1,000 people [10]. Indeed, hospital admissions for drainage of dental abscesses showed the greatest increase in socially disadvantaged areas [11].

We would suggest that poor access to dental care, particularly in deprived areas of the UK where other risk factors for community acquired pneumonia coexist, is likely to increase the burden of pneumonia and place further pressure on limited healthcare resources. In addition, dental services within hospitals and long-term care institutions need to improve as they are woefully inadequate in their current form and provide little more than a service to extract loose or severely decayed teeth. Investment in General Dental Services in the UK amounts to <2% of the NHS budget, even in our own institution the funds for the entire Dental School is less than the Critical Care Unit. Oral health needs to be established as a priority not only in the community but also for hospital patients or institutionalised adults in much the same way as deep-vein thrombosis prophylaxis. Investment in promotion of oral hygiene is likely to prove an extremely cost-effective long-term measure.

M.P. Wise*, D.W. Williams[#], M.A. Lewis[#], J.G. Thomas[†] and P.J. Frost*

*Adult Critical Care, University Hospital of Wales, [#]Dept of Oral Surgery, Medicine and Pathology, Cardiff University, Dental School, Cardiff, UK, and [†]Depts of Pathology and Periodontics, West Virginia University Schools of Medicine and Dentistry, Morgantown, WV, USA.

STATEMENT OF INTEREST

A statement of interest for M.A. Lewis can be found at www.erj.ersjournals.com/misc/statements.shtml

REFERENCES

- 1 Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS. Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. *Chest* 2005; 128: 3854–3862.
- 2 Birnbaum HG, Morley M, Greenberg PE, Cifaldi M, Colice GL. Economic burden of pneumonia in an employed population. *Arch Intern Med* 2001; 161: 2725–2731.
- 3 Colice GL, Morley MA, Asche C, Birnbaum HG. Treatment costs of community acquired pneumonia in an employed population. *Chest* 2004; 125: 2140–2145.
- 4 Fry AM, Shay DK, Holman RC, Curns AT, Anderson LJ. Trends in hospitalizations for pneumonia among persons aged 65 years or older in the United States, 1988–2002. *JAMA* 2005; 294: 2712–2719.
- 5 Almirall J, Bolibar I, Serra-Prat M, et al. New evidence of risk factors for community-acquired pneumonia: a population-based study. *Eur Respir J* 2008; 31: 1274–1284.
- 6 Laheij RJ, Sturkenboom MC, Hassing RJ, Dieleman J, Stricker BH, Jansen JB. Risk of community-acquired pneumonia and use of gastric acid-suppressive drugs. *JAMA* 2004; 292: 1955–1960.
- 7 Paju S, Scannapieco FA. Oral biofilms, periodontitis, and pulmonary infections. *Oral Dis*. 2007; 13: 508–512.
- 8 Adachi M, Ishihara K, Abe S, Okuda A, Ishikawa T. Effect of professional oral health care on the elderly living in nursing homes. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; 94: 191–195.
- 9 Thomas SJ, Atkinson C, Hughes C, Revington P, Ness AR. Is there an epidemic of admissions for surgical treatment of dental abscesses in the UK? *BMJ* 2008; 336: 1219–1220.
- 10 Boulous MN, Philipps GP. Is NHS dentistry in crisis? “Traffic light” maps of dentists distribution in England and Wales. *Int J Health Geogr* 2004; 3: 10.
- 11 Moles DR. Epidemic of dental abscesses?: Dental abscesses have increased most among poorer people. *BMJ* 2008; 336: 1323.

DOI: 10.1183/09031936.00096808

Reproducibility of exhaled breath condensate markers

To the Editors:

The letter by ROSIAS *et al.* [1] highlights that individual biomarkers in exhaled breath condensate (EBC) require specific methods for optimal collection. Denominators need to be determined (such as time, total breath collection, protein, electrolytes, conductivity or other more stable markers) and, critically, researchers should consider collecting whole or global EBC, which may enhance reproducibility.

To determine the reproducibility of simple ions and more complex molecules, such as proteins and eicosanoids in different disease categories, we used the EcoScreen® (Erich Jaeger GmbH, Hochberg, Germany) to study the within-day variability of EBC biomarkers from 11 subjects with asbestosis, 14 with pulmonary fibrosis (PF) and 22 healthy normal subjects (tables 1 and 2).

