Rossi G, et al. Preanalytical variables affecting the measurement of serum paraoxonase-1 activity in horses

Supplementary Table 1. Intra- and inter-assay precision of paraoxonase-1 (PON-1) activity measured using paraoxon-ethyl and p-nitrophenyl acetate as substrate.

	Paraoxon-ethyl			p-nitrophenyl acetate		
Pool	High	Medium	Low	High	Medium	Low
Intra-assay						
Mean (U/L)	131.8	91.2	66.7	1,908	1,429	1,019
SD (U/L)	1.4	0.4	0.5	13	11	100
CV (%)	1.1	0.4	0.7	0.7	0.7	9.8
Inter-assay						
Mean (U/L)	ND	108.9	64.1	ND	1,496	1,215
SD (U/L)		4.1	5.6		89	93
CV (%)		3.7	8.7		5.9	7.7

CV = coefficient of variation; ND = not determined; SD = standard deviation.

Supplementary Table 2. PON-1 activity measured in an equine serum pool sequentially diluted to assess the accuracy by the linearity under dilution (LUD) test.

	Paraoxon-ethyl			p-nitrophenyl acetate		
LUD	Observed PON-1 activity (U/L)	Expected PON-1 activity (U/L)	% Recovery	Observed PON-1 activity (U/L)	Expected PON-1 activity (U/L)	% Recovery
100%	66.8	66.8	0.0	1,174	1,174	0.0
90%	60.9	60.0	–1.4	1,036	1,056	1.9
80%	51.0	53.4	4.4	862	939	8.2
70%	44.3	46.7	5.2	778	822	5.3
60%	41.5	40.0	–3.8	690	704	2.1
50%	34.0	33.4	–1.8	480	587	18.1
40%	28.9	26.7	–8.3	419	469	10.7
30%	20.9	20.0	–4.5	341	352	3.1
20%	16.0	13.3	–19.6	263	235	12.1
10%	10.3	6.7	–54.3	94	117	20.3
Mean			–8.4			8.2

% Recovery = measured concentration/expected concentration × 100%.

Supplementary Table 3. PON-1 activity measured in an equine serum pool with low PON-1 activity spiked with another pool with high PON-1 activity to assess the accuracy by the spiking recovery test (SRT).

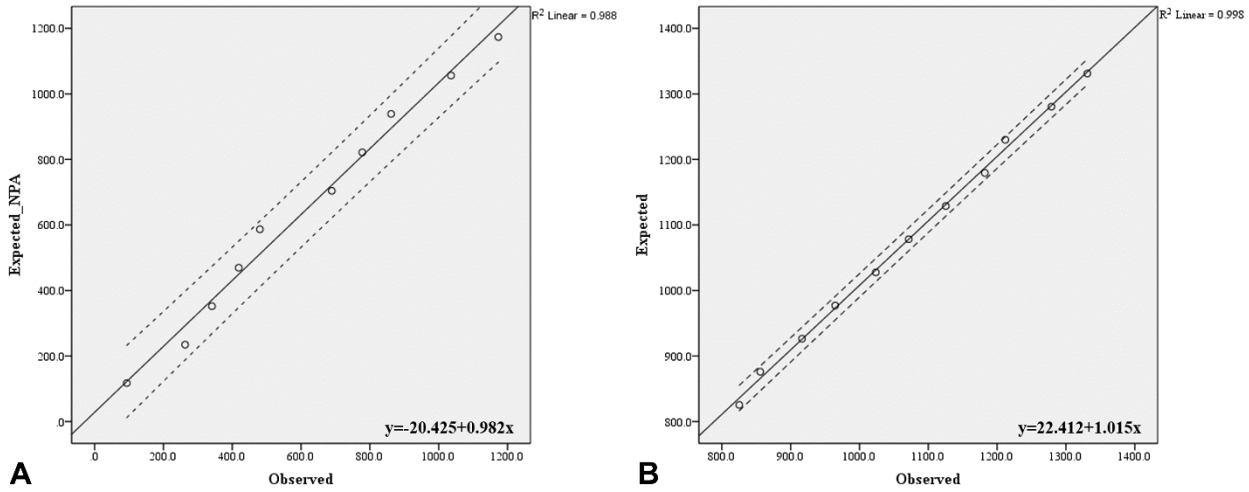
	Paraoxon-ethyl			p-nitrophenyl acetate		
SRT	Observed PON-1 activity (U/L)	Expected PON-1 activity (U/L)	% Recovery	Observed PON-1 activity (U/L)	Expected PON-1 activity (U/L)	% Recovery
0%	30.8	30.8	0.0	825	825	0.0
10%	34.5	36.0	4.2	856	86	2.3
20%	44.3	41.28	–7.2	917	927	1.1
30%	54.4	46.52	–16.8	965	977	1.3
40%	59.4	51.76	–14.8	1,024	1,028	0.4
50%	64.3	57	–12.8	1,072	1,078	0.6
60%	69.1	62.24	–11.1	1,125	1,129	0.3
70%	75.1	67.48	–11.2	1,182	1,179	–0.2
80%	79.0	72.72	–8.6	1,212	1,230	1.5
90%	83.2	83.2	0.0	1,279	1,281	0.1
Mean			–7.8			0.7

