# Quantification of proteins in whole blood, plasma and DBS, with element-labelled antibody detection by ICP-MS

HOGELING, Shanna M., COX, Michael, BRADSHAW, Robert <http://orcid.org/0000-0003-1533-2166>, SMITH, David <http://orcid.org/0000-0001-5177-8574> and DUCKETT, Catherine J <http://orcid.org/0000-0002-6845-1890>

# Supplementary Materials

## A. Gold memory effect

### Short introduction and methods

NANOGold® is known to have affinity for the tubing of the sample introduction system [1,2], which leads to carryover of gold into other samples. To eliminate the gold memory effect, wash solutions were compared. The first wash solution consisted of 1% HCl, 1% $HNO_3$ and 2% thiourea diluted in ultra-pure water. The second wash method consisted of two solutions of which the first solution contained 5% *aqua regia* and the second solution consisted of 10 mM L-cysteine.

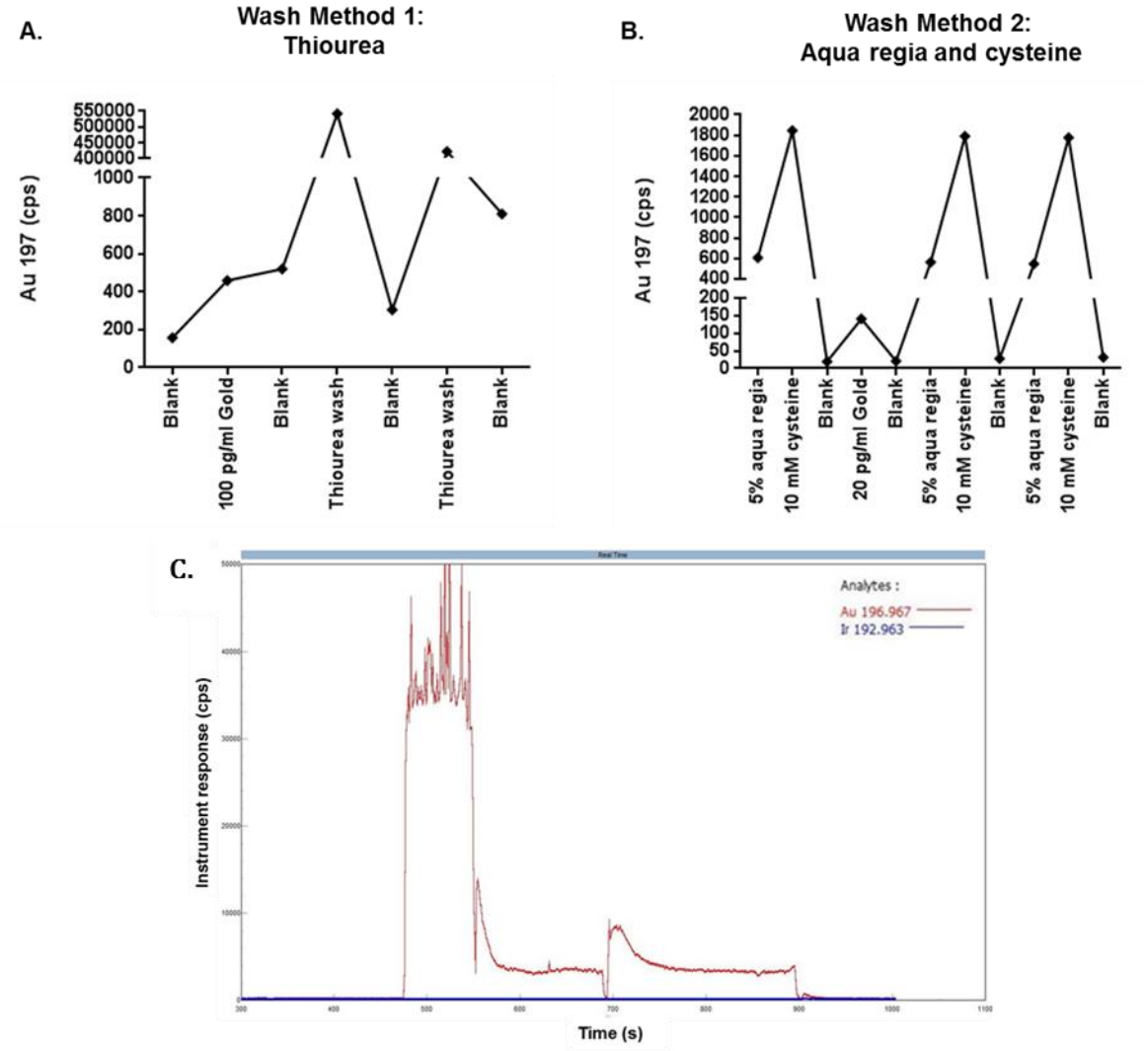


***Figure A1.*** *Comparison of wash method 1 (1% $HNO_3$, 1% HCl, 2% Thiourea) and wash method 2 (5% aqua regia and 10 mM Cysteine). A and B) Effectiveness of both methods after incorporation of gold into the ICP-MS system. C) Instrument response after flow injection of 0.01 µg/ml gold and both wash solutions 5% aqua regia and 10 mM cysteine.*

## Gold memory effect results

To eliminate the gold memory effect two different wash methods were tested. A blank sample is described hereafter as a sample that has been prepared as a blank and used to illustrate the carryover issue; not intentionally containing gold. In Figure A1.A the gold memory effect is visible; when after injection of 100 pg/ml gold, a higher gold count was measured in the blank sample in comparison with the gold sample. With the use of a thiourea wash the gold counts of the blank samples did not decrease and a subsequent rinse caused the gold counts to increase. Furthermore, the thiourea wash produced high gold counts itself, which might influence readings within the blank samples. The second wash method is divided into two solutions, which were inserted into the system successively. Both wash solutions produced gold counts as well, similar to what was observed in the thiourea wash (Figure A1.B). However, gold counts in the blank samples were similar with every measurement. Surprisingly, the gold counts in the blank after insertion of 20 pg/ml gold were not increased, which might indicate that the effect of the washes remained for a longer time. In Figure A1.C the effectiveness of the second wash method was indicated by injection of 0.01 µg/ml gold after a blank sample, followed by both wash solutions. After the wash solutions, the blank was measured again and the gold counts in the sample were similar to the first measurement. To determine whether the high counts in the 5% *aqua regia* and 10 mM cysteine washes stabilized over time, multiple measurements were performed (Figure A2.A and A2.B). Both washes showed stability of gold counts per second after two to three washes, thus indicating that all the gold is released from the sample introduction system.

