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CORRESPONDENCE

Collecting exhaled breath condensate from non-ventilated preterm-born infants: a modified method

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INTRODUCTION

Exhaled breath condensate (EBC) collection is a non-invasive, safe method for measurement of biomarkers in patients with lung disease. Other methods of obtaining samples from the lungs, such as bronchoalveolar lavage, are invasive and require anaesthesia/sedation in neonates and infants. EBC is particularly appealing for assessing biomarkers in preterm-born infants, a population at risk of ongoing lung disease.[1] Previous small studies of EBC from ventilated, preterm-born neonates identified potential biomarkers of respiratory disease.[2,3] A method for EBC collection in non-ventilated infants may allow for longitudinal monitoring of lung disease biomarkers beyond the intensive care unit and potentially identify infants most at risk of ongoing disease. However, EBC collection is more challenging in infants compared to older children and adults. The 2017 European Respiratory Society (ERS) technical standard for exhaled biomarkers in lung disease highlighted the importance of standardising EBC collection methods and recommended the development of EBC collection systems suitable for infants and preschool children.[4] To date, EBC collection in infants has used custom-made devices.[5,6] However, we are unaware of successful EBC collection in non-ventilated, preterm-born neonates and infants using a commercially available system. We aimed to adapt a commercially available system to collect EBC in non-ventilated preterm-born neonates and infants.

METHODS

Modification of a commercial system

EBC was collected using an R-Tube collection device (Respiratory Research Inc., Charlottesville, VA) adapted by reduction of equipment dead space. The mouthpiece and 'Tee' section of the R-Tube was removed and replaced with a three-dimensional (3D)-printed connector (polylactic acid plastic), with three connection points (Fig. 1). The polypropylene condensation tube of the R-Tube device was attached to one connection point. The second point connected to a blue duckbill silicone rubber valve from another R-Tube device, which served as a one-way inspiratory valve. The third connection point was attached to a neonatal nasal mask (size 11, EME Ltd, Brighton, UK) or infant mask (size 0/1, 12 mL effective dead space, Laerdal Medical AS, Stavanger, Norway). All connections were sealed tightly to prevent air leak. Room air was inspired through the duckbill valve connection and exhaled into the condensation tube through the existing one-way valve. Both duckbill valves were checked prior to and during collection to ensure they were not stuck and were opening during the breathing cycle. An insulating cover and aluminium sleeve (−20 °C) was placed around the condensation tube during collection. Care was taken to maintain the temperature throughout the collection period in neonates, with replacement of the aluminium sleeve with another cooled to −20 °C halfway through collection. New polypropylene condensation tubes were used for each collection, with connectors and duckbill valves sterilised between each use with laboratory-grade disinfectant.

Validation in preterm infants

Measurements were performed as a substudy of the Preterm Infant Clinical and Functional Outcome (PIFCO) study (ACTRN126130010627181) and approved by the Women and Newborn Health Service Human Research Ethics Committee (20130193EW), the Child and Adolescent Health Service Human Research Ethics Committee (2014083EP) and the University of Western Australia (RA/3/1/5942). Infants were eligible for the substudy if they were born <32 weeks' gestation and informed parental written consent had been obtained. EBC was obtained with the infant in the supine position. The device was handheld upwards manually at a 90° angle to the infant's face. Collections of EBC in preterm neonates were obtained at the infant's bedside in the neonatal intensive care unit, at 35–40 weeks' postmenstrual age, over 20–30 minutes during natural sleep.

Collections from preterm-born infants at 12–16 months corrected postnatal age took 15 minutes, and were obtained after lung function testing under sedation with chloral hydrate (80–100 mg/kg). All collections took place when infants were clinically stable and oxygen saturations and heart rate were monitored throughout collection. Baseline minute ventilation was measured in the infants prior to EBC collection using an ultrasonic flow meter (Ecomedics AG, Duernten Switzerland) and calculated using analysis software (Wbreath v3.2.0, Ndd Medizintechnik AG, Zurich, Switzerland). Condensate was collected using the R-Tube plunger device and stored at −80 °C. Successful collection was defined as volumes greater than 100 µL based on immuno-assay sample requirements.

