

## Supplementary material

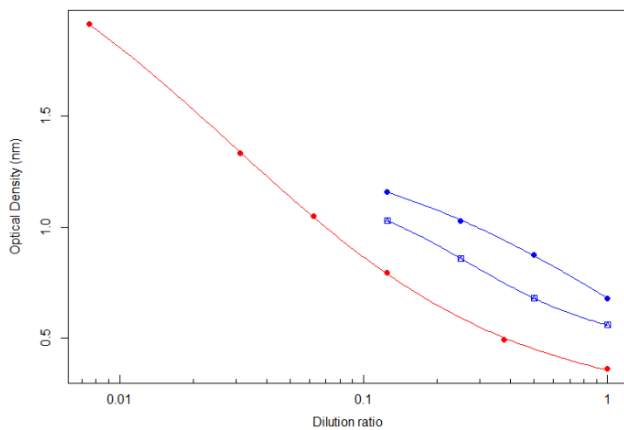
Hall et al. Determining pregnancy status in harbour seals using progesterone concentrations in blood and blubber

### Parallelism assays

Six plasma and three blubber samples were used for the assessment of the performance of the assay. Optical density (nm) was modelled against the dilution factor using a 4-parameter logistic-log model. Statistical comparisons were carried out using the 'drm' function in the package 'epicalc' in the statistical program R (R Development Team, 2018). Examples are shown in Figure S1. There was no significant difference between the plasma or blubber dilution curves and the standard curves ( $p > 0.1$ ) providing evidence that for parallelism, supporting the assumption that the binding characteristics allow reliable measurement of progesterone in seal blood and blubber using this ELISA kit.

Figure S1. a) Example plots of the progesterone ELISA parallelism validations for two diluted plasma samples. b) Plot of the progesterone ELISA parallelism validations for the diluted blubber sample extracts. The standard curve is indicated by the red line circles, while the plasma dilutions are indicated by the blue lines.

(a)



(b)

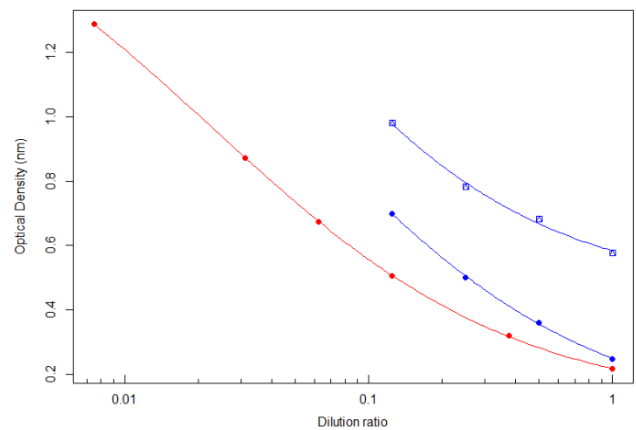


Table S1 Inter and intra assay coefficients of variation for plasma and blubber samples.

Plasma Inter assay	ID	Plate			
6.0015	584	1		Mean	7.6267
8.6274	584	2		SD	1.2635
7.2491	584	3		CV	0.1657
8.6288	584	4		CV%	16.5670
Plasma intra assay					
16.8265	D102	3		Mean	15.9604
16.3741	D102	3		SD	0.8948
16.1975	D102	3		CV	0.0561
15.9350	D102	3		CV%	5.6062
14.4690	D102	3			
Blubber inter assay					
5.8858	327	2		Mean	5.6177
6.4171	327	1		SD	0.6558
5.0147	327	3		CV	0.1167
5.1531	327	4		CV%	11.6745
Blubber intra assay					
388.0282	D038	2		Mean	376.2199
399.2588	D038	2		SD	25.8734
377.9130	D038	2		CV	0.0688
339.6795	D038	2		CV%	6.8772