

## Defining the Optimal Maneuver for the Measurement of Hydrogen Cyanide in Breath – the Nose is Best\*

Hydrogen cyanide (HCN) is present in normal human breath at concentrations in the low parts per billion (ppb) range. HCN is also known to be produced by *P. aeruginosa*, an important respiratory pathogen, and increased levels in exhaled breath may be a biomarker of *P. aeruginosa* infection.

The mouth is a known reservoir of HCN due to the oxidation of thiocyanate by salivary peroxidase, and this source may alter exhaled breath levels. An appropriate breathing maneuver must therefore be determined to allow optimal sampling of HCN exhaled from the lower respiratory tract. This may require exclusion of the mouth reservoir of HCN and/or control of exhalation flow.

The Selected Ion Flow Tube – Mass Spectrometry (SIFT-MS) technique offers the opportunity for on-line analysis of HCN in exhaled breath at concentrations in the low ppb range. Synchronous pneumotachometer measurements of exhalation flow and volume allow exploration of the effects of these variables on HCN concentration.

The aims of this study were to:

1. Determine HCN concentrations in the oral and nasal cavities.
2. Study the effects of oral and nasal breathing on the HCN concentration in exhaled breath.
3. Study the effects of exhalation flow and volume on the HCN concentration in exhaled breath.
4. Determine the optimum breathing maneuver for the measurement of HCN concentration in exhaled breath.

### Experimental Method

The SIFT-MS instrument (Voice200®, Syft Technologies Ltd) and pneumotachometer were configured to make simultaneous exhalation measurements: a mouthpiece or nasal mask, a respiratory filter, a disposable cardboard roll and the pneumotachometer were attached in series. A side port was created in the disposable cardboard roll, 2 cm distal to the respiratory filter and, using a purpose-built adaptor, air was sampled by the SIFT-MS instrument inlet. The flow rate at the pneumotachometer was displayed on a screen during exhalation so that a subject could exhale at a target flow. The data acquired from the SIFT-MS instrument and the pneumotachometer were synchronized so that the concentration of HCN in an exhaled breath could be plotted against the fraction of exhaled volume.

Six healthy non-smoking subjects completed multiple exhalation maneuvers with permutations of 2 minutes of oral or nasal pre-test tidal breathing, an oral or nasal inhalation to total lung capacity, and then an oral exhalation of vital capacity into a mouthpiece or a nasal exhalation into a nasal mask, at a target flow of 10 or 20 L min<sup>-1</sup>. Reservoir HCN concentrations in the oral and nasal cavities were determined by direct sampling. Comparisons of oral vs. nasal inhalation and exhalation maneuvers, high vs. low flows, and direct sampling of mouth vs. nose were performed by analysis of variance and paired *t*-test.

**Results**

The mouth and nose reservoir HCN concentrations of six normal subjects were determined using SIFT-MS. The concentration sampled directly (without exhalation) from the mouth was higher than HCN sampled from the nose (mean: 66.5 vs. 23.5 ppb,  $p < 0.01$ ), as shown in Figure 1.

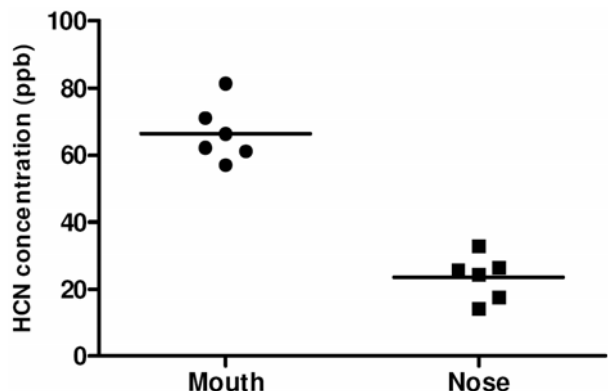


Figure 1. HCN concentrations sampled directly from the mouth and nose of six normal subjects.

For the six normal subjects, HCN concentrations were higher in oral vs. nasal exhalations (mean: 5.0 vs. 2.6 ppb,  $p < 0.01$ ; end-exhaled: 4.5 vs. 2.4 ppb,  $p < 0.01$ ). There was a decay in HCN concentration the first 20% of exhalation by volume for oral exhalations, this was not observed for nasal exhalations (6.9 vs. 3.0 ppb,  $p < 0.01$ ).

Breathing via the mouth or nose for 2 minutes prior to breath testing did not significantly affect the mean or end-exhaled HCN concentrations measured during the breath test as seen in Figure 2. Mean HCN concentrations in exhaled breath were not significantly different for the combinations of 2 min of pre-test tidal mouth or nose breathing then inhalation, followed by mouth or nose exhalation of vital capacity, at a target exhalation flow of 10 L min<sup>-1</sup>.

After 2 minutes of breathing via the nose, a single inhalation via the mouth immediately prior to breath testing gave a slightly higher nasally exhaled mean HCN concentration (2.4 vs. 2.1 ppb,  $p = 0.01$ ) than a nasal inhalation. There was, however, no difference in end-exhaled HCN concentration with the different inhalations (Figure 3).

