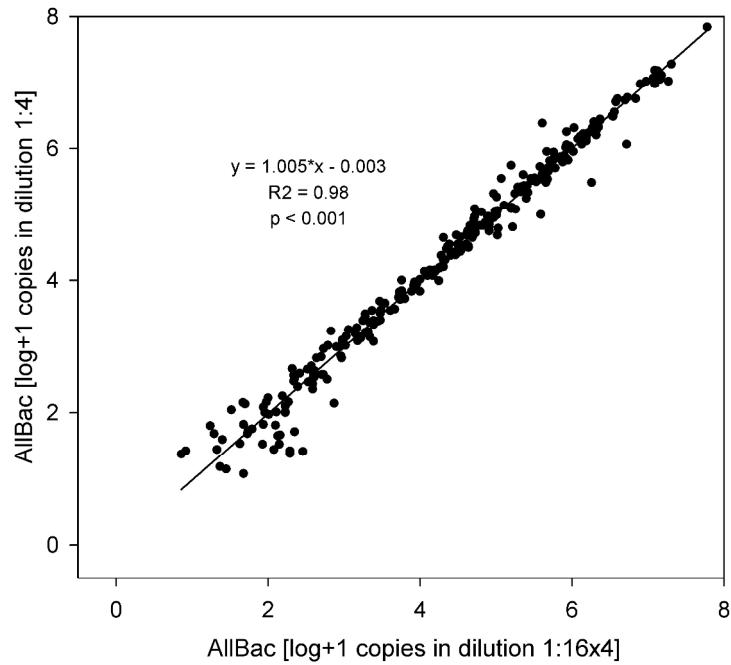


Figure S2 – Scatterplot showing the AllBac marker concentrations measured in the 1:4 dilution, and the 1:16 dilution multiplied by 4, respectively, of the 280 samples included in further analysis. Solid line shows linear regression, equation and coefficient of determination for the regression given in the plot.