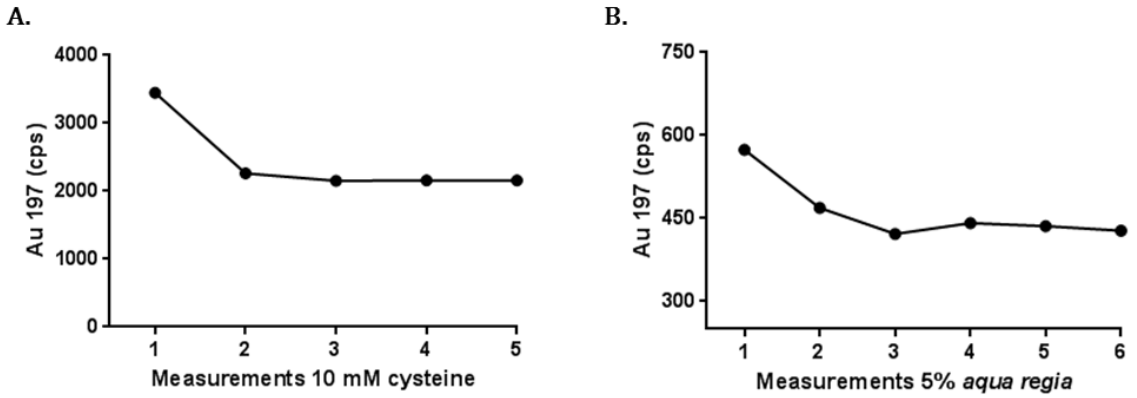


***Figure A2.*** *A) Multiple measurements of 10 mM cysteine wash. B) Multiple measurements of 5% aqua regia wash.*

## Conclusion

Based on these results it was decided to continue with the use of the second wash method consisting of 5% *aqua regia* and 10 mM cysteine. Though both wash solutions produce gold counts, this suggests the stabilization of Gold counts after two to three washes that gold is present in the wash solutions itself. If this wash method were to be used for clinical diagnosis or forensic investigations, compounds used for the wash solutions should be free of gold to ensure no possible carry-over of gold by the wash solutions. This would as well reduce the wash time that is needed to rinse the ICP-MS instrument before sample analysis.

## B. Development of IgG ELISA

### Short introduction and methods

For quantification of IgG levels in plasma, whole blood and dried blood spots, an IgG ELISA needs to be developed based on DELFIA systems from PerkinElmer. For optimization of an IgG ELISA a Nunclon™ Delta Surface 96 wells plate (ThermoFisher Scientific) was coated with 0.2, 0.4 or 0.8 µg/ml Goat-anti-human IgG (Sigma-Aldrich) in D-PBS for 2 hours at room temperature or overnight at 4°C. For testing the effect of a blocking step 3% BSA in D-PBS was used. Three sample matrices were tested for optimization of the IgG ELISA: 1x Diluent N (Abcam), Tris-buffered Saline with 0.05% Tween 20 (TBS-T) and D-PBS with 0.05% Tween 20 and 1% bovine serum albumin (BSA; Sigma-Aldrich). Pentaglobin® (Biotest, Dreieich, Germany) was used as an IgG standard and standards and samples were incubated for 90 minutes within each well of the 96 well plate. Mouse-anti-human IgG Eu-labelled antibody was used as a detection antibody with the following concentrations: 25, 50 and 100 ng/ml and 0.25, 0.5, 1.0 and 2.0 µg/ml. To release Europium from the antibody DELFIA® Enhancement solution (PerkinElmer) was incubated for 15 minutes and analysed with the Europium programme of the Wallac Victor2 1420 multilabel counter microplate reader.

### Development of IgG ELISA results

For the development of an IgG ELISA various capture and detection antibody concentrations were tested (Figure B1). To select the most suitable concentrations for this ELISA the signal to noise ratio was calculated for every concentration combination. A signal to noise ratio (S/N) of 5 or higher was taken to be sufficient. Furthermore, R squared values of every standard curve above $R^2 = 0.8$ were taken into account for the selection of the concentrations. Standard curves made with a capture antibody concentration of 0.2 µg/ml gave a S/N of 3 (Figure B1.A) or 2 (Figure B1.D, G and J; Table B1), which were not sufficient according to set requirements. R squared values of Figure B1.A and B1.D were found to be above 0.8. Figures B1.B, E, H and K were developed with a capture antibody of 0.4 µg/ml and gave insufficient S/N of 2, 2, 3 and 2, respectively (Table B1). Furthermore, R squared values of detection antibody concentration 0.25 and 0.5 µg/ml were found to be above 0.8. Standard curves developed with a capture antibody concentration of 0.8 µg/ml showed S/N of 3 (Figure B1.C), 4 (Figure B1.F and L) and 5 (Figure B1.I). In addition, a capture antibody of 0.8 µg/ml showed the best fitted standard curves in comparison to the other concentration combinations. Only the R squared value of Figure B1.F was below 0.8. After repeating the concentration combinations used in Figure B1.C, F, I and L gave for 0.25, 0.5, 1, and 2 µg/mL S/N of 5, 6, 4 and 4, respectively.

