Sputum processing methods in airway disease Which is best?

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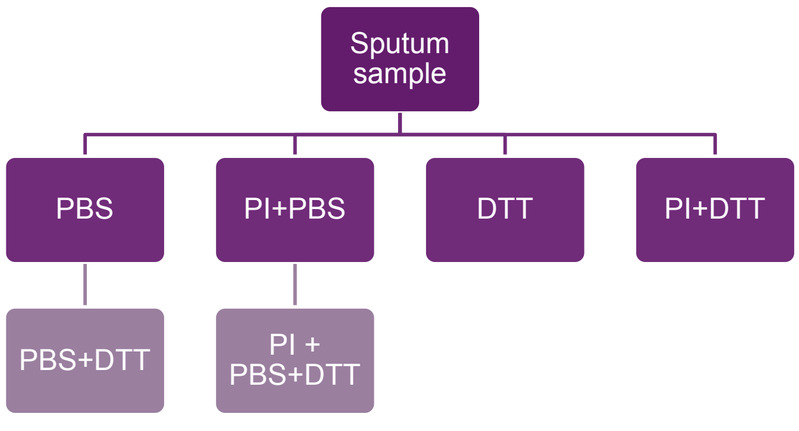
Introduction

Sputum Induction techniques preferentially sample a specific region of the lung. Sputum samples reflect events on the surfaces of the large bronchial/central airways (vs distal alveolar region). This is noteworthy since this is the site that is proximal to many respiratory diseases' pathology and therefore provides opportunities to better understand these diseases and ultimately provide precision therapeutic target areas.

The analysis of cytokines and mediators in sputum supernatants may be affected by endogenous proteases and the processing of sputum with dithiothreitol (DTT). Sputum extraction with PBS or the addition of protease inhibitors has been suggested to improve the recovery of mediators from sputum supernatants. Depending on the antibodies used, the effect of these interfering factors may also vary with the analytical platforms. In this study we focused on a number of potent pro-inflammatory mediators and their expression following several processing methods in CF, COPD, asthma and healthy controls using single-plex ELISA (Life Technologies, UK) and multi-plex RANDOX investigator platforms.

Methodology -Sputum processing

Sputum was processed according to the following methods:



Methodology - Sputum induction

CF patients offered a spontaneous sputum sample. Sputum was induced in all other subject groups using the following procedure. Hypertonic saline aerosols (NaCl 3,4,5%) were generated at room temperature by an Omron NE-U17 ultrasonic nebulizer [Omron Ltd, UK] and each subject inhaled each concentration for 5 min. After each inhalation, subjects were asked to blow their nose, to rinse their mouth and throat with water and to expectorate sputum into a container by coughing.

Methodology - Sputum cytokine analysis

- IL-8 expression measured using IL-8 single plex ELISA from CF, COPD and pooled HV smokers and non-smokers
- Sputum pools - CF, COPD, Asthma, HV smokers, HV non smokers - RANDOX multiplex High Sensitivity Array – IL-8, VEGF, IL-6, TNFa, IL-1a, IL-1b, MCP-1 and EGF
- Samples in our study were analysed in assays where the assay diluents mimicked the sample matrix

Table 1 Subject demographics and FEV1 % pred

	Age	M:F	FEV_1 % pred
Cystic Fibrosis	30 (+/-6.8)	5:5	41.4 (+/-14.0)
COPD	55 (+/-18.9)	5:3	65 (+/-15.8)
Asthma	36 (+/-8.6)	8:2	90 (+/-17.0)
HV smoker	33 (+/-16.4)	5:3	92 (+/-32.1)
HV non-smoker	24 (+/-4.8)	3:3	103 (+/-16.3)

Summary and conclusions

- No one processing protocol stood out in particular for pooled cytokines but for individual cytokine expression the use of DTT+PI appeared not to have such detrimental effects on cytokine expression as previously reported.
- Each mediator assay and specific analysis platform must be validated before use – you cannot make assumptions based on previously published sputum results
- Further investigations are required to understand the impact on cellular out-comes
- Multiple analytical platforms will need to be assessed against larger patient numbers.

