OBJECTIVES: The aim of the study was to compare expression of six common ISGs (IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1) and the corresponding IS in RNA derived from two commonly used whole blood RNA collection systems (PAXgene and Tempus).

Methods: Whole blood was collected from ten healthy individuals (median age 25.5 years) in sodium heparin tubes and incubated without or with recombinant human interferon alpha 2b (rhIFNα, 2 IU/ml, 4 hrs, 37°C, 5% CO₂). Next, samples were divided between PAXgene (PreAnalytix, 2 IU/ml, 4 hrs, 37°C recombinant human interferon alpha 2b (rhIFNα, 2 IU/ml, 4 hrs, 37°C, 5% CO₂)) and Tempus (Applied Biosystems) tubes and RNA was isolated according to the manufacturer’s protocols. cDNA was synthesized (500 ng input RNA; qScript cDNA synthesis kit) and ISG expression measured on a QuantStudio 6 Real-Time PCR instrument using a TaqMan Fast Advanced Assay. For each ISG, expression was normalized against the geometric mean of two housekeeping genes (18s rRNA and HPR1) and calculated using the formula 2^(-ΔCt). Relative gene expression is reported as the normalized expression of each ISG divided by the median of normalized expression of the same ISG in unstimulated samples. The median relative expression of all six ISGs was used to calculate the IFN score for each sample.

Results: There was no statistically significant difference in the normalized expression of any of the six ISGs in either the rhIFNα-stimulated or unstimulated samples derived from PAXgene or Tempus tubes. The greatest difference in mean normalized expression in both unstimulated and stimulated samples was observed for ISG15 (difference in mean normalized expression was 0.0034 and 0.11, respectively). Overall there was a strong correlation of the IFN score between PAXgene and Tempus tubes for both the unstimulated (R² = 0.9117, p<0.0001) and rhIFNα-stimulated samples (R² = 0.8529, p<0.0001).

Conclusion: Despite reported differences in gene expression patterns associated with samples collected in PAXgene versus Tempus tubes, our results demonstrate that 6-gene interferon scores do not differ significantly between RNA samples obtained with these two systems. These results suggest that health care and research centres can use either tubes for IFN score determination using these 6 ISGs and results can be directly compared irrespective of the RNA collection system employed.

REFERENCES:

Disclosure of Interests: None declared