Figure S3

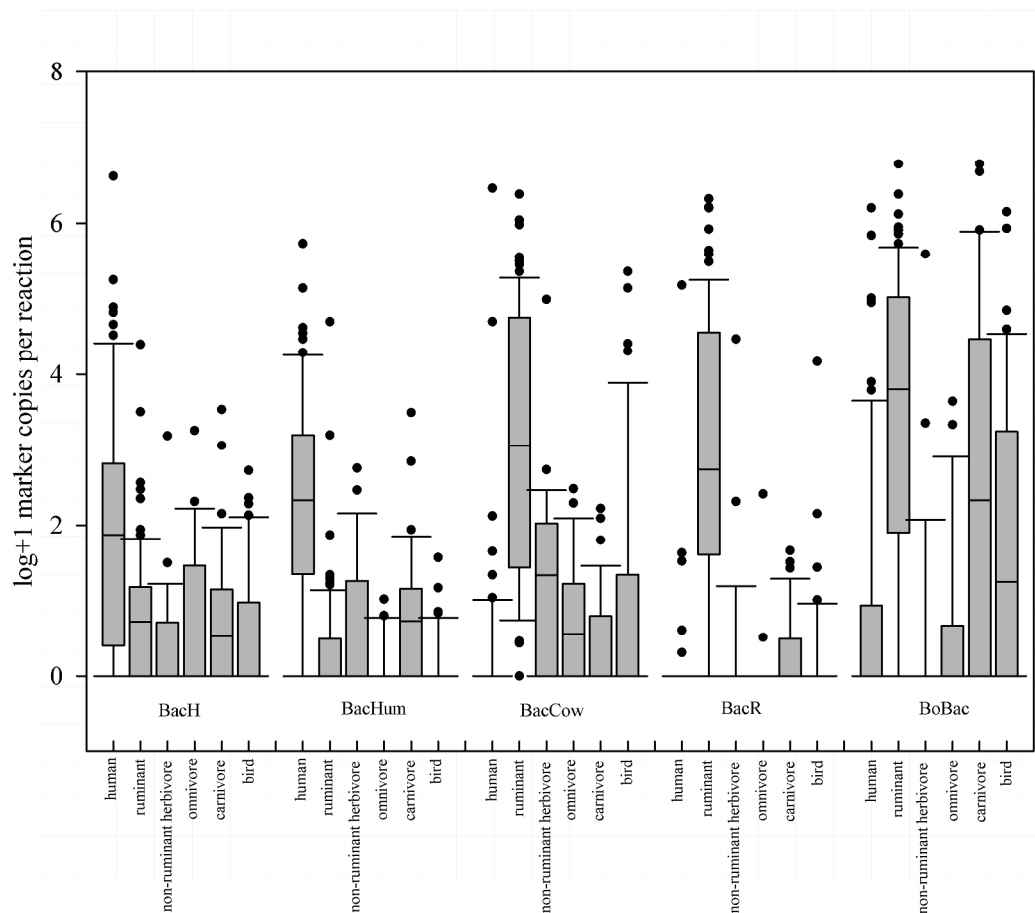


Figure S3 – Marker concentrations of tested assays in fecal DNA extracts from the source groups humans (n=61), ruminating mammals (n=79), non-ruminating herbivorous mammals (n=28), omnivorous mammals (n=28), carnivorous mammals (n=29) and birds (n=39). Results were measured in the 1:4 dilution of the DNA samples and transformed into logarithmic format after addition of 1 to each value. Boxes, 25th and 75th percentile; lines within the boxes, median; whiskers, 10th and 90th percentile, respectively; n, number of samples in each category.

Figure S4 – Quantitative relation of marker concentrations of BacH vs. BacHum (S4a), BacCow vs. BacR (S4b), BacCow vs. BoBac (S4c), BacR vs. BoBac (S4d) in target samples (black dots) and non-target samples (empty dots). Solid lines are the 1<sup>st</sup> median.

