# Caution in Using Proteins Extracted from Exhaled Breath Condensate of Intubated Patients for Pediatric ARDS Studies

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**Research note**

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# Abstract

**Objective** We sought to analyze the quality of proteins extracted from the humidified moisture exchange filter (HMEF) of pediatric acute respiratory distress syndrome (PARDS) patients undergoing invasive mechanical ventilation.

**Results** Proteins were extracted from ten filters from one control and six PARDS subjects. Between 0.69-1.69 mg of protein was extracted from each. Silver stain of these extracts identified only one discrete band compared to many discrete bands in mouse BALF. Liquid chromatography- mass spectrometry of this band and a corresponding band in mouse bronchoalveolar lavage fluid identified them as human and mouse albumin respectively. Multiple other non-degraded proteins were obvious in mouse bronchoalveolar lavage fluid but not proteins extracted from HMEFs.


# 1. Introduction

The identification of subtypes and endotypes in critical care medicine in general and acute respiratory distress syndrome (ARDS) in particular has generated hope that the area of personalized medicine in critical care may be dawning. To date, subtypes and endotypes in ARDS have been defined by injury mechanism, physiologic parameters, and serum biomarkers (1). Serum biomarker identification of hypo- and hyper-inflammatory subtypes have been well described (2; 3). Three prior publications have reported proteomic analysis of exhaled breath condensate in mechanically ventilated adults (4; 5; 6) indicating that direct proteomic assessment of the lower airways in ARDS may be possible.

# 2. Main Text

## 2.1 Methods

We sought to determine the feasibility of analyzing proteins extracted from the humidified moisture exchange (HME) filters (Pall Corp, #1602292) of intubated pediatric ARDS patients after 12 hours of use (Cincinnati Children's Hospital Institutional Review Board Approval #2017 – 1345). After cutting open the casing, the cellulose filter material was extracted from 10 HME filters from 6 PARDS and 1 control subjects. The filter material was placed in the top compartment of a 15 kDa centrifugal filter unit (Millipore, UFC900308) and 10 mL of PBST was added to extract and concentrate proteins in exhaled breath condensate.

## 2.1 Results

The total protein extracted varied from 0.1 to 1.7 mg with a noticeable discoloration of ARDS extracts compared to control (Fig. 1A). However, silver stain of these specimens (Bio-Rad, Hercules, CA) identified only weak ~ 65 kDa bands. As a positive control, these bands were compared to C57BL/6 bronchoalveolar lavage fluid with total protein extracted being 0.1 mg (Fig. 1B). Human and mouse 65 kDa bands were analyzed by MALDI-TOF and were identified as human and murine albumin respectively. Densitometry of discrete bands vs. total lane intensity as an estimate of intact, high-

abundance proteins demonstrated 4–5% intact protein for HME ventilator specimens vs. 37% for mouse BAL fluid. We concluded that, at least in our hands, the proteins extracted from the HME filters of intubated PARDS subjects were substantially degraded.

### 2.3 Discussion

Despite there being at least three previous studies assessing the protein in exhaled breath condensate of ARDS subjects, to our knowledge, this study is the first to assess whether extracted proteins are intact by evaluating them by silver stain before analysis. McNeil, *et al* digested HME and lung edema fluid proteins to show correlation between the two by Liquid Exhaled Breath Condensate Proteins Collected from HME Filters. (A) The exhaled breath condensate of intubated pediatric ARDS patients was qualitatively different than that of a control subject although the total protein extracted was comparable. (B) Loading 20 micrograms of protein per lane, exhaled breath condensate had only one distinct protein band which was human albumin by MALDI-TOF. Mouse bronchoalveolar lavage fluid was used as an airway fluid protein comparison and the corresponding band was identified as mouse albumin.

Chromatography-Mass Spectrometry (LC-MS) with correlation of several inflammatory cytokines by enzyme-linked immunosorbent assay (ELISA) (5). Zhou, *et al* developed an inline gas chromatography mass chromatography device to directly analyze proteins in exhaled breath (6), and van der Zee, *et al* collected exhaled breath condensate using an exhalation limb elbow with analysis by Luminex (4). Comparison of reported analyte concentrations vs. manufacturer assay specifications showed that the McNeil study was near and the van der Zee study below the lower limit of detection for most of the assays reported.

The human airway is an environment rich in proteases and other defense-related proteins, and it is perhaps not surprising that proteins collected from exhaled breath condensate would show a high degree of degradation. Most quantitative assays are antibody based, and depending on assay characteristics (polyclonal vs. monoclonal capture and detection antibodies, specific assay chemistry, etc.) low levels of degradation might artificially increase and higher levels of degradation artificially decrease the amount of analyte quantified. Perhaps more importantly, differences in degradation might confound comparisons between samples. These differences could be due factors such as patient disease status, filter type, storage conditions, and protein extraction methods, none of which were examined in this study. Nonetheless, our findings indicate that protein degradation can be a substantial problem in studies of exhaled breath condensate and should be accounted for in experimental design.

## 3. Limitations

We analyzed protein content of the only HMEF approved for use at our institution. It may be that non-cellulose filters or other brands of cellulose material filters could have lesser degrees of protein degradation. We did not directly centrifuge filters as McNeil, *et al* (5) did as the safety of doing so in a bucket rotor centrifuge was uncertain.

