Development and evaluation of in vitro ciliary transport assays under air–liquid interface and submerged conditions for the assessment of (muco-)ciliary activity

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1. Abstract

Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterized by impaired ciliary motility, leading to chronic respiratory symptoms. Although more than 60 genes have been associated with PCD, genetic confirmation is possible in only about two-thirds of patients, making diagnosis challenging and highlighting the need for additional functional diagnostic approaches. This study evaluated two in vitro particle transport assays under air–liquid interface (ALI) and submerged conditions using primary human nasal epithelial cells (hNECs). Particle transport was analyzed by live imaging and tracking using FIJI TrackMate. Transport velocities were compared, and reference ranges were established using samples from healthy donors. PCD samples showed absent or severely reduced directed particle transport, whereas non-PCD samples demonstrated measurable transport. Both assays effectively identified functional impairment of mucociliary clearance (MCC) and showed good agreement with established diagnostic classifications. These findings support the potential of particle transport assays as complementary functional tools for PCD diagnosis, particularly when integrated with genetic and established diagnostic methods.

2. Introduction

PCD is a rare genetic disorder characterized by impaired motile cilia function. In the respiratory tract, coordinated ciliary beating drives MCC, removing mucus, pathogens, and inhaled particles from the airways. Impaired MCC can therefore lead to recurrent respiratory infections and progressive chronic lung disease. PCD diagnosis remains challenging, as no single diagnostic method reliably identifies all affected individuals. Current approaches include nasal nitric oxide measurement, high-speed video microscopy, transmission electron microscopy, immunofluorescence, and genetic testing, but do not always directly assess cilia-driven transport function. Particle-based in vitro transport assays provide a functional approach by measuring particle transport on differentiated human nasal epithelial cell cultures. This study aimed to optimize transport assays under air–liquid interface (ALI) and submerged conditions and evaluate their potential for functional PCD detection. [1]

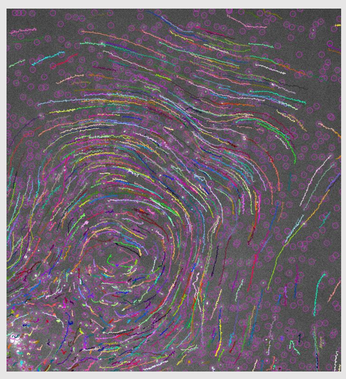


Figure 1 Sumerged Transportassay (Obrinja, 2026)

3. Aims and leading questions

Aim 1: Evaluation & optimization of fluorescence particle-based ciliary transport assays under ALI and submerged conditions.

Optimization parameters:

- Washing interval
- Incubation interval
- Particle concentration
- Transwell format

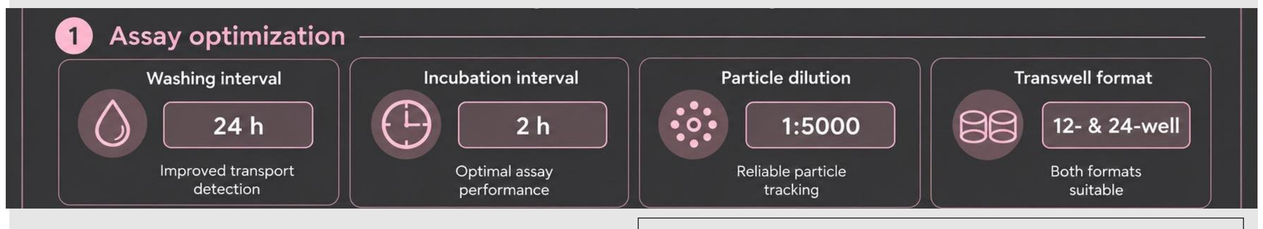
Aim 2: Evaluation of transport assay performance under ALI and submerged conditions for PCD identification

- Can PCD cases be detected using the in vitro ciliary transport assays?

4. Material and Methods

hNECs were obtained by nasal brushing from healthy donors and patients undergoing diagnostic evaluation for suspected PCD. Cells were expanded and differentiated in Transwell inserts under air–liquid interface (ALI) culture conditions.
Two fluorescence particle-based in vitro ciliary transport assays were evaluated: an ALI assay, using a minimal volume of particle suspension on the apical surface, and a submerged assay, using a larger liquid volume. Assay conditions were systematically optimized, including washing and particle incubation intervals, particle concentration, and Transwell insert size. Fluorescent 2 µm particles were applied to differentiated cultures and their cilia-driven transport was recorded using an inverted Olympus IX73 microscope equipped with a TRITC fluorescence filter and a high-speed camera at 30 frames per second. Particle trajectories and transport velocities were subsequently quantified using FIJI TrackMate. The optimized assays were then applied to healthy donor and diagnostic patient samples to evaluate their ability to detect functional impairment associated with PCD.

5. Results



12-well: Comparable transport under ALI and submerged conditions.
24-well: Higher transport velocities under submerged conditions.