Two EBC samples, obtained over exactly 10 min, were collected on the same day from each subject at 09:00 h and 13:00 h. The mean $\pm$ SD EBC volumes collected at 09:00 h and 13:00 h were similar ($1,831 \pm 498$ versus $1,831 \pm 586$ μ L). The coefficient of variation (CV) for within-day EBC volume for all subjects was 14.8%. The mean CV for within-day EBC volume was increased at 23.0% in subjects with PF, possibly due to their more severe, restrictive lung function. Patients with PF had significantly reduced forced expiratory volume in one second (% predicted) and forced vital capacity (% pred) compared with normal subjects (73.2 ± 17.7 versus 97.1 ± 14.7 and 72.9 ± 17.5 versus 98.7 ± 13.1 at 09:00 h and 13:00 h, respectively; both $p < 0.0001$, ANOVA and multiple comparison *post hoc* tests) and those with asbestosis (73.2 ± 17.7 versus 83.2 ± 14.2 ($p = 0.14$) and 72.9 ± 17.5 versus 85.3 ± 16.8 ($p = 0.09$), respectively).

TABLE 1 Demographics of normal controls and patients with asbestosis and pulmonary fibrosis

	Normal controls	Asbestosis	Pulmonary fibrosis
Patients n	22	11	14
Age yrs	46.4±17.3	71.5±5.8	60.0±11.9
Male/female	11 (50)/11 (50)	11 (100)/0 (0)	8 (57)/6 (43)
Lung function			
FEV ₁ pred %	97.1±14.7	83.2±14.2	73.2±17.7 [#]
FVC pred %	98.7±13.1	85.3±16.8	72.9±17.5 [#]
FEV ₁ /FVC %	99.5±14.5	90.0±10.4	85.3±10.7***

Data are presented as mean±sd or n (%), unless otherwise stated. Statistical significant difference between subjects with pulmonary fibrosis and normal individuals was observed. #: p<0.0001; ***: p<0.001 (ANOVA and multiple comparison *post hoc* tests).

As demonstrated in previous studies [2, 3], EBC pH was the most reproducible marker with the best overall CV for within-day variability, whether measuring the pH at the time of collection (3.4%) or after 8 weeks when stored at -80°C (1.8%). Moreover, there was no significant difference between the mean CVs (within-day) of these three groups of subjects.

Total nitrogen oxides (NO_x) showed an overall mean (range) within-day CV of 38.0 (2.4–111.2)%, while the mean CV for duplicates was 4.9%. Hydrogen peroxide demonstrated a within-day CV of >40% for all groups. A previous study [2] demonstrated that siliconised glass tubes might be better for monitoring EBC NO_x and H₂O₂ than other collecting media, such as glass, the EcoScreen® or the RTube® (Respiratory Research Inc., Charlottesville, VA, USA).

In addition, we estimated the within-day CV for more complex molecules including eicosanoids (leukotriene B₄ (LTB₄) and 8-isoprostane) and proteins (total protein, endothelin-1 (ET-1), interleukin (IL)-8, 3-nitrotyrosine (3-NT) and transforming growth factor-β₁). Excluding ET-1, all CVs for within-day variability were, in general, >40% which suggests that the EcoScreen® is not a satisfactory method of collection for these biomarkers; further refinements are required. As ROSIAS *et al.* [4] suggested, a condenser system with a silicon or glass coating is more efficient for the measurement of 8-isoprostane or albumin in EBC. However, the variation in ET-1, IL-8 and 3-NT is also likely to have been a result of technical limitations of the ELISA analysis as, although the duplicates for the standard curves showed excellent agreement, the mean CVs for the EBC duplicates in ET-1, IL-8 and 3-NT assays were as high as 19.2, 30.9 and 31.7%, respectively (data not shown), and levels were close to the detection limit of the assays. Using an ELISA Signal Amplifier (Vicgene Biotechnology, Mountain View, CA, USA) for the 3-NT assay did not improve the sensitivity (unpublished data). Apart from 8-isoprostane, there was no significant difference between the mean CVs for normal controls and patients with asbestosis or PF.

Overall, we found that reproducibility was poor and varied according to the biomarker studied when the EcoScreen® was used, but different disease groups had similar reproducibility, except for 8-isoprostane. Where samples lay well within the linear range of assays, results were more likely to be reliable. Improving the standardisation of breathing (*e.g.* by monitoring tidal or minute volume) and perhaps collecting total or global breath [1] may assist in both reproducibility and reliability. Therefore, an optimal device should be selected for each particular biomarker so as to maximise the volume of exhaled breath condensate to be collected and to analyse the mediators of interest within the linear range of each assay, which may require concentrating the sample or using signal amplification.

