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)

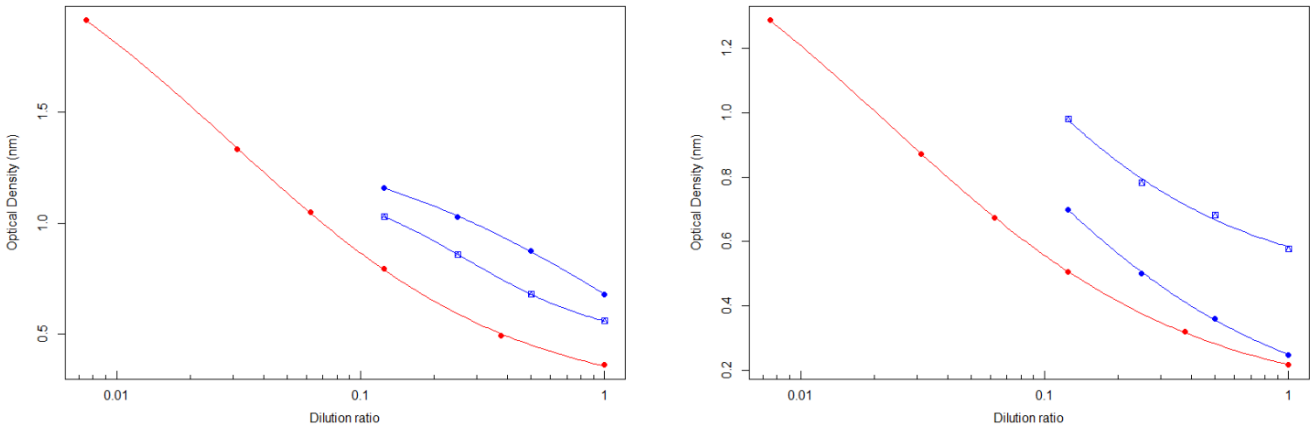

Table S1 Inter and intra assay coefficients of variation for plasma and blubber samples.

| 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 |