Based on these results further attempts to improve the IgG ELISA were done with a capture antibody concentration of 0.8 µg/ml and a detection antibody concentration of 0.25 and 0.5 µg/ml. To lower the background values, a blocking step was introduced in the protocol (Figure B2.A and B), which resulted in a lower S/N in comparison to the same concentration combinations shown in Figure B2. Furthermore, different sample matrices were tested to improve signal to noise values of the ELISA. Figure B2.C and D show standard curves developed in TBS-T and Figures B2.E and F show standard curves made with 1x Diluent N. Both sample matrices led to a signal to noise ratio of 1 and low R squared values. Furthermore, europium counts of standard 4 (4000 ng/ml) were lower in comparison to standard 3 (2000 ng/ml) for Figures B2.A, B and F. In addition, Europium counts for Figures B2.C, D and E were flattened. This decrease and flattening of europium counts might suggest that the detection antibody concentrations are too high.

In Figure B3 standard curves were developed with lower detection antibody concentration in comparison to the concentrations used for Figure B2. In addition, based on previous results it was decided to exclude a blocking step from the protocol. New detection antibody concentrations were tested in two sample matrices: TBS-T (Figure B3.A, B and C) and PBS with 1% BSA and 0.05% Tween 20 (Figure B3.D, E and F). Figure B3.A, B, C, D and E gave signal to noise ratio values of 2, 2, 1, 4 and 3, respectively. Furthermore, the standard curves gave a R squared value below 0.8. Figure B3.F showed a R squared value of 0.9986 and gave a signal to noise ratio of 7 suggesting an antibody concentration combination of 0.8 µg/ml for the capture antibody and 100 ng/ml for the detection antibody might be most suited for this ELISA. This was confirmed after a repeat of this experiment with the suggested antibody concentrations.