RESULTS

Our modified device reduced the dead space of the system from 30 to 4 mL. EBC was successfully collected in the neonatal period via nasal mask in 14/19 attempts at mean (SD) age of 35.8 (1.7) weeks' postmenstrual age. Three of these neonates met the criteria for mild chronic lung disease defined as requiring supplemental oxygen for at least 28 days after birth but not at 36 weeks postmenstrual age. None of the infants were receiving respiratory support or oxygen supplementation at the time of EBC collection. Insufficient EBC volume was collected in five neonates. Collection was successful in two neonates who had nasogastric feeding tubes in situ while breathing through nasal masks. EBC was collected successfully in all 32 infants at 12–16 months of age, nine of whom had a neonatal diagnosis of mild chronic lung disease. All infants remained clinically stable throughout EBC collection.

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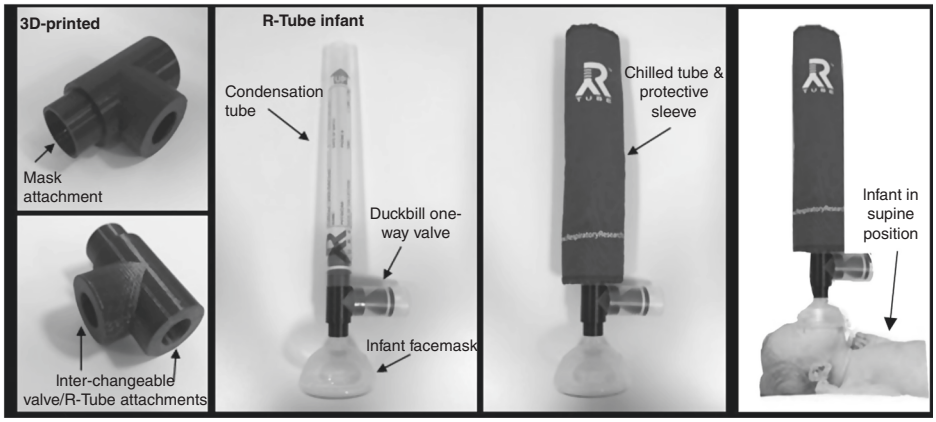

Fig. 1 Configuration of the 3D-printed connector and setup of the infant face mask, 3D-printed connector and R-Tube attachment for the collection of EBC in preterm-born infants. The 3D-printed connector model can be found on the NIH 3D Print Exchange website: https://3dprint.nih.gov/discover/3dpx-013974. The infant nasal or face mask fits into the mask attachment on the 3D-printed connector. For EBC collection in infants in the supine position, the duckbill one-way valve is placed on the perpendicular connection point on the 3D-printed connector with the duckbill pointed inwards towards the 3D-printed connector. The condensation tube is then placed on the remaining connection point, with the red 'Up' arrow pointing away from the 3D-printed connector. Both duckbill valves are prepared for use by gently squeezing the surrounding tubing to ensure they are not stuck. The chilled aluminium tube and protective sleeve can then be placed over the condensation tube ready for EBC collection. The device is then handheld and the nasal or face mask placed over the infant's nose and mouth taking care to ensure no air leak for the duration of EBC collection. The duckbill valves are checked again to ensure they open during the breathing cycle of the infant.

Table 1. Participant details and EBC volumes collected from neonates and infants.

Demographics	Neonates ($n = 14$)	Infants ($n = 32$)
Male (*n*, %)	9 (64 %)	25 (78%)
Gestation at birth (w)	28.2 ± 2.4	27.8 ± 1.3
Age at collection (w PMA/m cPNA)	35.8 ± 1.7	14.4 (13.7–15)
Weight at collection (kg)	2.5 ± 0.4	10.0 ± 1.4
EBC volume collected (μL)	100 (100–163)	425 (300–500)
Tidal volume (mL)	14.4 ± 4.7	78.4 ± 9.5
Minute ventilation (mL/min)	1030 ± 254	2395 ± 295

Parametric data are reported as mean ± SD, non-parametric data as median (IQR). *PMA* postmenstrual age of neonates, *cPNA* corrected postnatal age of infants.

