In vitro and *in vivo* functional residual capacity comparisons between multiple breath nitrogen washout devices.

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Online supplement

Description of MBNW devices:

Custom built in-house device (WIMR)

WIMR measures flow via a standard mesh-type pneumotachograph (Hans Rudolph® Inc, Shawnee, KS, USA, model no. 3700) and pressure transducer (Sensortechnics GmbH, Puchheim, Germany, model no. HCLA02X5EB) (3).

N_2 is measured directly via a side-stream N_2 analyser (Medgraphics Corporation, St Paul, MN, USA, model no. 762033-001). A fixed orifice valve (Bird Precision, Waltham, MA, USA, Ruby type model no. RB82453) was incorporated which served to improve the linearity response of the N_2 analyser. The pre-capillary dead space of the set up, which comprised the mouthpiece (Jaeger silicon adult size), bacterial filter (Respigard II 303E), fixed orifice valve attachment and the pneumotachograph flowhead, was 35 ml. The post-capillary deadspace was 17.67 ml.

Daily calibration and verification of gas and flow signals was performed as per a standardised protocol for the in-house device (3). Flow verification was performed using a 1-L Hans Rudolph® calibration syringe connected to the flow head via a standard bacterial filter. Verification was performed over five full syringe strokes and measured within ± 3% accuracy, respectively. A two-point N_2 calibration was performed by blocking the expiratory port in order to direct all bias flow gas to the N_2 analyser, with room air and 100% O_2 gas as reference points.

A fixed delay of 50 ms was used to align the flow and N_2 channels. This delay was calculated by injecting a bolus of 100% O_2 gas using a syringe attached to the entrance of the fixed orifice valve (i.e. the gas measurement point), resulting in a step increase in N_2. The delay was then defined as the difference between time at 50% of the step change in flow and 50% of the step change in N_2.

ECO MEDICS AG Exhalyzer® D device (EM)

EM measures N_2 indirectly via a main-stream infra-red CO_2 sensor (CapnostatH 5®, Respironics Novametrix LLC, Wallingford, CT, USA) and a side-stream laser oxygen sensor (Oxigraf, Inc, Mountain View, CA, USA) (2). Since the concentrations of both O_2 and CO_2 are obtained, the concentration of N_2 is then simply calculated using Dalton's law (see Equation 1).

$$f_{N2} + f_{O2} + f_{CO2} + f_{H2O} + f_{Ar} = 1 \qquad \text{(Equation 1)}$$

with assumptions regarding the partial pressures of water vapor and Argon (2). The pre-capillary deadspace of the setup, which comprised the mouthpiece (VacuMed thermoplastic adult size), bacterial filter (Respigard II 303E), and half the internal volume of the ultrasonic flowmeter with a dead space reducer insert (adult size #3), was 58 ml. The post-capillary deadspace was 22 ml.

Calibration of flow and gas channels were performed daily, as outlined below. Flow was calibrated as per manufacturer's guidelines for the adult set up, using a 1-L Hans Rudolph® calibration syringe connected to the flow head via a standard bacterial filter. Ten full syringe strokes were performed and measured within ± 3% accuracy. Two-point gas channel calibration of the O_2 sensor and zero calibration of the CO_2 sensor were conducted as per manufacturer's guidelines and as previously described (2).

Synchronization of the flow and gas channels was performed monthly, again as previous described (2). The delays between the flow and respective gas channels were calculated and verified based on the 50% rise time in the step response in both gases seen when post-capillary dead space was reinspired,

and averaged over 10 breaths in a typical human subject. These delays were used to synchronise the gas channels to the flow channel.

ndd EasyOne Pro® LAB device (ndd)

The ndd measures N_2 indirectly via a side-stream ultrasonic transducer for sampling of side-stream molar mass (MMss), with an additional infra-red carbon dioxide (CO_2) analyser (ndd Medizintechnik AG, Switzerland). The pre-capillary deadspace of the setup, which comprised the spirette, FRC barriette, and half the internal volume of the ultrasonic flowmeter, was 24.75 ml. The post-capillary deadspace was 15.85 ml.

The previous clinical software ("ndd old") used the concept of a prototype expirogram, derived from the shape of the molar mass versus expired volume curve in the early breaths of the washout (1). The expired N_2 volume for each breath is then determined by scaling the prototype expirogram to match the end-expiratory N2 concentration for that breath, and integrating the flow with the scaled expirogram.

In the updated software ("ndd new"), the N_2 concentration is determined by solving Dalton's law (Equation 1 above) and Equation 2 below simultaneously for the respective fraction of N_2 and O_2, i.e. f_{N2} and f_{O2}:

$$f_{N2}.MM_{N2} + f_{O2}.MM_{O2} + f_{CO2}.MM_{CO2} + f_{H2O}.MM_{H2O} + f_{Ar}.MM_{Ar} = MM \quad \text{(Equation 2)}$$

where MM is the molar mass measured in the side stream

as all other quantities are either measured (f_{CO2} and f_{H2O}), known (molar masses of all the gas constituents, MM_{xx}), or assumed (f_{Ar}). The fraction of Argon is assumed to be related to the N_2 concentration ($f_{Ar} = 0.0093 * f_{N2}/0.7809$) as it is not absorbed by the body. Two Nafion sampling tubes are used to ensure the gas in the molar mass sensor has the same humidity as ambient.

The molar mass sensor was two-point calibrated automatically by software using room air and 100% O_2 as reference points. CO_2 gas sensor calibration was conducted automatically using a single point reference point, i.e. room air, prior to each trial. The calibration was manually verified prior to each testing session as follows. Flow verification was performed using a 1-L Hans Rudolph® calibration syringe connected to the flow sensor via the manufacturer's calibration adapter. One full inspiratory pump stroke was performed followed by an expiratory pump stroke at moderate speed. Flow verification was completed after three full trials were performed and measured within ± 3% accuracy.

The new software also introduces changes to the estimation of delay between the flow and gas measurement points. In ndd old, delay was calculated automatically from the time-based cross correlation between the mainstream and sidestream molar mass signals, at the start of each trial. In contrast, ndd new uses a volume-based cross correlation method to estimate the delay, which are more robust to variation in breathing patterns particularly very brief pauses in flow (personal communication with the manufacturer).

References

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