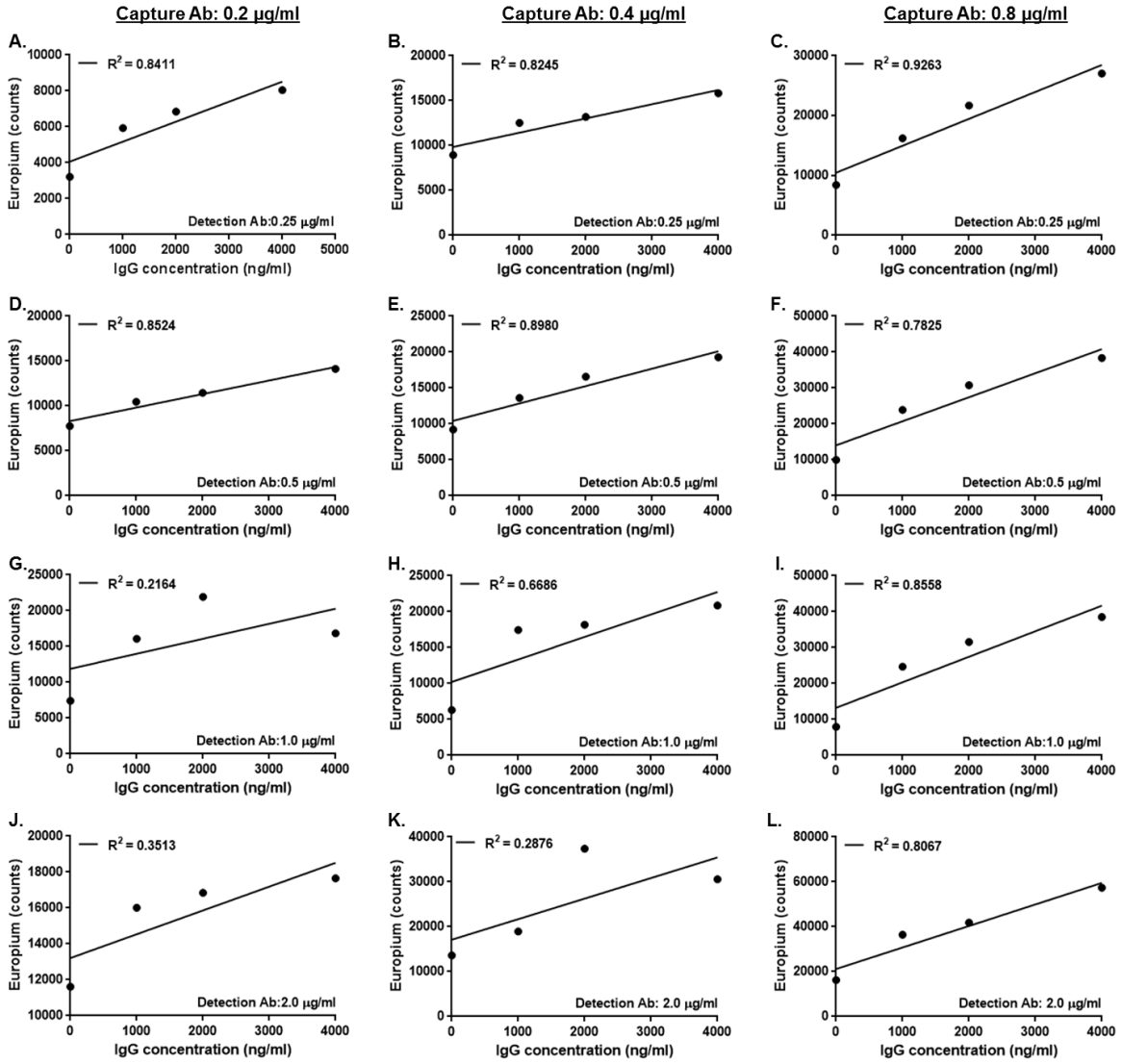


***Figure B1.*** *IgG calibration curves made with various capture and detection antibody concentrations for development of an IgG ELISA with an Europium labelled detection antibody. A, D, G and J were developed with a capture antibody concentration of 0.2 µg/ml and detection antibody concentrations of 0.25, 0.5, 1.0, 2.0 µg/ml, respectively. B, E, H and K were developed with a capture antibody concentration of 0.4 µg/ml and detection antibody concentrations of 0.25, 0.5, 1.0, 2.0 µg/ml, respectively. C, F, I and L were developed with a capture antibody concentration of 0.8 µg/ml and detection antibody concentrations of 0.25, 0.5, 1.0, 2.0 µg/ml, respectively. Ab = antibody.*

| Figure 9 | Capture antibody | Detection antibody | Signal to noise |
|---|---|---|---|
| **A** | 0.2 µg/ml | 0.25 µg/ml | 3 |
| **B** | 0.4 µg/ml | 0.25 µg/ml | 2 |
| **C** | 0.8 µg/ml | 0.25 µg/ml | 3 |
| **D** | 0.2 µg/ml | 0.5 µg/ml | 2 |
| **E** | 0.4 µg/ml | 0.5 µg/ml | 2 |
| **F** | 0.8 µg/ml | 0.5 µg/ml | 4 |
| **G** | 0.2 µg/ml | 1.0 µg/ml | 2 |
| **H** | 0.4 µg/ml | 1.0 µg/ml | 3 |
| **I** | 0.8 µg/ml | 1.0 µg/ml | 5 |
| **J** | 0.2 µg/ml | 2.0 µg/ml | 2 |
| **K** | 0.4 µg/ml | 2.0 µg/ml | 2 |
| **L** | 0.8 µg/ml | 2.0 µg/ml | 4 |

***Table B1.*** *Signal to noise values of standard curves Figure B1 Values calculated by dividing Europium counts of highest standard by the lowest standard.*