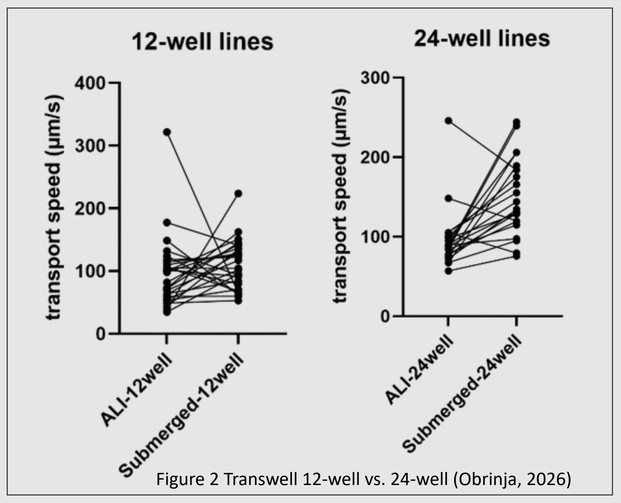


Figure 2 Transwell 12-well vs. 24-well (Obrinja, 2026)

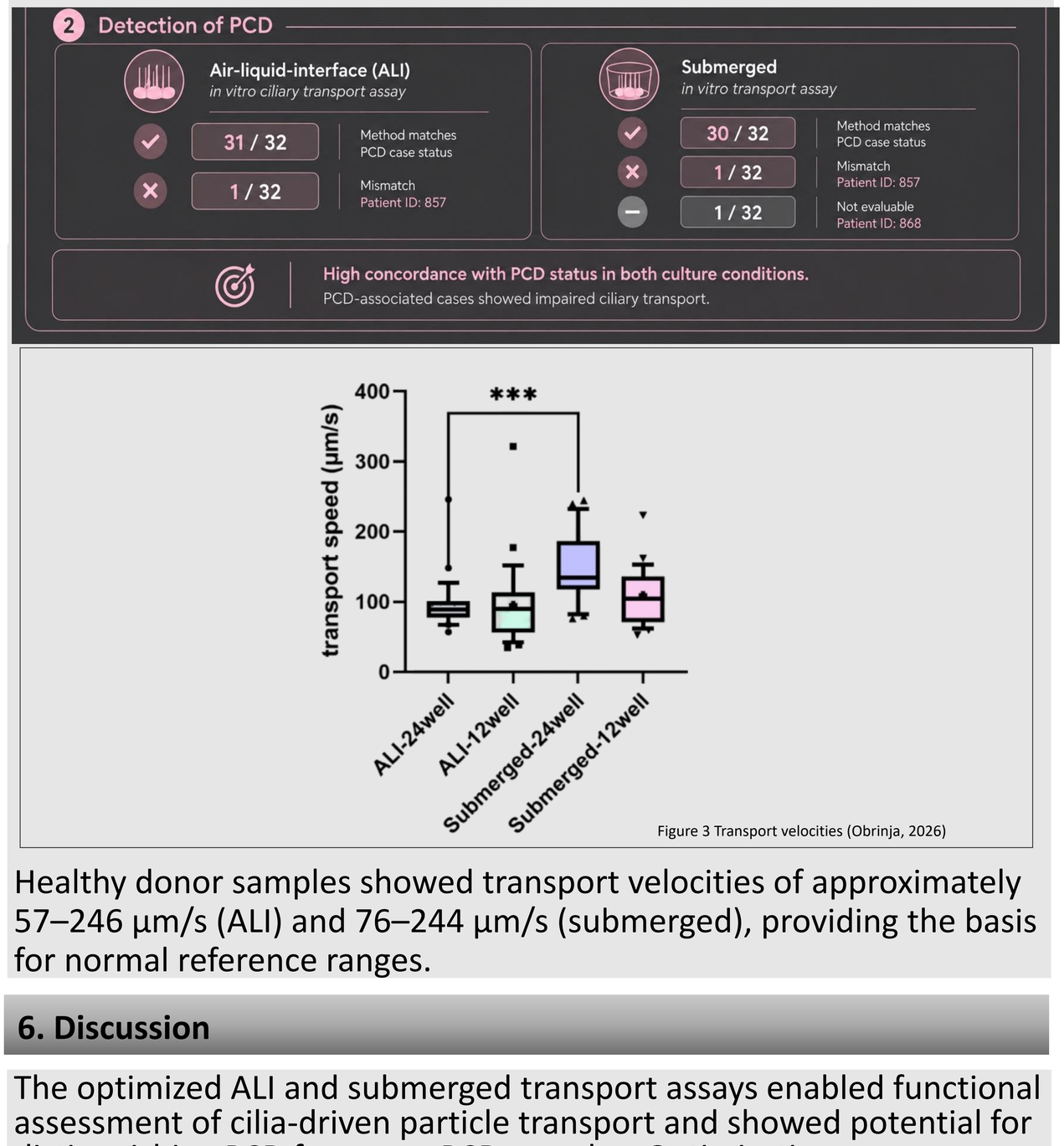


Figure 3 Transport velocities (Obrinja, 2026)

Healthy donor samples showed transport velocities of approximately 57–246 µm/s (ALI) and 76–244 µm/s (submerged), providing the basis for normal reference ranges.

6. Discussion

The optimized ALI and submerged transport assays enabled functional assessment of cilia-driven particle transport and showed potential for distinguishing PCD from non-PCD samples. Optimization demonstrated that assay conditions, including washing interval, incubation time, particle concentration, and Transwell format, substantially influence assay performance. The transport assays provide a direct functional readout of (muco-)ciliary activity, complementing established diagnostic methods that primarily assess ciliary structure, beating patterns, or genetic alterations. However, assay reliability strongly depends on the quality and differentiation of ALI cultures, representing an important limitation. Overall, the findings support the use of particle-based transport assays as a complementary functional approach in PCD diagnostics. Further standardization and validation in larger patient cohorts are required before implementation into routine diagnostic workflows.

Refrences
[1] Shoemark et al., 2025
Figures
Figure 1 Obrinja, N. (2026). *Submerged Transportassay, DBMR*
Figure 2 Obrinja, N. (2026). *Transwell 12-well vs. 24-well, DBMR*
Figure 3 Obrinja, N. (2026). *Transport velocities, DBMR*