Median (IQR) successful EBC collection volumes are shown in Table 1. There was a weak positive correlation between baseline minute ventilation and EBC volume collected (Spearman correlation; $r = 0.387$, $p = 0.029$). The volume of EBC collected was independent of weight or age in either subgroup.

DISCUSSION

We adapted a commercially available system to collect EBC in preterm-born neonates and infants during natural sleep by minimising dead space as recommended by the ERS.[7] Collection was well-tolerated in all infants, yielding sufficient sample for most targeted assays in 89% of studies.

The ERS/American Thoracic Society (ATS) Workforce specifications for equipment used in infant pulmonary function testing recommend equipment dead space <2 mL/kg. According to these guidelines, the 30 mL dead space in the original commercial EBC collection system is unsuitable for infants below 15 kg. With a dead space of 4 mL, and the use of a nasal mask that fits snugly over the nose (no effective dead space), our adapted collection system enables EBC collection in infants and neonates as small as 2 kg, in accordance with the ERS/ATS guidelines. While the use of a nasal mask allowed collection during quiet sleep and removed any potential salivary contamination,[8] some studies suggest variability of certain biomarkers between nasal and mouth breathing, which may confound longitudinal measurement or comparisons to older populations.[4]

Our adapted device paired with the use of a face mask with 12 mL effective dead space (size 1, Laerdal Medical AS, Stavanger, Norway) brings the total effective dead space to 16 mL, making this combination suitable for EBC collection in infants as small as 8 kg. This setup allowed successful EBC collection in all attempts made in infants aged 12–16 months.

The small tidal volume of neonates resulted in collection times of approximately 20 minutes to produce sufficient EBC volumes for downstream analyses, such as with targeted enzyme-linked immuno-assays and metabolomics previously used in EBC analysis, which typically require a minimum sample volume of 50–100 μL.[9,10] Further analyses should investigate if longer collection times increase risk of metabolite oxidation or alterations in sample pH.[4] Collection time in the 12–16-month-old infants is similar to that in adults. Although infants generate smaller EBC volumes, the sample yield is still sufficient for multiple assays which previous studies have conducted.[2,11–13] Minute ventilation correlates with condensate volume in term infants and older subjects.[6] We found no correlation between baseline minute ventilation and sample volume. Despite the absence of this correlation, the EBC volumes collected using our device are comparable to those reported in studies of infant EBC collection using homemade devices.[6] Cooling and maintaining the collection device at lower temperatures comparable to other studies (−40 to −80 °C) which report greater EBC volumes from ventilated neonates[2,3,13] may be an important consideration to increase EBC volume in future studies.[14] The feasibility of EBC collection in non-sedated infants beyond the neonatal stage remains unknown. However, other lung function tests such as the forced oscillation technique and multiple breath washout are achievable via face mask in sleeping infants beyond the neonatal period.[8] It is therefore likely that EBC collection is also probable during quiet sleep.

In conclusion, we demonstrate that EBC collection is feasible and can be collected safely using an adapted commercial device

in non-ventilated preterm-born neonates and infants. For a population particularly susceptible to chronic lung disease, a non-invasive approach of measuring biomarkers in the airway may allow early identification of and intervention in lung disease.

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AUTHOR CONTRIBUTIONS

R.U. collected and analysed the data, interpreted the data, drafted the initial manuscript, performed literature search, drafted the figures and approved the final manuscript as submitted. B.S. collected and analysed the data, reviewed and revised the manuscript, and approved the final manuscript as submitted. B.H. developed the design for the adapted R-Tube and 3D-printed the custom connector, reviewed and revised the manuscript; and approved the final manuscript as submitted. J.J.P. was the principal investigator of the PIFCO study, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. G.L.H. contributed to the overall study design and reviewed and approved the final manuscript as submitted. S.J.S. contributed to the overall study design, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Consent statement: This study took place as part of the Preterm Infant Clinical and Functional Outcome (PIFCO) study (ACTRN126130010627181) and was approved by the West Australian Women and Newborn Health Service Human Research Ethics Committee (20130193EW), the Child and Adolescent Health Service Human Research Ethics Committee (2014083EP) and the University of Western Australia (RA/3/1/5942). Informed parental written consent was obtained for all participants.

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