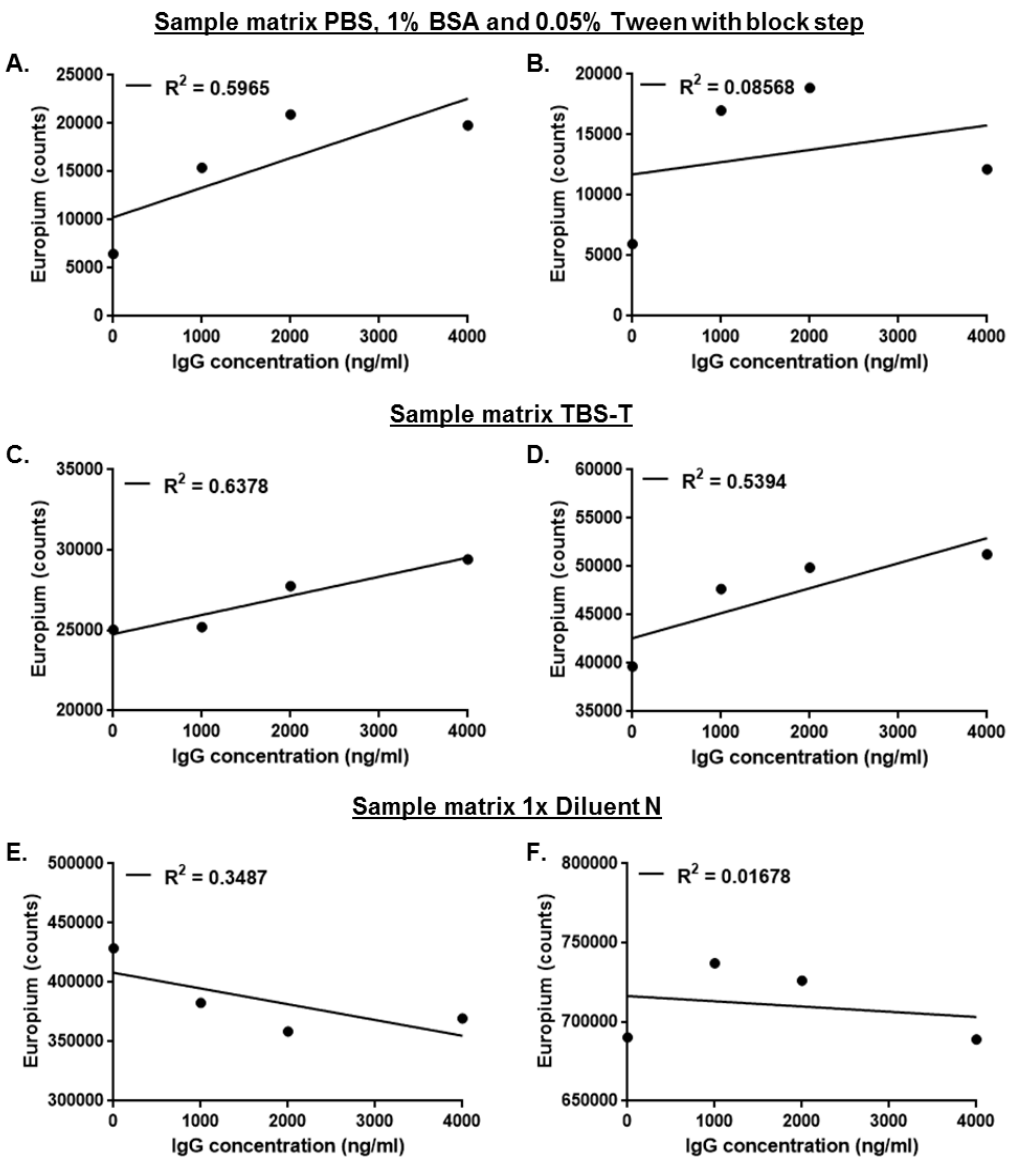


***Figure B2.*** *IgG calibration curves for the development of an IgG ELISA with an Europium labelled detection antibody. A concentration of 0.8 µg/ml was used for the capture antibody and A, C and E were developed with a detection antibody concentration of 0.25 µg/ml and 0.5 µg/ml was used for B, D and F. A and B) Calibration curves were developed in a sample matrix of 1% BSA and 0.05% Tween 20 in PBS and to decrease background a block buffer consisting of 3% BSA in PBS was used. C and D) A sample matrix of Tris buffered Saline with 0.05% Tween 20 was used. E and F) Calibration curves were developed with a sample matrix of 1x Diluent N, which is the sample matrix from the Albumin Human ELISA kit.*

