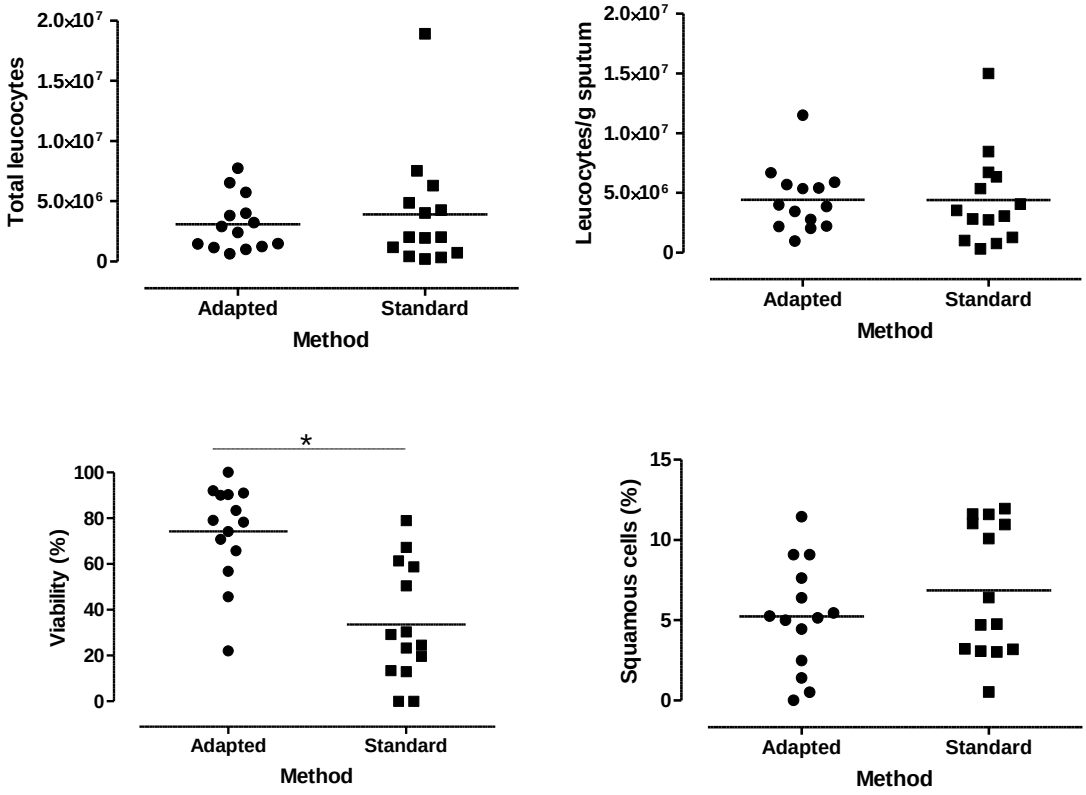

Supplementary figure 1: Comparison of standard (0.1% DTT) and adapted (0.05% DTT) sputum processing techniques and the effects on sputum cell counts and cell viability n=14

Sputum samples were processed using either a standard sputum processing technique utilising 0.1% DTT to liquefy the sample, or a technique adapted for intracellular STAT phosphorylation analysis containing a reduced DTT concentration; 0.05%.

Importantly there were no differences found in the cell counts in sputum processed by the two methods. The differential cells counts showed no difference in the number of macrophages, neutrophils or eosinophils when processed differently indicating that the cellular makeup of the samples was maintained regardless of the processing method used. The cell viability was significantly higher in sputum processed using the adapted method compared to the standard method, with mean values of 74% ± 6 and 34% ± 7 for adapted and standard methods respectively ($p=0.0016$). Cell viability is a key requirement for the analysis of STAT phosphorylation and activation of sputum cells. Samples with low viability may lead to poor results or indistinct cell populations during flow cytometric analysis.

The new adapted method of sputum processing allows for sputum leucocytes to be separated from the mucus and, due to higher viability, these cells can then be subjected to further experimentation which may not be possible with cells isolated from the standard sputum processing method.