Public Abstract
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Title:Development of a novel immunoassay for simultaneous quantification of endocrine parameters

Since the advent of the radioimmunoassay in the 1950s, numerous immunologically-based methods have evolved for sample analysis. Although each immunological method possesses unique assets and liabilities, all share limited abilities in range of concentrations detectable and the number of analytes resolvable simultaneously; most procedures are limited to one analyte determined per replicate per sample. The objective of this study was to adapt technology evolving from the genomics “revolution” for multiplexed hormonal analysis in livestock. We sought to complete this objective using the following sequencing technologies and tools: Illumina BeadXpress, Luminex xMAP, and quantitative immuno-PCR. The Illumina BeadXpress and Luminex xMAP both share similar characteristics and each of these proprietary platforms consists of a laser spectrum analyzer and a bead-set. Each bead-set contains microscopic beads and each bead-set has unique identifying signatures. As a test of proof of concept, the surface of a bead set was conjugated to an LH antibody. Using these technologies, we were able to establish an assay for LH on the Luminex platform. We were unable to develop an analyte detection assay on the Illumina platform. The restrictive proprietary constraints of both the Luminex and Illumina platforms however, greatly limited assay flexibility. Therefore, we chose to establish an assay for LH using quantitative immuno-PCR (iPCR). Quantitative immuno-PCR exploits PCR signal amplification in combination with antibody capture and specificity. Briefly, a sandwich immunoassay format is performed with capture antibody immobilized to a PCR plate. A second detection antibody, which is conjugated to an oligonucleotide, is then added to the solution phase for binding. After a series of washes to remove the unbound oligo-antibody conjugate, the plate contents are subjected to quantitative PCR (qPCR). Detection of LH was achieved, but background binding contributed to significant interference. Subsequently, to simplify the design and demonstrate proof of concept, a biotinylated oligonucleotide was competed with unlabeled biotin in streptavidin coated polystyrene plates and then detected for oligonucleotide via qPCR. The results of this latter experiment demonstrated that the iPCR methodology could detect unlabeled biotin across three orders of magnitude. In conclusion, background binding contributed to significant interference with quantitative immuno-PCR but one that is believed to be resolvable. Moreover, our observations suggest that immuno-PCR has the potential to improve detection capabilities of current hormonal assays with three or more orders of magnitude in sensitivity, and ultimately provide multiplex capabilities, with the conjugated oligonucleotide serving as both a quantitative detection label and as a barcode for later identification of analyte. The technological leap in capabilities provided by successfully multiplexing can greatly aid in our efforts to understand the complex interaction of endocrine and metabolic signals in the dynamically changing animal and greatly reduce sample throughput investments.