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Multiplex flow cytometric immunoassay for serum biomarker profiling

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Introduction

Serum biomarker profiling is a promising approach to detect administration of growth promoters in cattle. The multiple biomarkers can be detected simultaneously by a multiplex flow cytometric immunoassay (FCIA) with colour-encoded microspheres. Serum biomarkers used to detect rbST treatment are insulin-like growth factor 1 (IGF-1), IGF binding protein 2 (IGFBP2), osteocalcin and antibodies induced by rbST.

Method

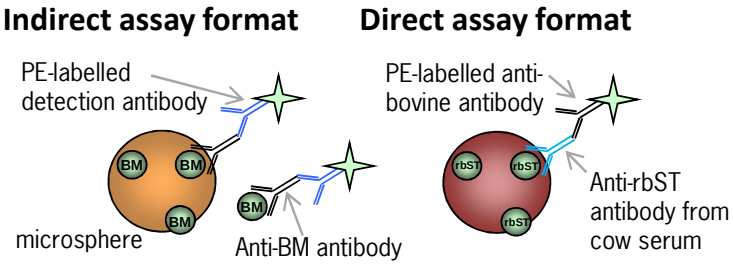

Figure 1: *Assay format of multiplex FCIA. Indirect assays are performed for biomarkers (BM) IGF-1, IGFBP2 and osteocalcin. Direct assay is performed for antibodies against rbST.*

- Analysis in flow cytometer: red laser to determine type of assay biomarker (colour of microsphere); green laser to measure biomarker concentration (amount of fluorescence per microsphere)
- Animal experiment with 4 rbST-treated and 3 blank cows for assay validation

Results

- Sensitive assay for each biomarker (Figure 2) allows detection of changes in biomarker concentrations in relevant range

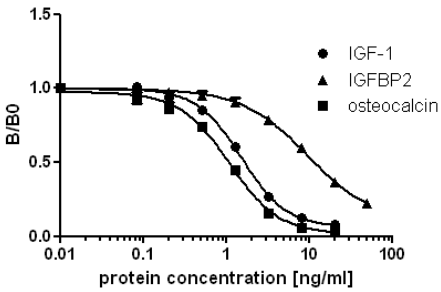

Figure 2: *Standard curves obtained for IGF-1, IGFBP2 and osteocalcin in serum-matched buffer*

- Short-term response upon treatment for IGF-1 and IGFBP2, long-term response for anti-rbST antibodies and osteocalcin (Figure 3)
- Discrimination in biomarker profiles of treated and untreated animals can be shown with principal component analysis (PCA) shows (Figure 4)
- Statistical analysis with k-nearest neighbours (KNN) gives a 89 % true-positive rate and 100% true-negative rate (Table 1)

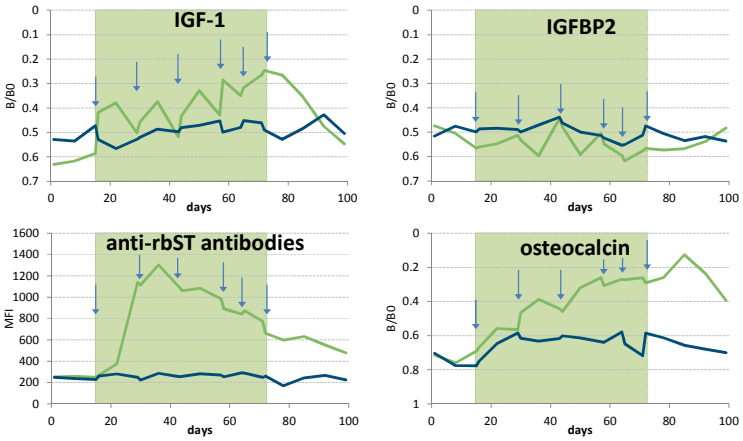

Figure 3: *Time courses of signal responses for each biomarker (IGF-1, IGFBP2, anti-rbST antibodies, osteocalcin). Signals of a rbST-treated cow (green) are compared to signals of blank cow (blue). rbST treatment period is indicated with a green rectangle and treatment time points with blue arrows.*

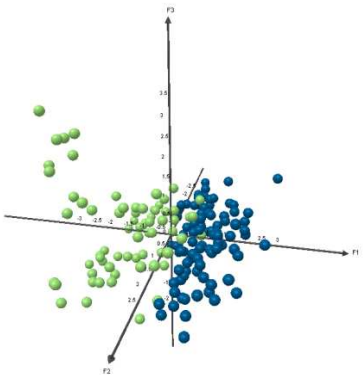

Figure 4: *Distribution of rbST-treated (green) and blank samples (blue) after performing PCA.*

Table 1: *Classification table after performing KNN (log10 transformation and auto scaling)*

	predicted as	
treatment	blank	rbST treated
blank	100 %	0 %
rbST treated	11 %	89 %

Conclusion

- Responses for each biomarker show differences between rbST-treated an blank animals
- Statistical analysis on combined data makes discrimination of treated and blank group possible

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