% Recovery = measured concentration/expected concentration × 100%.

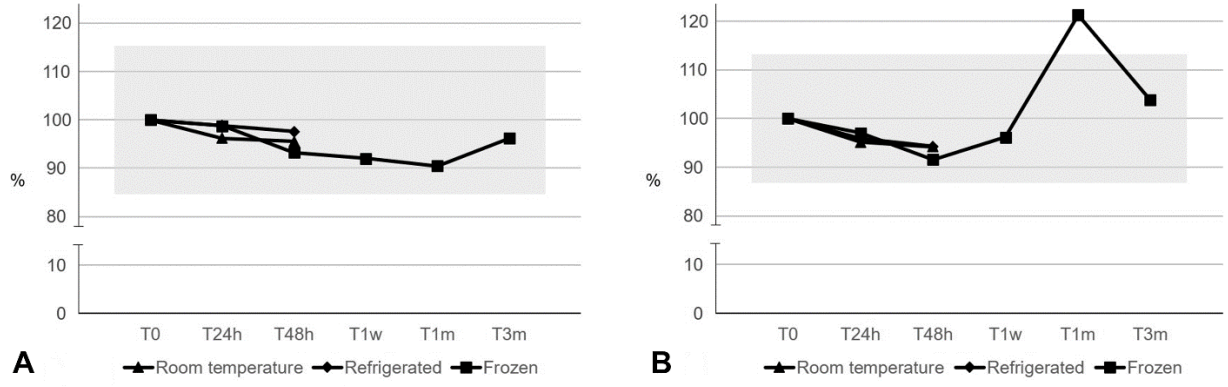
Supplementary Table 4. Results of PON-1 activity in equine serum samples collected monthly and assessed using 2 different substrates.

