

Online supplement 4.

Internal Validation

Disease-specific VOCs or VOC patterns (breathprints) are usually identified in cross-sectional studies with internal validation [1,2,3]. Although internal validation is considered an adequate method to limit over fitting when training and validation sets are from the same population are used, it has implicit limitations in predicting how a model will fit in an independent population. Especially when this technique is used for feature selection for the model, as is often the case in VOCs studies. Examples of internal validation methods are: split-half, cross-validation, x-fold analyses, x-fold cross validation and bootstrapping [4-6].

External Validation

Definitive confirmation of disease-specific VOCs or VOC patterns, fit for inclusion in prospective diagnostic trials, can only be provided by validation in entirely independent patient cohorts. As breathomic profile (the “breathome”) may potentially be influenced by genetics [7], environment, and diet, these validation cohorts should be preferably multi-centre and multi-national.

Whilst there have been many exploratory studies performed in breath analysis, often with internal cross validation (usually overestimating accuracy), there have been far fewer external validation studies, due to the need for multi-centre involvement and the attendant increase in financial and administrative requirements.

External validation has been provided in single centre and multi-centre settings [8, 9-13]. This has shown external validity of breath profiles that discriminated asthma (including those with fixed airflow obstruction) from COPD by using a validation dataset from a different hospital to that used for the model generation [8]. Likewise, a discriminatory model for COPD vs. controls based on 6 VOCs (GC-MS) was externally validated with patients from a different hospital [9]. In acute respiratory distress syndrome (ARDS) GC-MS and e-Nose data have been successfully externally validated at a single site [11,12]. Recently published data

showing than diagnostic accuracy cannot be estimated precisely from the training set, not even after internal validation, external validation is recommended for such studies [13].

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