# Abbreviations

ARDS Acute Respiratory Distress Syndrome

ELISA Enzyme-linked immunosorbent assay

HMEF Humidified Moisture Exchange Filter

LC-MS Liquid Chromatography-Mass Spectrometry

PARDS Pediatric Acute Respiratory Distress Syndrome

# Declarations

### Data Availability

Primary data in this manuscript can be obtained by contacting the primary author.

### Ethics Approval and Consent to Participate

This study was approved by the Cincinnati Children's Hospital Institutional Review Board Approval #2017-1345. Written informed consent was obtained from the subjects' parents or legal guardians.

### Consent to Publish

All authors have provided their consent to publish.

### Availability of Data and Materials

All data is available from the corresponding author upon request.

### Competing Interests

The authors declare no conflicts of interest.


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### Authors Contributions

MO and RJ designed and performed experiments and participated in manuscript revision.

BMV conceptualized the project, wrote, and revised the manuscript.


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# Figures

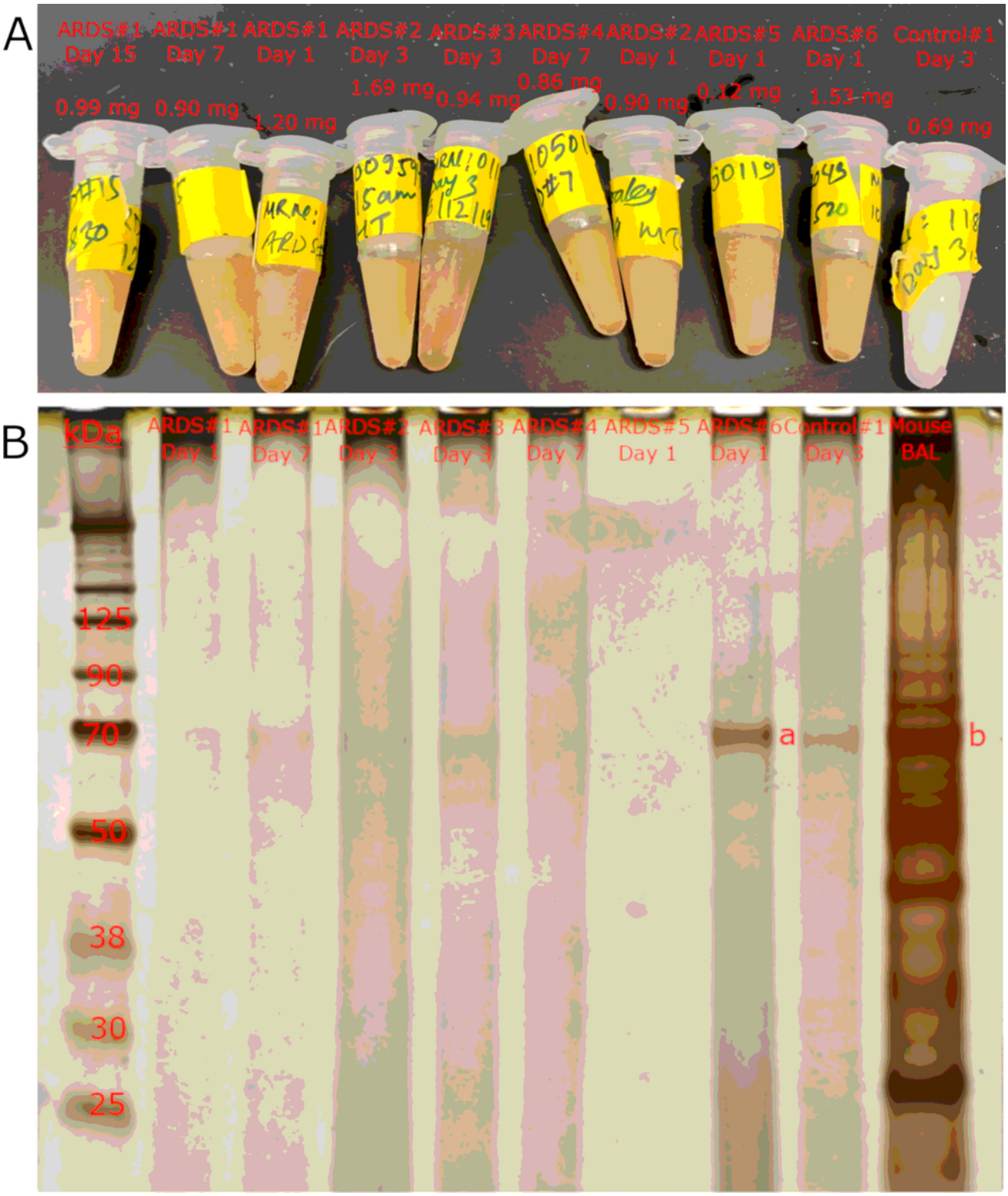


**Figure 1**

Exhaled Breath Condensate Proteins Collected from HME Filters. (A) The exhaled breath condensate of intubated pediatric ARDS patients was qualitatively different than that of a control subject although the total protein extracted was comparable. (B) Loading 20 micrograms of protein per lane, exhaled breath condensate had only one distinct protein band which was human albumin by MALDI-TOF. Mouse

bronchoalveolar lavage fluid was used as an airway fluid protein comparison and the corresponding band was identified as mouse albumin.