TABLE 2 Within-day variability of exhaled breath condensate

	Normal controls	Asbestosis	Pulmonary fibrosis	Overall	Subjects n
Volume µL	10.9	12.7	23.0	14.8 (0–112.2)	45
pH					
Bedside	2.1	4.7	4.3	3.4 (0–14.3)	47
Laboratory	2.1	1.6	1.5	1.8 (0–8.2)	46
ET-1 fmol·mL⁻¹	43.6	23.9	34.8	36.1 (2.9–86.5)	46
NO_x µM	39.5	33.9	39.5	38.0 (2.4–111.2)	38
LTB₄ pg·mL⁻¹	54.8	30.9	47.8	46.6 (1.3–132.8)	43
H₂O₂ µM	50.5	40.5	64.2	51.5 (3.4–120.0)	40
8-isoprostane ng·mL⁻¹	66.0	31.4*	56.4	54.8 (4.6–132.7)	45
Protein µg·mL⁻¹	78.6	68.5	67.5	72.9 (0–140.8)	45
IL-8 pg·mL⁻¹	83.7	70.9	59.6	73.2 (0–141.3)	41
3-nitrotyrosine ng·mL⁻¹	84.9	81.9	82.0	83.1 (0–141.3)	36
TGF-β₁ pg·mL⁻¹	116.7	63.5	90.5	96.4 (1.1–141.4)	47

Data are presented as coefficient of variation % or mean (range), unless otherwise stated. Exhaled breath condensate samples were collected at two sampling times: 09:00 h and 13:00 h. ET: endothelin; NO_x: nitrogen oxides; LTB₄: leukotriene B₄; IL: interleukin; TGF: transforming growth factor. *: p<0.05 was considered significantly different between subjects with asbestosis and normal subjects (ANOVA and multiple comparison *post hoc* tests).

S. Chow*, D.H. Yates[#] and P.S. Thomas*,[†]

*Dept of Pathology, School of Medical Sciences, Faculty of Medicine, University of New South Wales, [#]Dept of Thoracic Medicine, St Vincent's Hospital and Research and Education Unit, Workers' Compensation (Dust Diseases) Board, and [†]Dept of Respiratory Medicine, Prince of Wales Hospital, Randwick, Sydney, Australia.

STATEMENT OF INTEREST

None declared.

REFERENCES

1 Rosias PP, Jobsis Q, van de Kant K, *et al.* Global condensation: a "climate change" towards better standardisation of

exhaled breath condensate measurements. *Eur Respir J* 2008; 31: 684–685.

2 Liu J, Conrad DH, Chow S, Tran VH, Yates DH, Thomas PS. Collection devices influence the constituents of exhaled breath condensate. *Eur Respir J* 2007; 30: 807–808.

3 Prieto L, Ferrer A, Palop J, Domenech J, Llusar R, Rojas R. Differences in exhaled breath condensate pH measurements between samples obtained with two commercial devices. *Respir Med* 2007; 101: 1715–1720.

4 Rosias PP, Robroeks CM, Niemarkt HJ, *et al.* Breath condenser coatings affect measurement of biomarkers in exhaled breath condensate. *Eur Respir J* 2006; 28: 1036–1041.

DOI: 10.1183/09031936.00085408

Exhaled breath condensate is not suitable to detect EGFR somatic mutations

To the Editors:

Somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) are molecular targets of great clinical interest in lung cancer. In particular, its ability to permit clinical efficacy of specific therapies with tyrosine kinase inhibitors (*i.e.* gefitinib or erlotinib) has been stressed [1, 2].

Interestingly, our group has already shown that the exhaled breath condensate (EBC) can be a useful and noninvasive way to collect biological samples derived from high up and deep within the airways [3, 4]. EBC has been demonstrated to be informative on molecular alterations of nonsmall cell lung cancer (NSCLC) and to characterise the oxidative and inflammatory process in the airways/lung [5, 6]. In particular, we recently demonstrated an overlapping microsatellite signature from EBC and tumour tissue of the same patient showing that it already contained genetic markers potentially useful for early lung cancer diagnosis [4].

Our aim was to verify the possibility to research EGFR mutations in EBC and, therefore, to use them again as a noninvasive diagnostic tool for lung cancer. For this reason, 23 patients with histological evidence of NSCLC were enrolled in the study. As several reports have shown that the most frequent mutations are in-frame deletions in exon 19, point mutation in exon 21 (L858R) or amino acid substitution in exon 20 (T790M) which are implicated in the tyrosine kinase inhibitors sensitivity [2], exons 18–21 of EGFR were sequenced on DNA extracted from paraffin tissues of all cases and from EBC of 10 patients.

EGFR mutations in tumour tissues were analysed first. Only one patient presented with a pathological deletion in exon 19,

FIGURE 1. Sequencing of epidermal growth factor receptor exon 19 in DNA from a) exhaled breath condensate and b) tumour tissue of the same patient. The pathological deletion 745_750del is clearly evident in the tissue but not in the exhaled breath condensate.