Figure S4a

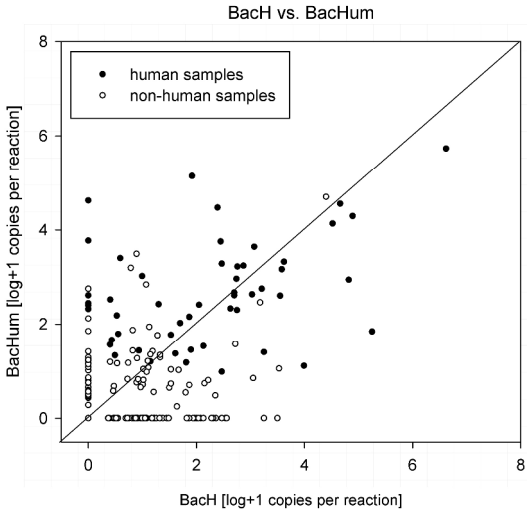


Figure S4b-d

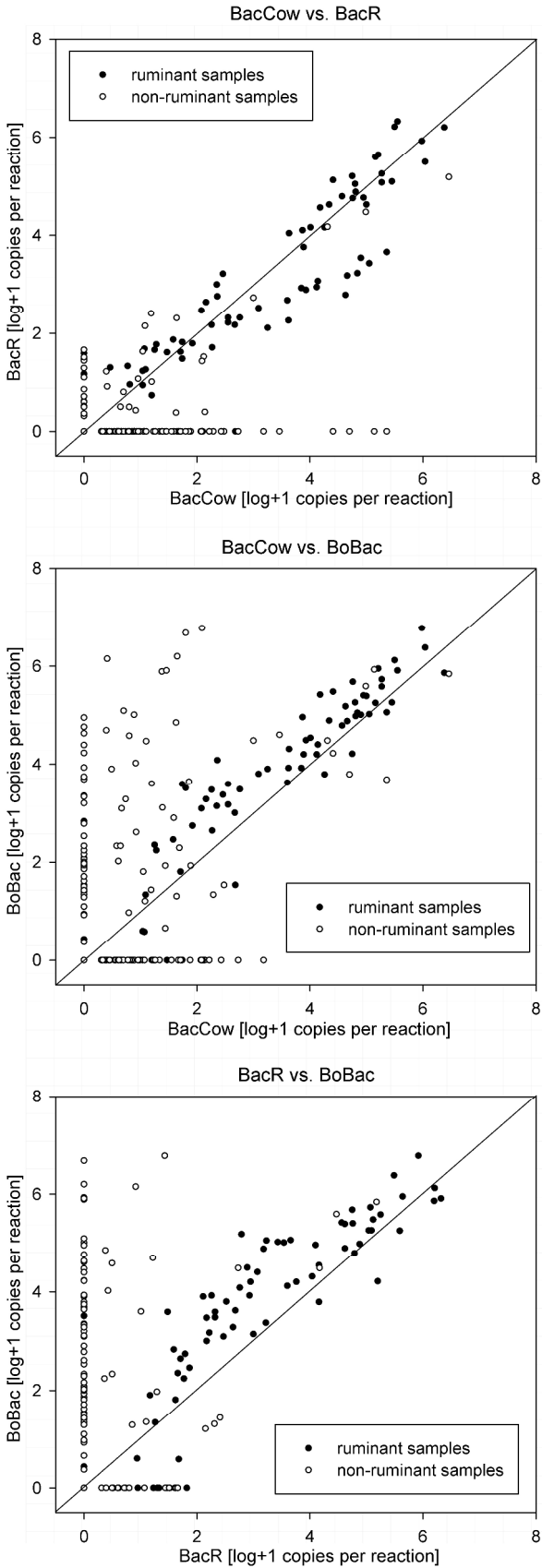




Figure S5 – Marker concentrations of tested assays in target and non-target fecal DNA extracts. Results are given as marker copies per ng of extracted DNA and transformed into logarithmic format after addition of 1 to each value. Boxes, 25<sup>th</sup> and 75<sup>th</sup> percentile; lines within the boxes, median; whiskers, 10<sup>th</sup> and 90<sup>th</sup> percentile, respectively; n, number of samples in each category.

Figure S5

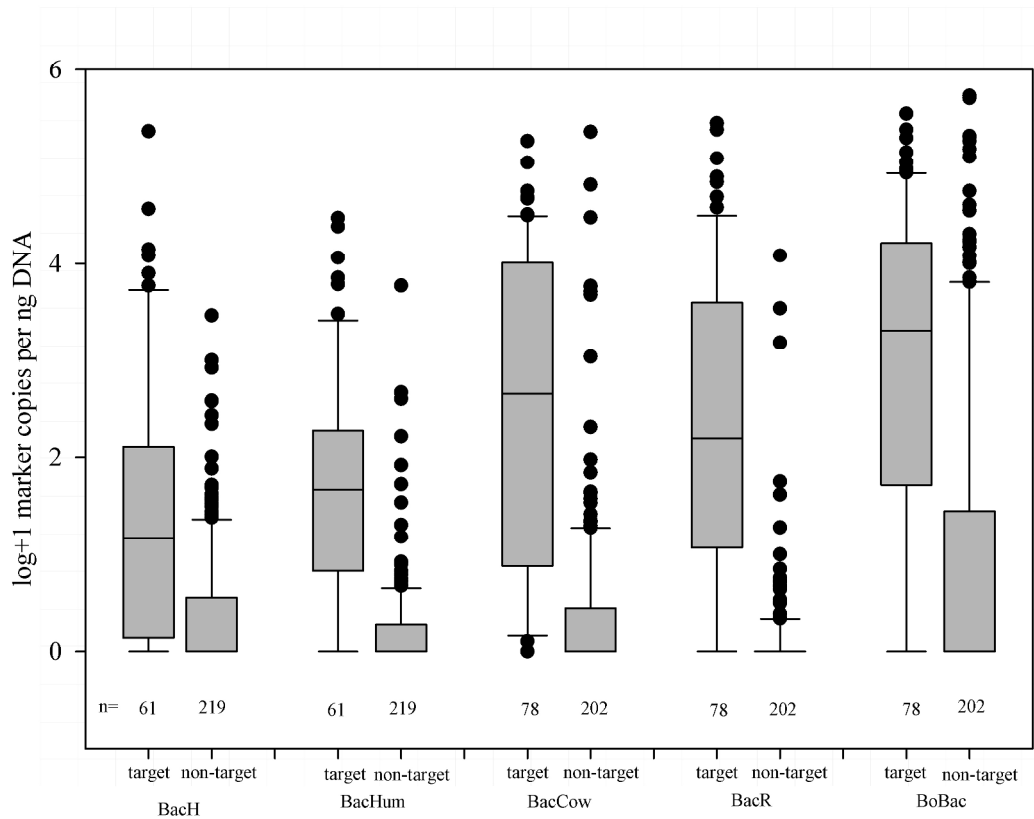


Figure S6a-e – Quantitative relation of AllBac concentration vs. BacH (S6a), BacHum (S6b), BacCow (S6c), BacR (S6d), and BoBac (S6e) in target samples. All data given as marker copies per ng of extracted DNA. The figures only include samples yielding positive results with both assays compared in a plot; dashed lines denote the 1<sup>st</sup> median; solid lines are linear regression curves, equations and coefficients of determination for regressions are given in the figures.