Results summary

- Using Repeat-measures ANOVA there were significant differences in key cytokines IL-6, IL-8 and VEGF when processing protocols were compared in all subject groups ($p<0.05$)
- There were significant differences in IL-8 expression when comparing PI+DTT vs PBS and PI+PBS ($p<0.05$) in CF and COPD patients

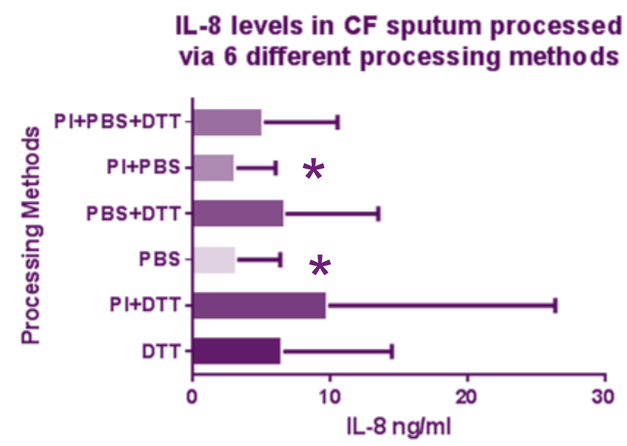
Table 2 Processing method ranking for IL-8 ELISA

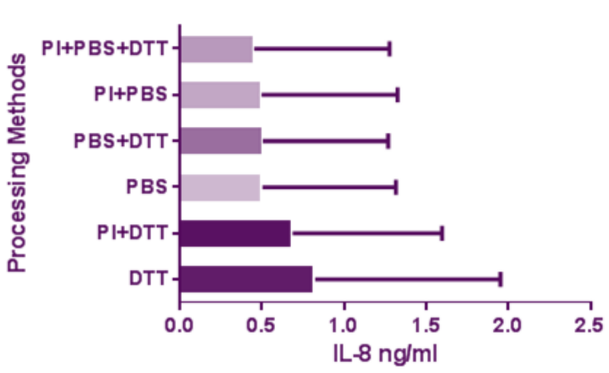
Processing protocol	SCORE	No. of samples	Score / sample	Rank
PBS	106	37	2.86	3
PI + PBS	112	37	3.03	4
DTT	93	36	2.58	2
PBS + DTT	183	36	5.08	5
PI +DTT	78	36	2.17	1
PI+PBS+DTT	186	36	5.17	6

Table 3 Disease specific ranking for all cytokines using RANDOX: –IL-8, VEGF, IL-6, TNFa, IL-1a, IL-1b, MCP-1 and EGF.

Protocol	CF	COPD	Asthma	HV Smoker	HV non smoker
PBS	4	2	3=	2	2
PI+PBS	6	5=	5	5	6
DTT	1=	4	3=	4	3
PBS+DTT	5	5=	4	6	5
PI+DTT	1=	3	2	1	4
PI+PBS+DTT	3	1	1	3	1

Figure 1 Single Plex IL-8 expression in CF, COPD and Healthy non smokers* <0.05 vs PI+DTT





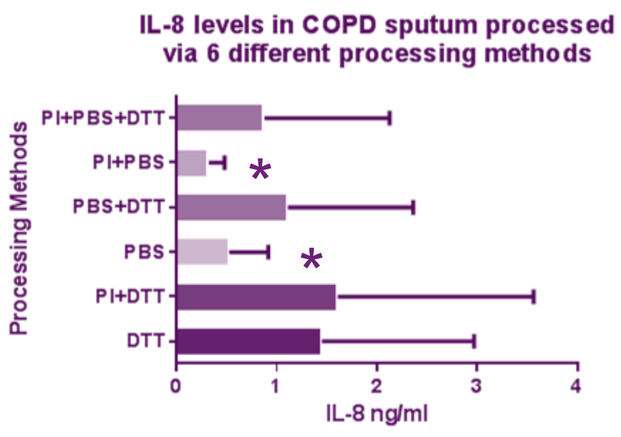


Figure 2 Pooled IL-6 and VEGF expression in COPD, asthma, healthy smokers and healthy non smokers using different processing methods