Development of a dried blood spot assay for the detection of GM-CSF autoantibodies to aid in the diagnosis of autoimmune pulmonary alveolar proteinosis

Eagappanath Thiruppathi[1], Joannah Kim[1], Rico Djidotor[1], Aamir Ali[1], Brenna C. Carey[2], Bruce C. Trapnell[2]

[1]TrilliumBiO, Rockville, MD, United States;[2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

INTRODUCTION

Background:

Autoimmune pulmonary alveolar proteinosis (autoimmune PAP) is a rare lung disease characterized by the abnormal accumulation of surfactant within the alveoli. This buildup impairs normal lung function, leading to symptoms such as progressive shortness of breath, fatigue, and increased susceptibility to lung infections – all impacting patients' quality of life[1]. Autoimmune PAP is caused by autoantibodies against granulocyte-macr(GM-CSF), a cytokine essential for the differentiation and function of alveolar macrophages that ophage colony-stimulating factor clear surfactant from the lungs. These autoantibodies block normal GM-CSF signaling, which leads to malfunctioning macrophages, abnormal accumulation of surfactant and reduced gas transfer between lung and blood.

Rationale:

Timely and accurate detection of GM-CSF autoantibodies is critical for diagnosis of autoimmune PAP. Conventional testing requires serum collection via venipuncture, which can be inconvenient, and limits testing accessibility in some clinical settings.

Objective:

This study was conducted to develop and validate a novel laboratory assay to detect GM-CSF autoantibodies from dried spot obtained using the ADX100 serum separator card. This approach facilitates easy transport, and storage of patient samples, potentially expanding access to testing.

METHOD

Assay Development:

A particle-based flow cytometry immunoassay was developed for the detection of GM-CSF autoantibodies using microspheres coated with recombinant GM-CSF. Serum from autoimmune PAP patients was eluted from whole blood via ADX100 serum separator cards. The eluted serum was incubated with coated particles to enable specific antibody binding. Detection was carried out using a biotin-labeled secondary antibody specific for human IgG, followed by streptavidin-phycoerythrin (PE) conjugation.

Fluorescence Detection and Quantification:

Fluorescence from PE-bound complexes was measured on a Luminex 200 instrument, with data acquired via xPONENT v4.3 software. Results were reported as median fluorescence intensity (MFI). GM-CSF autoantibody concentrations were quantified by comparing MFI values to a custom 8-point standard curve generated using monoclonal GM-CSF antibody. Values, reported in micrograms per milliliter (µg/mL), were calculated using a modified 4-parameter logistic (4PL) calibrator enhancing precision and accuracy. A clinical threshold of 8 µg/mL for GM-CSF autoantibodies was used to differentiate between positive and negative cases of autoimmune PAP2.

Sample Preparation:

For dried serum processing, an optimized assay buffer was developed to effectively release the analyte from the ADX100 card. A novel buoyancy-based extraction method enabled rapid and reproducible analyte recovery within 15 minutes, compared to the conventional one-hour incubation method.

Assay Performance:

To assess assay performance, a series of experiments were conducted using dried serum samples. Sixty serial dilutions of GM-CSF autoantibody–positive sera from autoimmune PAP patients were spiked into healthy donor serum and applied to ADX100 cards. To evaluate method agreement under simulating clinical sample collection, paired venous and capillary-derived dried serum samples were collected from 28 individuals. Precision was assessed using 40 replicate serum-derived ADX-100 samples, prepared by spiking aPAP serum into healthy serum across a range of concentrations. Additionally, the sensitivity and specificity of the assay were systematically evaluated to confirm its clinical utility.

RESULTS

A strong linear correlation was observed between GM-CSF autoantibody levels measured in serum from venipuncture and those measured using serum-derived ADX100 samples, with a Pearson correlation coefficient of 0.99 and a coefficient of variation (CV) below 13% (**Figure 1A**). Similarly, capillary-derived samples collected using the ADX100 demonstrated high concordance with paired serum samples, also achieving a Pearson correlation of 0.99 and a CV of 15% (**Figure 1B**).

Bland-Altman analysis demonstrated strong agreement between serum samples obtained via venipuncture and those measured using serum-derived ADX100 samples, with all data points falling within the predefined limits of agreement. Similarly, comparison between serum-derived and capillary-derived ADX100 samples showed that 89% of data points were within these limits. These findings indicate no significant bias between serum and ADX100-based measurements of GM-CSF autoantibody levels (**Figures 2A** and **2B**).

Further validation of assay performance was supported by a comparative correlation study between ADX100 and serum samples, which yielded an area under the curve (AUC) of 1.0—corresponding to 100% sensitivity and specificity for GM-CSF autoantibody detection (**Figure 3**). The lower limit of quantitation (LLOQ) for GM-CSF autoantibodies was determined to be 0.98 ng/mL. Precision assessments showed strong reproducibility, with an inter-run CV of 6.2% (n = 20), and high repeatability, with an intra-run CV of 2.4% (n = 60). Variability analyses across punch-to-punch and card-to-card replicates produced CVs of 5.88% and 14.97%, respectively, supporting the assay's robust analytical precision.