Figure S6a-e

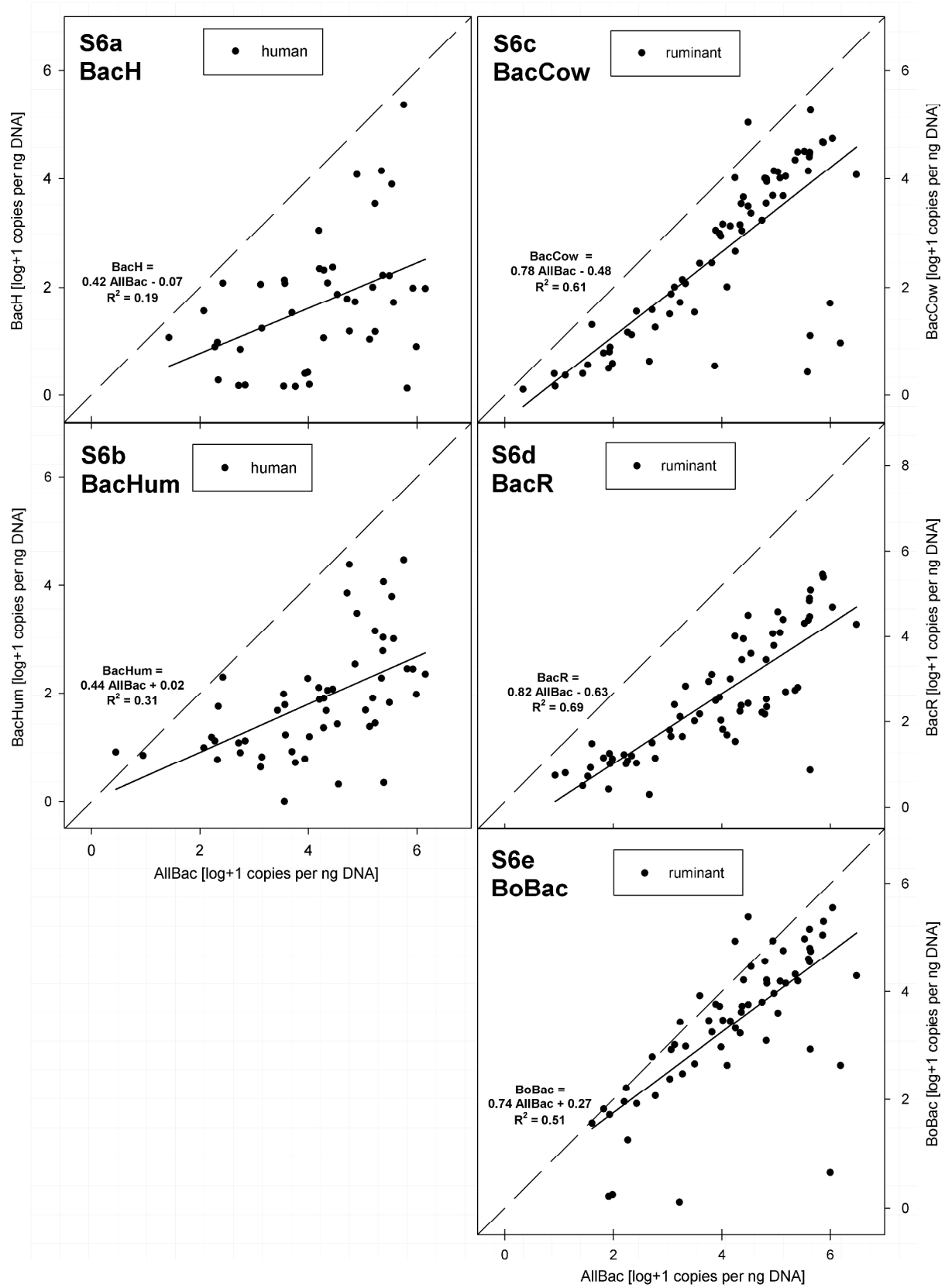


Figure S7a-d – Quantitative relation of marker concentrations of BacH vs. BacHum (S7a), BacCow vs. BacR (S7b), BacCow vs. BoBac (S7c), BacR vs. BoBac (S7d) in target samples (black dots) and non-target samples (empty dots). Solid lines are the 1<sup>st</sup> median. All measurements are given as marker copies per ng of extracted DNA.