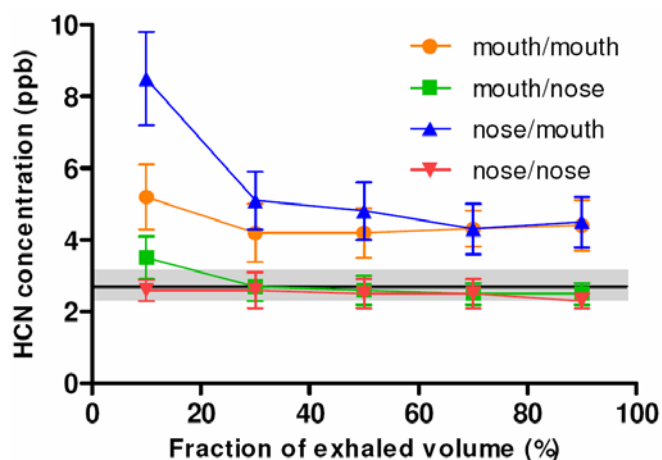


Figure 2. Mean (SE) HCN concentrations in exhaled breath for the four permutations. Maneuvers are described as (tidal breathing, inhalation)/(exhalation). Mean (SE) ambient HCN concentration is given as black line (grey area).

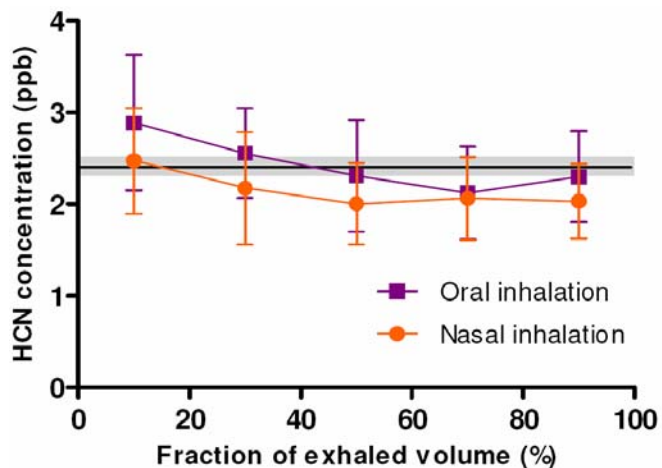


Figure 3. Mean (SE) HCN concentrations in exhaled breath for 2 min of pre-test tidal breathing via the nose, then inhalation to TLC via the nose, followed by exhalation of vital capacity, at a target exhalation flow of 10 or 20 L min<sup>-1</sup>, via the nose. Mean (SE) ambient HCN concentration is given as black line (grey area).

Target flows of 10 and 20 L min<sup>-1</sup> with nasal pre-test breathing and exhalations via the nose, gave significantly different mean HCN concentrations of 2.1 and 1.8 ppb respectively (p=0.05). There was no difference in end-exhaled HCN concentration with the different flow rates as shown in Figure 4.

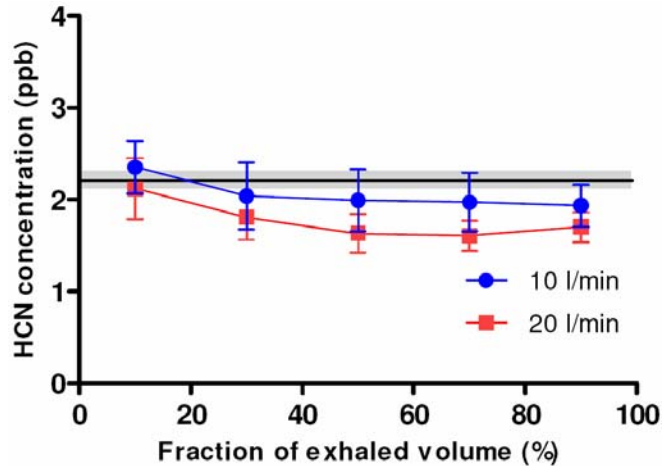


Figure 4. Mean (SE) HCN concentrations in exhaled breath for 2 min of pre-test tidal breathing via the nose, then inhalation to TLC via the mouth or nose, followed by exhalation of vital capacity, at a target exhalation flow of 10 L min<sup>-1</sup>, via the nose. Mean (SE) ambient HCN concentration is given as black line (grey area).

## Conclusion

The mouth reservoir of HCN can contaminate breath maneuvers causing decay in the first 20% of the breath. Nasal inhalation and exhalation limits this contamination, allowing improved sampling of the lower airways. Pre-test breathing does not alter the contamination effect; consistent results were achieved with both nasal and oral pre test breathing.

An optimized maneuver of nasal inhalations and exhalation with a flow rate between 10 and 20 L min<sup>-1</sup> will give reproducible end exhalation HCN concentrations. This optimized maneuver can now be used to determine the utility of exhaled HCN as a biomarker of *P. aeruginosa* in the lower airways. The SIFT-MS technique provides a very useful means for analyzing HCN on breath.

An appropriate breathing maneuver must be determined to allow optimal sampling of any VOC of interest exhaled from the lower respiratory tract. This may require exclusion of the mouth reservoir, and/or control of exhalation flow.

For more information about the unique SIFT-MS technology or this research, please contact your nearest Syft Technologies office or visit [www.syft.com](http://www.syft.com).

\* Adapted from a poster presented by J. Dummer, M. Storer, R. Finn, J. Scotter, C. Frampton, M. Swanney and M. Epton at The Thoracic Society of Australia and New Zealand annual scientific meeting, Brisbane, Australia, 19-24 March 2010.

Ethics approval for this study was granted by the Upper South A regional ethics committee, Ministry of Health, New Zealand (URA/06/03/023).