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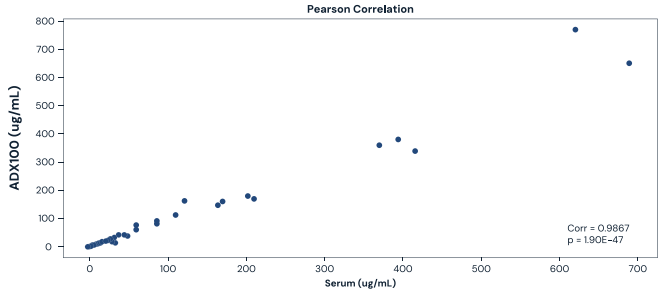
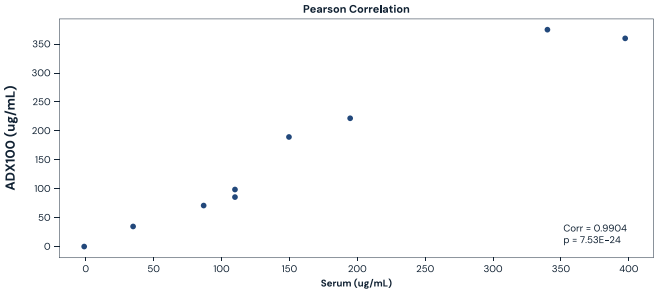
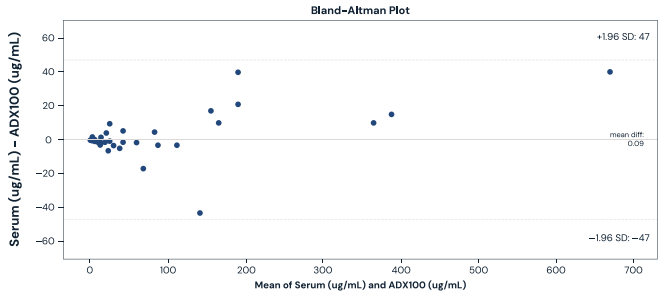


(A) Pearson correlation between GM-CSF autoantibody levels measured in serum from venipuncture and serum-contrived ADX100.

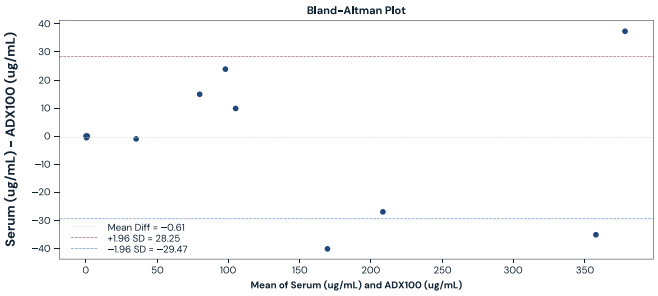


(B) Pearson correlation between serum from venipuncture and capillary-derived ADX100 samples.

Figure 1. Correlation Analysis Between Serum and ADX100 Samples



(A) Serum-contrived ADX100 vs. Serum from venipuncture



(B) Capillary-derived ADX100 vs. Serum from venipuncture

Figure 2. Bland-Altman Analysis of Serum and ADX100 Samples

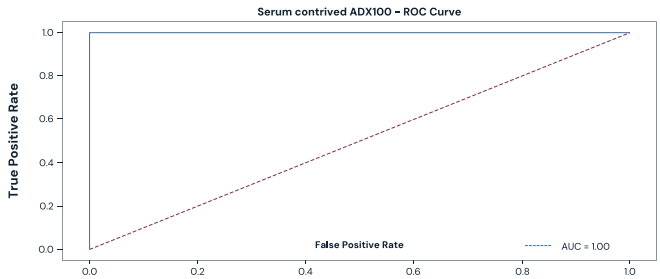


Figure 3. Receiver Operating Characteristic (ROC) Curve for GM-CSF Autoantibody Detection

CONCLUSION

Our particle-based flow cytometry immunoassay, incorporating a custom standard curve and calibrators, demonstrates high precision and sensitivity for the detection of GM-CSF autoantibodies in dried blood spot samples. Use of the ADX100 serum separator card ensures reliable recovery and accurate quantification. This approach offers a practical and convenient tool to help rule-out or confirm autoimmune PAP, representing a significant advancement in the detection of this rare and serious lung disease.

ACKNOWLEDGEMENT

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The Association of Pulmonary Advanced Practice Providers (APAPP), Nashville, Tennessee June 26 - 28, 2025
The European Respiratory Society (ERS) Congress, Amsterdam, Netherlands, September 27 - October 1
The American College of Chest Physicians (CHEST) 2025 Annual Meeting, October 19 – October 22, Chicago, Illinois