sensitive proteomics methodologies overcomes these issues remains to be seen.

E. Sapey*, D. Bayley, A. Ahmad and R. Stockley#
*Dept of Medicine, University of Birmingham, , and #Queen Elizabeth Hospital, Queen Elizabeth Medical Centre, Birmingham, UK.

STATEMENT OF INTEREST

None declared.

REFERENCES

1 Rosias PP, RobroeksCM, Kester A, et al, Biomarker reproducibility in exhaled breath condensate collected with different condensates. *Eur Respir J* 2008; 31: 934–942.
2 Stockley RA, Bayley DL. Validation of assays for inflammatory mediators in sputum. *Eur Respir J* 2000; 15: 778–781.
3 Bayley DL, Abusriwil H, Ahmad A, Stockley RA. Validation of assays for inflammatory mediators in exhaled breathe condensate. *Eur Respir J* 2008; 31: 943–948.
4 Leung TF, Li CY, Yung E, Liu EK, Lam CW, Wong GW. Clinical and technical factors affecting pH and other biomarkers in exhaled breathe condensate. *Pediatr Pulmonol* 2006; 41: 87–94.

DOI: 10.1183/09031936.00088608

From the authors:

We read with great interest the letter from E. Sapey and co-workers on ''The validation of assays used to measure biomarkers in exhaled breath condensate''. Indeed, we agree with E. Sapey and co-workers that the variability of current assays can be further improved.

However, in our opinion, this does not imply that ''exhaled breathe condensate is not an ideal collection method for protein measurements''. One should be aware of the associated ambiguity. There are in fact two relatively separate methods that are involved in the technique of exhaled breath condensate. The first is the method used to collect the condensate of a patient and, subsequently, the method to analyse this condensate.

Hence, we want to highlight that the validation of an assay was not the aim of our study [1]. Moreover, the principal aim was to assess differences between the condensers, including the new glass condenser (*i.e.* to assess the reproducibility of exhaled breath condensate volume, hydrogen peroxide, 8-isoprostane and cytokine measurements using different condensers). Therefore, the coefficients of variation of the cytokines presented in our study [1] do not refer to the intra-assay variation, but to the total variability of the cytokines in exhaled breath condensate, which includes intra-assay variation, instrument variability and biological variability in healthy individuals. We hypothesised that exhaled breath condensate collection may be optimised by using a condenser with minimal adhesive properties, and as a result, this may improve the reproducibility of biomarker measurements in exhaled breath condensate. Logically, we had to use an assay to measure hydrogen peroxide, 8-isoprostane and cytokines and we acknowledged the limitations associated with the current assays. However, in order to minimise, or to equally disperse this analytical influence, we used only one type of assay for the measurement of one specific biomarker in exhaled breath condensate that was collected in different ways using different types of condenser. Using this method, we reported significant differences between different methods of condensate collection, in favour of the new glass condenser design [1].

We do not fully agree with E. Sapey and co-workers that the mean of the spiked samples in our study were in excess of measured values. In the case of 8-isoprostane, the mean concentration in exhaled breath condensate for the new condenser was 3.6 $pg \cdot mL^{-1}$, whereas the spiking concentrations were 3.9 and 7.8 $pg \cdot mL^{-1}$. For the cytokines, spiking was performed with a concentration of 10 $pg \cdot mL^{-1}$, whereas the mean values of cytokines ranged 0.7–6.3 $pg \cdot mL^{-1}$ (new condenser). Therefore, we feel that the intra-assay variation only accounts for a part of the total variability of biomarkers in exhaled breath condensate. The suggestion by E. Sapey and co-workers to define the lower limit of quantification for an assay is an interesting one.

By consequence, and in our opinion, this implicates that the potential or future value of exhaled breath condensate not only depends upon the validity of the analytical technique, but also depends upon the validity of the condensate collection technique, and both of these techniques are prone to improvement.

P.P. Rosias*# and E. Dompeling*
*Dept of Paediatric Pulmonology, University Hospital Maastricht, Care and Public Health Research Institute, Maastricht, and #Dept of Paediatrics, Maasland Hospital, Sittard, Utercht, The Netherlands.

STATEMENT OF INTEREST

None declared.

REFERENCES

1 Rosias PP, Robroeks CM, Kester A, *et al*. Biomarker reproducibility in exhaled breath condensate collected with different condensers. *Eur Respir J* 2008; 31: 934–942.

DOI: 10.1183/09031936.00101608