A novel point of care test for sputum sample stratification prior to the quantitative measurement of active neutrophil elastase

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Introduction

Active neutrophil elastase (NE) is a key biomarker of disease severity in respiratory diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and bronchiectasis[1,2,3].

Levels of active NE, however, are reported to vary broadly between patients (both basally and during exacerbations); therefore, the use of a Point of Care (PoC) test, that provides a rapid, qualitative measurement of NE, could greatly enhance resource management, by facilitating the appropriate selection of type of activity-based immunoassay (ABI).

The aim of this study was therefore to evaluate the utility of a Novel PoC test (NEATstik®) as a research tool in sample stratification, for subsequent quantitative measurement of active NE using an ABI.

Materials & Methods

Sputum samples (n=19), obtained from a research sample biobank, were initially interrogated for active NE, using the novel PoC test, NEATstik® (8 µg/ml threshold in unprocessed sputum). Briefly, sputum was diluted x 10 in running buffer, followed by gentle inversion of the sputum pot (10 times), prior to the transfer of 70 µl of diluted sputum into the sample port. Samples were allowed to run for 10 minutes, before evaluation of test-line intensity (scored from 0-10).

Sputum sol was then prepared from all 19 sputum samples, with each positive NEATstik® sample recommended for analysis on ProAxsis' Original ABI for NE (detection range of 15.6 ng/ml to 1000 ng/ml). Sample processing involved an initial x 5 dilution in PBS, gentle inversion for 1 minute, followed by centrifugation at 3,000 g for 30 minutes (4°C). A further x 100 dilution was performed prior to NE quantification, to mitigate matrix interference.

Any negative NEATstik® test samples were subsequently analysed using an alternative ABI for NE (ProteaseTag® Active NE Premium Immunoassay, ProAxsis Ltd), with enhanced sensitivity (detection range of 0.975 ng/ml to 62.5 ng/ml).

References

1. Mayer-Hamblett, N (2007) *American Journal of Respiratory and Critical Care Medicine*, 175: 822-828.
2. Sagel, S (2012) *American Journal of Respiratory and Critical Care Medicine,* 186 (9): 857-865.
3. Chalmers, JD (2016) *American Journal of Respiratory and Critical Care Medicine*, Epub

Results

Of the 19 sputum samples that were analysed using ProAxsis' NEATstik® device, 13 (68%) produced a positive test; with the remaining 6 samples yielding a negative test (Figs. 1A and 1B).

Sputum sol, produced from all positive samples, were then screened for active NE on the original NE ABI. NE was quantifiable in each of the 13 positive samples (100%). Active NE was measurable in 33% (2/6) of the 6 negative NEATstik® samples, that were assayed on the NE Premium ABI; with reported concentrations found to fall around the low limit of detection (0.975 ng/ml) (Fig.2).

Figure 3 illustrates the statistically significant correlation ($p<0.0001$) observed between active NE levels (µg/ml), and corresponding NEATstik® visual grades (Spearman r value=0.83).

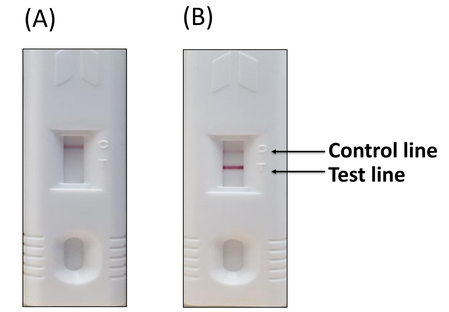

Figure 1. Representative negative (A) and positive (B) NEATstik® test, obtained with sputum samples.

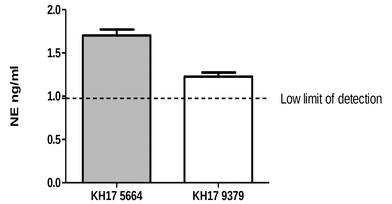

Figure 2. Analysis of sputum samples producing a negative NEATstik® test, using ProAxsis' NE Premium ABI

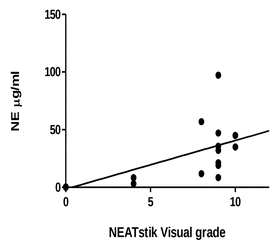

Figure 3. Observed correlation between reported active NE levels in sputum sol samples, and corresponding NEATstik® visual grades.

Discussion and Conclusion

Active NE was successfully quantified in all 13 positive (100%) NEATstik test samples, following analysis on the original NE ABI; with NE detectable in 33% of negative NEATstik test samples, when using the more sensitive ABI.

Taken together, this data highlights NEATstik® as a useful research tool for initial stratification of sputum samples into two cohorts; positive and negative test samples, which should then be assayed using ProAxsis' Original and Premium NE ABIs, respectively.