An innovative high sensitivity tool for active neutrophil elastase quantification

McGranahan C1, Johnston SJ2, Moffitt KL2, Ferguson TEG2, McCafferty DF1

1ProAxsis Ltd, Belfast, Northern Ireland

Introduction
The serine protease, neutrophil elastase (NE), is widely recognised as a key biomarker in respiratory disease; with its potential therapeutic value illustrated by the volume of anti-NE therapies currently undergoing clinical evaluation1-3. However, recruitment for these studies is constrained as many patients are ‘non-producers’ of sputum. Alternative sample types include nasal fluid but are reputed to contain lower NE levels. To address this issue, ProAxsis Ltd has harnessed its existing technology to produce an activity-based immunoassay (ABI) for active NE quantification, with enhanced sensitivity.

Materials & Methods

The key binding agents used within this high sensitivity ABI include the NE ProteaseTag®, PRX1138, and an anti-human NE monoclonal antibody. PRX1138, was synthesised using a combination of solid-phase peptide synthesis (SPPS), and solution-phase methodologies, whereas the NE antibody was developed using hybridoma technology.

The ABI protocol involves an initial incubation step to enable capture of PRX1138 onto streptavidin-coated plates, followed by addition of NE standard solutions. Detection of the complex was facilitated by the NE antibody, and subsequently a colorimetric substrate. Absorbance was measured at 450 nm.

Optimisation of various experimental parameters, including reagent incubation timings and antibody concentrations, was performed to maximise sensitivity.

Once the desired sensitivity had been achieved, a preliminary validation study was carried out. Specifically, omission experiments were performed to evaluate the degree of non-specific binding (NSB) between assay components. This involved the sequential exclusion of each reagent and then measuring the subsequent impact on overall assay signal.

The presence of a ‘hook’ effect was also examined i.e., signal suppression at analyte levels above that of the high standard. To do so, an extended NE standard curve was analysed with a top concentration of 960 ng/ml.

Results

A range of experiments were performed to identify the optimal conditions for the NE ABI; with an antibody dilution of 1:10,000 and an incubation time of 60 minutes deemed to be most suitable (Figs. 1A&B).

Upon completion of assay optimisation, a precise and reproducible measurement of NE was achieved in under 3 hours, with detection limits ranging from 0.47 ng/ml to 30 ng/ml; a >30 fold increase in sensitivity relative to ProAxsis’ original NE ABI (Figure 2).

Moreover, omission assays revealed negligible NSB between reagents (Fig. 3A), with no evidence of a ‘hook’ effect detectable upon testing higher concentrations of NE (Fig 3B).

Discussion and Conclusion

Here we report the development of a high sensitivity NE ABI (NE Premium), which raises the possibility of detecting NE in surrogate sample types such as nasal fluid and breath condensate; thereby, potentially improving patient recruitment for clinical trials.

References