# Impact of SARS-CoV-2 heat inactivation on active protease biomarkers in sputum sol

Ciaran T McGranaghan[1], Philip C Weir[1], Darragh F McCafferty[1], Kelly L Moffitt[1] and Timothy EG Ferguson[1]
[1]ProAxsis Ltd, Belfast, Northern Ireland

## Introduction

In December 2019, a novel coronavirus infection emerged in Wuhan, Hubei province, China [1]. The causative viral agent has been identified and labelled SARS-CoV-2 due to its high homology with the SARS coronavirus (SARS-CoV). The resulting coronavirus disease 2019 (COVID-19) which primarily affects the respiratory system has spread worldwide causing over 4.3 million deaths to date.

Increased handling of clinical samples, potentially contaminated with SARS-CoV-2, have led to the development of protocols to inactivate the pathogenic virus with the aim of enhancing laboratory safety. Reported methods include heat inactivation and chemical denaturation[2,3], however, there is little investigation of the effect of these methods on biomarker stability.

Here we report the impact of heat treatment on the quantification of key inflammatory biomarkers including proteases and cytokines routinely measured in sputum sol.

## Materials & Methods

**Sample Processing:** Expectorated sputum (n=20) was diluted (x5) with phosphate buffered saline (PBS). The diluted sputum was mixed by inversion (x10) then centrifuged x3000*g* for 30 minutes at 4°C. The sputum sol supernatant was subsequently aliquoted and stored at -80°C prior to biomarker analysis.

**Heat inactivation:** Prior to analysis, an aliquot of sputum sol was thawed on ice, then diluted in the appropriate assay buffer and subjected to heat treatment (56°C, 45 mins) with gentle shaking (600 rpm).

**Biomarker analysis**: Sputum sol samples (± heat treatment) were analysed for active protease biomarkers using Neutrophil Elastase (NE), Proteinase 3 (Pr-3) and Cathepsin G (Cat G) ProteaseTag® Activity-based Immunoassays (ProAxsis Ltd.). Samples were diluted (NE x100, Pr-3 x100 and Cat G x50) in the appropriate buffer supplied by the manufacturer. Interleukin-8 (IL8) was quantified using a Luminex Multi-analyte assay (R&D Systems) with samples diluted (x20) in sample diluent prior to analysis.

## References

1. Zhou P *et al*. *Nature* 2020; 579:270-273.
2. Juerka AS, Solvas JA and Basler CF. *Viruses* 2020; 12:622.
3. Pastorino B *et al. Viruses* 2020: 12:624.

## Results and Discussion

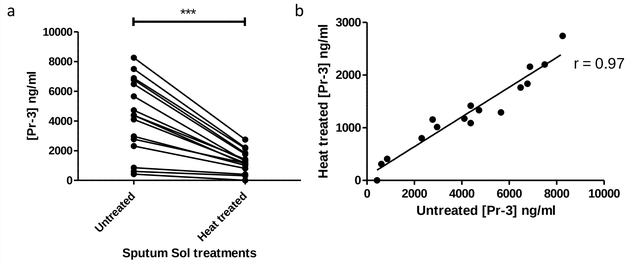


**Figure 1: a)** Comparison and **b)** correlation of Pr-3 levels in heat treated and untreated sputum sol samples

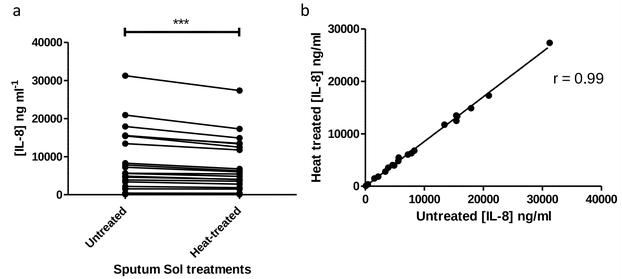


**Figure 4 : a)** Comparison and **b)** correlation of IL-8 levels in heat treated and untreated sputum sol samples

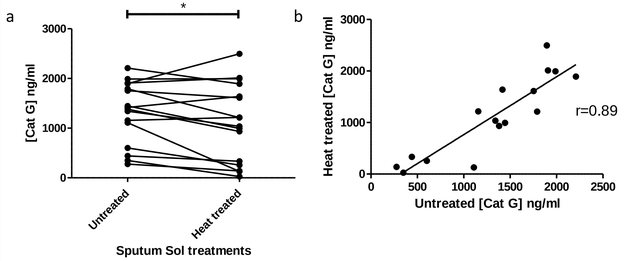


**Figure 2: a)** Comparison and **b)** correlation of Cat G levels in heat treated and untreated sputum sol samples

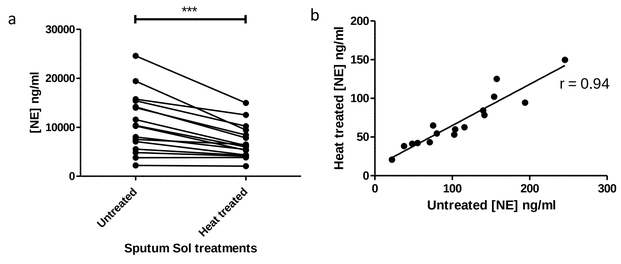


**Figure 3: a)** Comparison and **b)** correlation of NE levels in heat treated and untreated sputum sol samples

Sputum sol samples (n=20) were analysed for a range of active protease and cytokine biomarkers ± heat treatment (56°C, 45 mins) following dilution in the assay specific buffer.

Heat treatment led to a marked reduction in biomarker levels in sputum sol. Although still measurable, significant decreases in quantities of Pr-3 (**Figure 1a**, P<0.0001), Cat G (**Figure 2a**, P=0.0468), NE (**Figure 3a**, P=0.0001), and IL-8 (**Figure 4a**, P<0.0001) were observed.

However, quantified biomarker levels in heat treated samples, strongly correlated with untreated samples for Pr-3 (**Figure 1b,** r=0.97), Cat G (**Figure 2b**, r=0.89), NE (**Figure 3b**, r=0.94) and IL-8 (**Figure 4a**, r=0.99).

Despite the significant reduction in biomarker levels following heat treatment, analytes remain quantifiable and correlate with untreated samples.

## Conclusion

Heat treatment has a significant impact on levels of active protease and cytokine biomarkers within sputum sol. Nevertheless, protease biomarkers remain quantifiable and correlate with untreated samples when analysed using an activity-based immunoassay, and similarly for IL8 when analysed using a multi-analyte assay, thus offering a safer potential route for measurement in COVID-19 patients.