Figure S7a

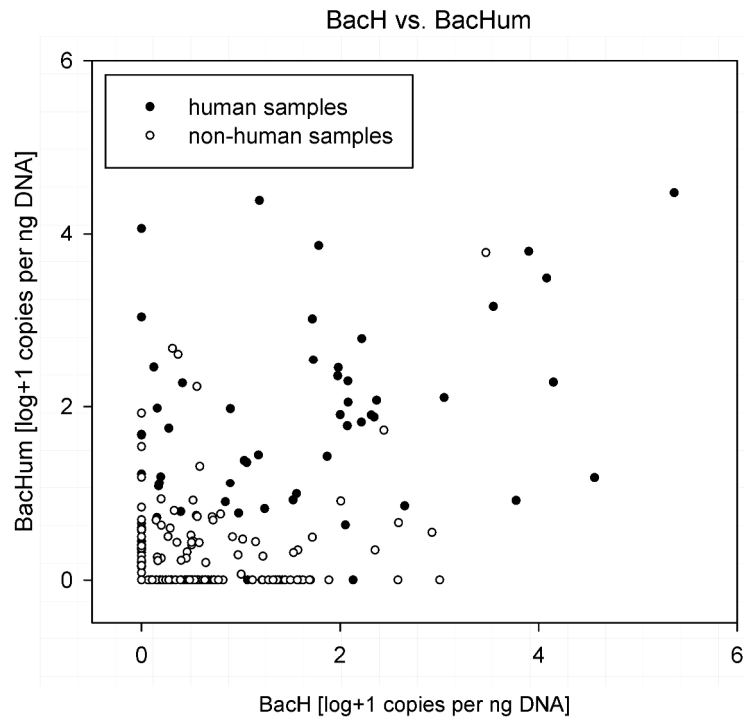


Figure S7b-d

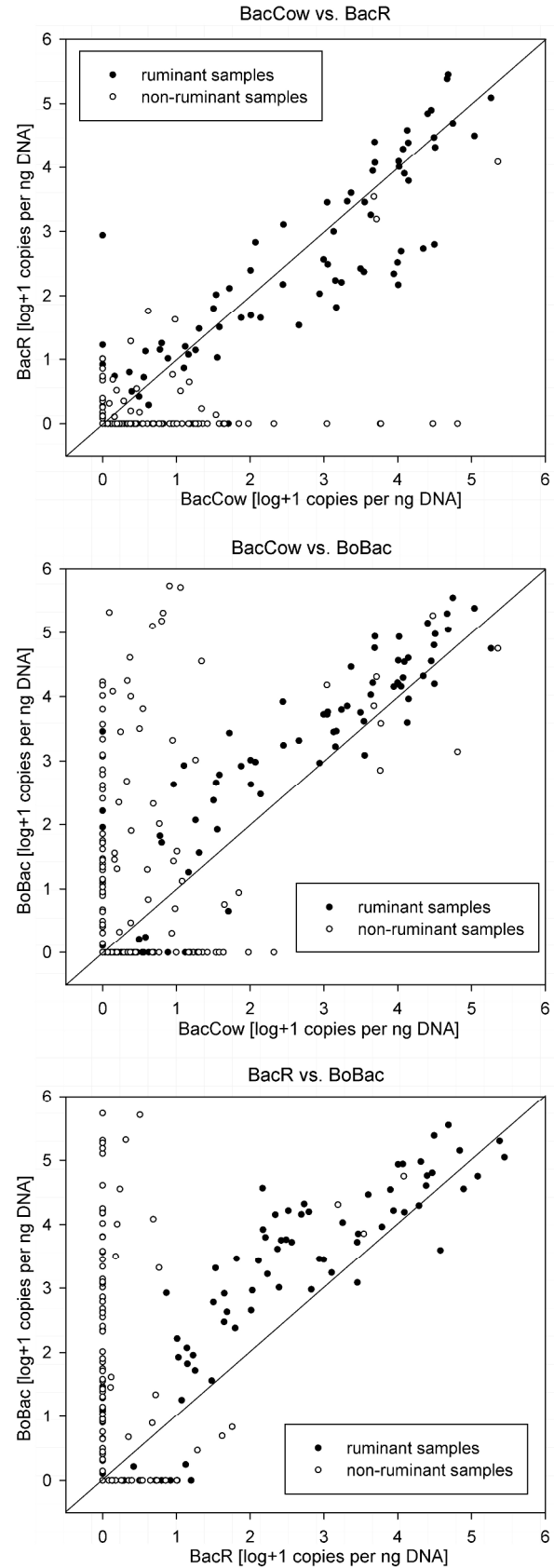


Figure S8

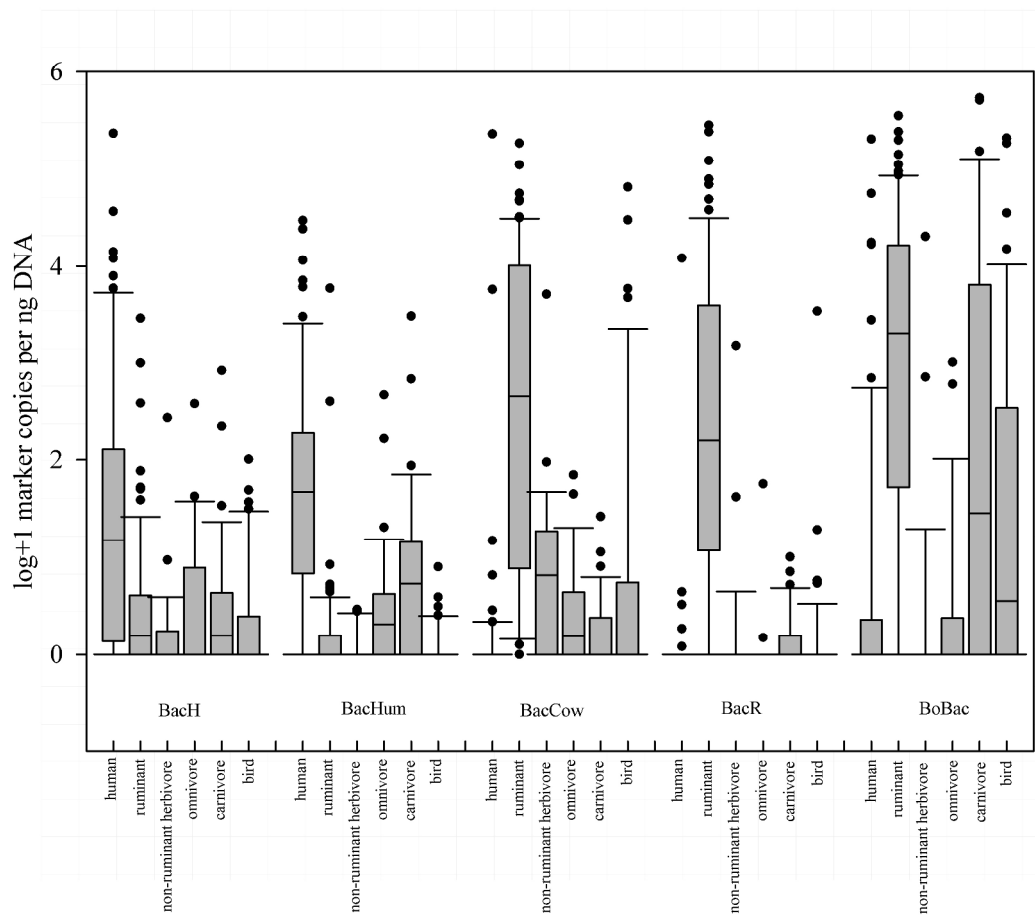


Figure S8 – Marker concentrations of tested assays in fecal DNA extracts from the source groups humans (n=61), ruminating mammals (n=79), non-ruminating herbivorous mammals (n=28), omnivorous mammals (n=28), carnivorous mammals (n=29) and birds (n=39). Results are given as marker copies per ng of extracted DNA and transformed into logarithmic format after addition of 1 to each value. Boxes, 25<sup>th</sup> and 75<sup>th</sup> percentile; lines within the boxes, median; whiskers, 10<sup>th</sup> and 90<sup>th</sup> percentile, respectively; n, number of samples in each category.

