Author's response to reviews

Title: Comparative analysis of selected exhaled breath biomarkers obtained with two different temperature-controlled devices

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Author's response to reviews: see over
1. EBC collection devices
Sampling of EBC is quite complex relying on multiple biochemical and physiochemical processes. At a given temperature breath condenser coatings affect recovery and measurement of biomarkers in EBC. It was shown that there was no difference in EBC protein concentration at a lower condensing temperature (-70°C vs. -20°C) but an increased sample volume suggesting that the lower condensing temperature not simply leads to a more efficient condensation of water vapour. One can expect that temperature alters the specific adhesion properties of a given material. Composition of the material is critical for EBC composition. Concerning ECoScreen2, a composition was investigated by FILT (FILT, Berlin, Germany) according to the temperature favoured using that device (-20°C). In the light of competition it is reasonable that there is no detailed information available concerning the specific properties, except that the material of the collection bags is composed of polyethylene. The exact composition and primary source of supply of the polyethylene plastic film are not known. A statement was inserted (page 6, line 7 ff). According to the information provided by the manufacturers this material was approved for the collection of exhaled biomarkers like prostaglandins and leukotrienes. That could be confirmed by our presented data.

So far most of the studies concerning EBC refer to the final condensate collected. Using ECoScreen2 there is the possibility to calculate the primary condensate as the collecting bags, where the condensation takes place as well, can be removed. We could not determine the composition of this “lost” portion after EBC harvest. However, we calculated the primary condensate volume according to the weight of the bags (weight after collection minus empty weight). When harvesting different portions of the calculated volume, no differences in pH or mediator concentration could be detected.

2. Variability of EBC biomarker measurements
Standardisation is an important topic. We appreciate the advice and data on repeatability are presented accordingly (methods, page 6, line 17 ff.; page 8, line 2 ff.; results, page 9, line 3 ff.; discussion, page 11, line 29 ff.; table 2, page 25).
3. Dilution effects of different collection devices

Dilution is a potential confounding factor and a subject of ongoing debate. We did not calculate dilutional effects. However, this important topic was addressed and discussed in detail (discussion, page 15, bottom and page 16, top).

4. Effects of randomized sequence of EBC collection

We chose an established design for evaluating the different devices in a comparative way. According to the reviewer’s comment, we present more data for the readers concerning interactions and possible washout effects (discussion, page 11, line 11 ff.).

Minor points

1. The text was revised accordingly (background, page 4, line 17 ff).

2. Figure 1 was deleted.

3. The questionnaire was not validated. The diseases addressed were lung, renal, hepatic, or cardiovascular disease as well as cancer (methods, page 5, line 10/11).

II.

Reviewer: Ildikó Horváth

Major points

1. Repeatability

Standardisation is an important topic. We appreciate the advice and data on repeatability are presented accordingly (methods, page 6, line 17 ff.; page 8, line 2 ff.; results, page 9, line 3 ff.; discussion, page 11, line 29 ff.; table 2, page 25).
2. cys-LT
Discussion on cys-LT results was adjusted (discussion, page 14, bottom and page 15, top).

**Minor points**

1. We hope that there are no more spelling mistakes.

2. Figure 1 was deleted. Instead, we present data according to Bland and Altman (Figure 1, page 22).

**III.**

**Comment on the report of Massimo Corradi**

The potential contamination of EBC by saliva is a central issue concerning the methodological aspects of EBC sampling. The design of the tested collection devices reliably prevents contamination of EBC through saliva. However, compared to EBC, the amount of different proteins in saliva is rather high [Gaber 2006] and during oral sampling, it can not be excluded that the exhaled breath is contaminated by the oral compartment. Comparing saliva and EBC composition in subnanogram amounts by high resolution two-dimensional electrophoresis, several proteins with very high abundance in saliva were never detected in the corresponding EBC. In addition, proteins with a low abundance in saliva could be consistently identified in EBC [Griese 2002]. One likely explanation for the detection of mediators in both matrices is that these mediators were independently released in both compartments. Metabolites of both cyclooxygenase and lipoxygenase could be detected in saliva. Interestingly, LTB₄ levels were predominatly detected compared with PGE₂ at all ages and the levels of these two mediators were not altered in saliva from smokers compared with non-smokers [Bäck 2007]. In our study we could demonstrate that various mediators known to be linked to inflammatory processes could be detected in EBC sampled by ECoScreen2. In this EBC matrix PGE₂ was predominately detected compared with LTB₄ in contrast to findings on saliva samples. Our data suggest a dominant lower airway source of the inflammatory biomarkers detected in EBC. Moreover, the observed variable changes in
biomarkers argue against EBC analysis representing a dominant saliva contamination recovered in the EBC.

References

