Materials
All biotinylated antibody reagents were obtained from Bac, part of Thermo Fisher Scientific. The antibody reagents used are 13 kDa camelid antibody fragments specific to each antigen. Recombinant insulin (Part no. cyt-270) and Factor VIII (Part no. pro-318) were both obtained from the manufacturer.

Protein Concentration Determination
The concentration determination on Octet requires the development of a standard curve. A reference standard curve is obtained using a purified standard sample of the respective protein. Known concentrations of the purified standard sample are spiked into the matrix of interest and are serially diluted (often two-fold) within the desired concentration range using an antibody-depleted assay matrix.

A biosensor step using biospecies that blocks non-reacted epitopes of interest or using BSA in the binding matrix/buffer may be necessary post-antibody capture to prevent non-specific binding (BSA). In addition, the use of detergents in the sample diluent to prevent potential non-specific hydrophobic interactions is recommended especially when dealing with glucoproteins. NSB refers to either the binding of the analyte to a biosensor surface not coated with the specific antibody or to an observation of an acceptable binding of the protein. In this way one can examine the contribution of free biotin to the antibody-coated biosensor surface and should be investigated and eliminated for each assay during development. If blocking is performed, a short baseline step should be established in the analyte sample matrix prior to sample detection.

Data Analysis
Data collected with the Octet RED60 and RED48 instrument were analyzed and processed using Octet Data Analysis software. The software allows one to use a combination of two binding rate analysis models: a double exponential or a 4-parameter logistic function. The resulting baseline data is then fit to one of several models (linear equation, 4-PL or 5-PL equations) to establish a standard curve from which the unknown analyte’s concentration is determined.

Octet System Comparison to HPLC
To compare the performance of Octet systems with HPLC, recombinant insulin was used as an example. Samples were analyzed side by side at Auran Biocinogen using the Octet RED60 instrument and HPLC using a Zorbax 300SB-C18 RP-HPLC column. Insulin samples were serially diluted two-fold in either PBS or DMEM media at 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 μg/mL. Half of the prepared samples were used for the RP-HPLC study while the other half were used to run blank crucible array-based analysis. For the Octet system, the assay used was as described prior, and for RP-HPLC the following conditions were used:

Sample flow rate: 1 mL/min
Borate: 0.1 M
Buffer: 0.5 M NaOH in water, pH 9.0
Gradient: 35-50% B in 8 minutes
Detection: 280 nm

The results suggest that HPLC exhibits excellent sample recovery at high insulin concentrations but significantly overestimates concentrations at the lower end of the dynamic range. The Octet systems on the other hand have a more linear limited dynamic range of quantitation for insulin. However, the systems exhibit excellent recovery of low concentration insulin samples with better sensitivity (Table 3). It also takes significantly less time to run the same number of samples on an Octet system as it does via HPLC, providing significant time savings that can lower overall analysis costs.

Conclusions
The simple Dip and Read® approach on the Octet platform in combination with High Precision Streptavidin biosensors (SAX) provide an easy to use, time-saving technique that enables high-throughput, accurate quantification of recombinant proteins. The LOD for insulin concentration when determined on the Octet system was 2.0 ng/mL, in both DMEM and TTX Kinetic Buffer, while it was much higher (25 μg/mL) in both media when determined on RP-HPLC. Moreover, while it takes more than 10 minutes to assay one sample on RP-HPLC, Octet systems take approximately the same time to assay as many as 8 samples (Octet RED60), 16 samples (Octet RED48) and 64 samples (Octet HTX). This technique is a simple replacement for HPLC and as it is more robust to various culture media, and can be used both upsteam and downstream in the therapeutic protein drug development process. In addition, Octet platform is equipped with GARP-compliant features enabling the development of robust methods that can be quickly validated for assay transfer to QC laboratories.

Acknowledgements
1 Auran Biocinogen for Octet system HPLC comparison data
2 Perkin Elmer (Thermo Fisher Scientific) for providing VHH antibody fragments

Author for correspondence: david_apiyo@pall.com