	Paraoxon-ethyl			4-nitrophenyl acetate		
Month	*n*	PON-1 activity (U/L median)	*p* value	*n*	PON-1 activity (U/L median)	*p* value
January	35	71.1 (49.0–125.0)	Jan vs Feb $p = 0.003$ Jan vs May $p = 0.001$ Jan vs Jun $p = 0.003$ Jan vs Aug $p = 0.010$ Jan vs Sep $p = 0.000$ Jan vs Oct $p = 0.019$	34	1,193 (676–1,538)	Jan vs Feb $p = 0.003$ Jan vs May $p = 0.001$ Jan vs Jun $p = 0.003$ Jan vs Jul $p = 0.019$ Jan vs Aug $p = 0.010$ Jan vs Sep $p = 0.000$ Jan vs Oct $p = 0.019$
February	35	70.3 (50.4–110.7)	Feb vs Apr $p = 0.006$ Feb vs Nov $p = 0.000$	34	1,314 (1,107–3,190)	Feb vs Apr $p = 0.006$ Feb vs Nov $p = 0.000$
April	35	76.9 (58.2–133.8)	Apr vs May $p = 0.000$ Apr vs Jun $p = 0.001$ Apr vs Jul $p = 0.003$ Apr vs Aug $p = 0.001$ Apr vs Sep $p = 0.011$ Apr vs Oct $p = 0.017$	34	1,223 (890–2,036)	Apr vs May $p = 0.000$ Apr vs Jun $p = 0.001$ Apr vs Jul $p = 0.003$ Apr vs Aug $p = 0.001$ Apr vs Sep $p = 0.011$ Apr vs Oct $p = 0.017$
May	35	86.4 (53.0–137.5)	May vs Jun $p = 0.003$ May vs Jul $p = 0.000$ May vs Aug $p = 0.006$ May vs Oct $p = 0.008$ May vs Nov $p = 0.000$ May vs Dec $p = 0.000$	34	1,310 (978–3,296)	May vs Jun $p = 0.003$ May vs Jul $p = 0.000$ May vs Aug $p = 0.006$ May vs Oct $p = 0.008$ May vs Nov $p = 0.000$ May vs Dec $p = 0.000$
June	35	87.0 (56.8–132.4)	Jun vs Nov $p = 0.000$ Jun vs Dec $p = 0.024$	34	1,287 (1,047–1,685)	Jun vs Sep $p = 0.045$ Jun vs Nov $p = 0.000$ Jun vs Dec $p = 0.024$
July	35	80.4 (51.4–121.0)	Jul vs Sep $p = 0.007$ Jul vs Nov $p = 0.000$	34	1,260 (1,016–1,678)	Jul vs Sep $p = 0.007$ Jul vs Nov $p = 0.000$
August	35	85.5 (60.8–133.5)	Aug vs Nov $p = 0.000$ Aug vs Dec $p = 0.001$	34	1,283 (1,057–2,327)	Aug vs Nov $p = 0.000$ Aug vs Dec $p = 0.001$
September	35	83.8 (53.3–144.5)	Sep vs Nov $p = 0.000$ Sep vs Dec $p = 0.020$	34	1,305 (212–2,126)	Sep vs Nov $p = 0.000$ Sep vs Dec $p = 0.020$
October	35	75.1 (46.6–122.9)	Oct vs Nov $p = 0.000$	34	1,224 (902–2,060)	Oct vs Nov $p = 0.000$
November	35	80.5 (48.5–129.3)	Nov vs Dec $p = 0.003$	34	1,202 (833–1,508)	Nov vs Dec $p = 0.003$

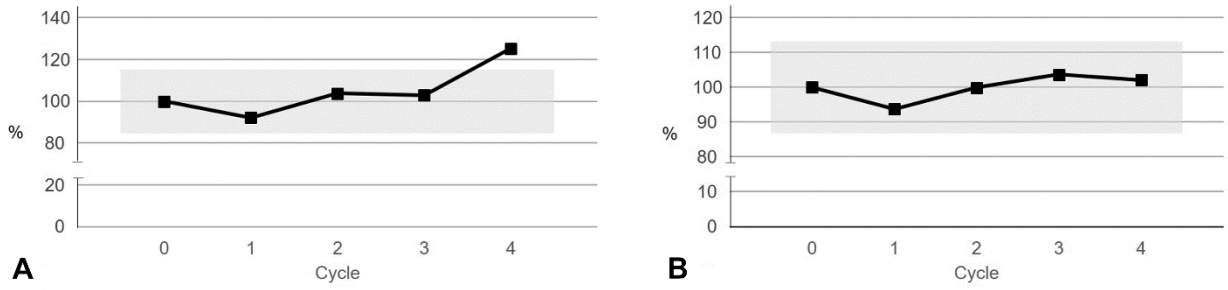
Minimum–maximum in parentheses.



Supplementary Figure 1. A. Linearity under dilution (LUD) and **B.** spiking recovery test (SRT) of PON-1 activity using p-nitrophenyl acetate as substrate. Each data point indicates the mean of a triplicate measurement. The solid line indicates the linear correlation between the expect and observed values, dashed lines indicate the 95% CI.



Supplementary Figure 2. Changes of PON-1 activity, expressed as percentages, in equine serum samples stored under different conditions. **A.** PON-1 activity assessed using paraoxon-ethyl as substrate. **B.** PON-1 activity assessed using 4-nitrophenyl acetate. The gray areas are the total observed error for each method.



Supplementary Figure 3. Changes of PON-1 activity, expressed as percentages, in equine serum samples during repeated freeze–thaw cycles. **A.** PON-1 activity assessed using paraoxon-ethyl as substrate. **B.** PON-1 activity assessed using 4-nitrophenyl acetate. The gray areas are the total observed error for each method.