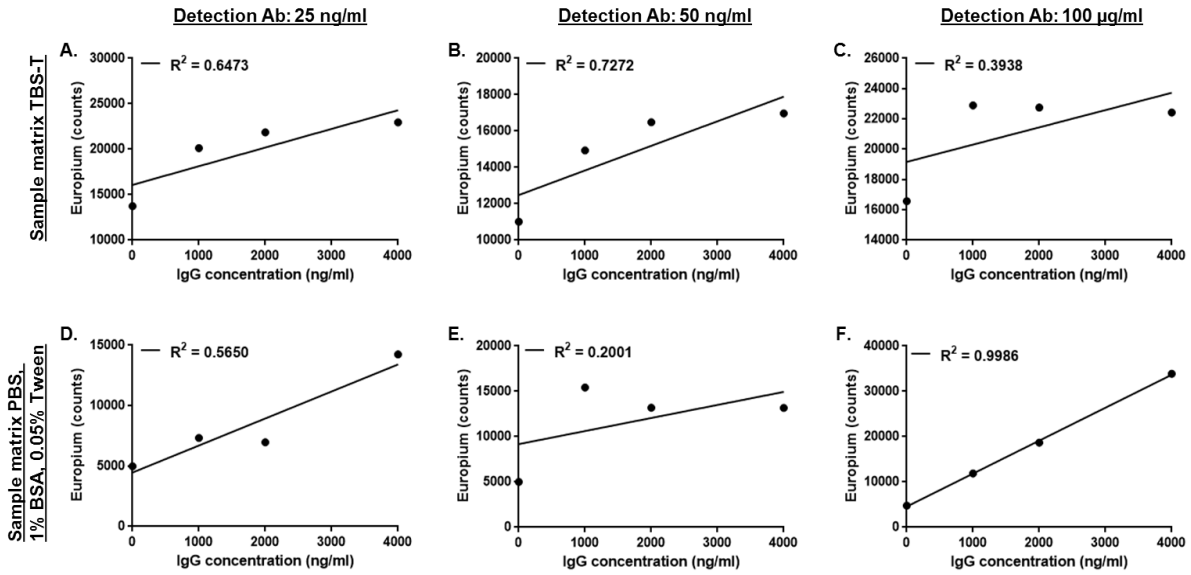


***Figure B3.*** *IgG calibration curves for the development of an IgG ELISA with an Europium labelled detection antibody. A concentration of 0.8 µg/ml was used for the capture antibody and a detection antibody concentration of 25 ng/ml, 50 ng/ml and 100 ng/ml was respectively used for A and D, B and E and C and F. A, B and C) IgG ELISA was developed without a block step and with a sample matrix of Tris buffered Saline with 0.05% Tween 20. D, E and F) IgG ELISA was developed without a block step and with a sample matrix of PBS with 1% BSA and 0.05% Tween 20. Ab = antibody.*

## Conclusion

Based on the results, a capture antibody concentration of 0.8 µg/ml and a detection antibody concentration of 100 ng/ml was used for further experiments. In addition, there was no blocking step in the protocol and a sample matrix of 1% BSA and 0.05% Tween 20 in PBS was used. The blocking step was not sufficient for this ELISA because the used BSA probably contained heavy metals, which can show high background levels in the assay [3].

Additional improvements that can be tested for this ELISA could be to change to a different type of 96 wells plate to decrease background signals of the plate. Furthermore, a blocking step could be introduced using purified BSA or a high grade of casein or gelatine.

## References

1. Kerr SL, PhD thesis, Loughborough University, 2008.
2. Managh AJ, Hutchinson RW, Riquelme P, et al., Laser ablation-inductively coupled plasma mass spectrometry: an emerging technology for detecting rare cells in tissue sections. *J. Immunol.,* 2014; **193**(5): 2600-2608. doi:10.4049/jimmunol.1400869.
3. DELFIA immunoassays: Guide to Converting ELISA Assays to DELFIA, http://www.perkinelmer.com/lab-solutions/resources/docs/MAN_DELFIA_ELISA_Conversion.pdf, (accessed July 2018).