NEATstik® - A Novel Point-of-Care Test for the Measurement of Active Neutrophil Elastase in Patients with Respiratory Disease

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Introduction

Sputum levels of active neutrophil elastase (NE) are frequently elevated in respiratory diseases, such as bronchiectasis, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF). Increased NE levels have been demonstrated to inversely correlate with pulmonary function[1,2], and risk of exacerbations[3]. Furthermore, active NE is recognised as a biomarker of subclinical infection, however, no tools currently exist for its routine monitoring at home or in the clinic.

ProAxsis have translated their ProteaseTag® technology into NEATstik®, a rapid point-of-care (PoC) test for the measurement of active NE in sputum. NEATstik® is a qualitative test, provides a result in less than 10 minutes and is complementary to the fully quantitative laboratory-based ProteaseTag® Active NE Immunoassay.

NEATstik® could facilitate routine monitoring of those patients at highest risk of upcoming exacerbations; enabling pre-emptive medical intervention, and mitigating the patient's risk of developing serious complications. In addition, for those patients who present with an ongoing exacerbation, the test may allow identification of patients most likely to respond to antibiotic therapy, as well as a tool for monitoring the efficacy of that therapy.

Materials & Methods

Clinical Samples: Sputum samples (n=19) were sourced from a biobank and the clinical status of each subject was unknown. Subject demographics were: Sex: M12, F7; Ethnicity: 10 White, 9 Black; Mean (sdv) age: 52 years (20.7).

Sample Processing: Sputum was split into two parts. One portion was diluted with 4 parts ice-cold PBS, gently mixed and centrifuged at 4°C for 30 minutes at 3000g. The supernatant (sol) was recovered and aliquoted. The second portion was diluted x10 using NEATstik® Sample Dilution Buffer and inverted to mix.

Active NE Quantification: Active NE in the processed sputum sol (portion 1) was quantified using the ProteaseTag® Active NE Immunoassay (ProAxsis Ltd) in accordance with manufacturers instructions.

NEATstik®: 70µL of diluted sputum (portion 2) was transferred onto the test sample port of the device and allowed to develop for 10 minutes. Signal intensity at the test line was visually graded (0-10). Results were recorded as positive (visual grade ≥2) or negative (visual grade <2).

References

1. Mayer-Hamblett, N et al (2007) Am J Respir Crit Care Med, 175: 822-828
2. Sagel, S et al (2012) Am J Respir Crit Care Med, 186 (9): 857-865
3. Chalmers, JD et al (2017) Am J Respir Crit Care Med, 195 (10): 1384-1393
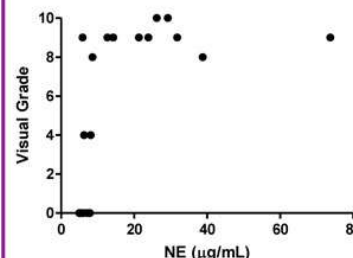
Results and Discussion

Active NE was detected in all sputum samples with a mean (sdv) of 17.75 (17.22) µg/mL and a range of 5.0-73.7 µg/mL. A highly significant correlation (Spearman r=0.7; p=0.0003) was found between active NE quantified by the ProteaseTag® Active NE Immunoassay and the visual grades observed on NEATstik® **(Figure 1)**.

13 sputum samples produced a positive result with visual grades ranging between 4-10. 6 samples were clearly negative with a visual grade of 0 **(Table 1)**. Representative images are shown in **Figure 2** of NEATstik® test-line intensity grades of 0, 4 and 10.

These results indicate that NEATstik® has a sputum active NE threshold of 8 µg/mL with sensitivity and specificity of 100% and 75% respectively. The positive predictive value is 85% and negative predictive value 100%.

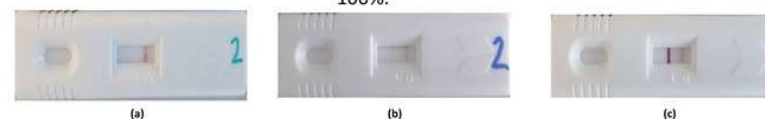Figure 1: Correlation between active NE (µg/mL) and visual grades on NEATstik®

Figure 2: NEATstik® test line intensity at grades (a) 0, (b) 4 and (c) 10

Table 1: Analysis of sputum samples with corresponding NEATstik® grades at the test line

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Active NE (µg/mL)	7.9	31.8	6.0	21.3	73.7	26.2	8.1	8.6	5.4	14.3	5.0	6.3	5.0	12.7	5.9	29.2	23.9	7.1	38.8
Visual Grade	0	9	0	9	9	10	4	8	0	9	0	4	0	9	9	10	9	0	8
Result	-	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	-	+

Conclusion

Our ProteaseTag® technology has been translated to NEATstik®, a highly sensitive and specific lateral flow device for the rapid detection of active NE in sputum which will enable proactive management of chronic respiratory diseases. Furthermore, it has utility in assisting in the identification of patients at highest risk of exacerbations, facilitating patient stratification and assessing antibiotic response.