



## Original Article

# Repeatability and reproducibility of the Forskolin-induced swelling (FIS) assay on intestinal organoids from people with Cystic Fibrosis<sup>☆</sup>

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## ARTICLE INFO

## Keywords:

Patient-derived intestinal organoids  
Cystic fibrosis  
Precision medicine  
Forskolin-induced swelling assay  
Repeatability  
Reproducibility

## ABSTRACT

**Background:** The forskolin-induced swelling (FIS) assay measures CFTR function on patient-derived intestinal organoids (PDIOs) and may guide treatment selection for individuals with Cystic Fibrosis (CF). The aim of this study is to demonstrate the repeatability and reproducibility of the FIS assay following a detailed Standard Operating Procedure (SOP), thus advancing the validation of the assay for precision medicine (theranostic) applications.

**Methods:** Over a 2-year period, FIS responses to CFTR modulators were measured in four European labs. PDIOs from six subjects with CF carrying different *CFTR* genotypes were used to assess the repeatability and reproducibility across the dynamic range of the assay.

**Results:** Technical, intra-assay repeatability was high (Lin's concordance correlation coefficient (CCC) 0.95–0.98). Experimental, within-subject repeatability was also high within each lab (CCCs all >0.9). Longer-term repeatability (>1 year) showed more variability (CCCs from 0.67 to 0.95). The reproducibility between labs was also high (CCC ranging from 0.92 to 0.97). Exploratory analysis also found that between-lab percentage of agreement of dichotomized CFTR modulator outcomes for predefined FIS thresholds ranged between 78 and 100 %.

**Conclusions:** The observed repeatability and reproducibility of the FIS assay within and across different labs is high and support the use of FIS as biomarker of CFTR function in the presence or absence of CFTR modulators.

## 1. Introduction

Cystic Fibrosis (CF) is a life-shortening autosomal recessive disease [1,2] caused by variants of the *CF transmembrane conductance regulator* (*CFTR*) gene [2]. More than 2100 *CFTR* variants have been reported (<http://www.genet.sickkids.on.ca>), of which 719 (34 %) are confirmed as CF-causing (<http://cftr2.org>) [3]. The *CFTR* variants have been grouped into seven classes, according to the mechanism by which CFTR production and function are disrupted, which impacts on the individual disease liability and potential benefit of CFTR modulating

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<https://doi.org/10.1016/j.jcf.2024.04.014>

Received 25 January 2024; Received in revised form 5 April 2024; Accepted 26 April 2024

Available online 14 May 2024

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pharmacotherapy (CFTR modulators) that target conformational defects of the CFTR protein [4].

Many people with CF (pwCF) carry (ultra-)rare *CFTR* variants that remain uncharacterized in terms of CFTR function and response to CFTR modulators. This hampers individual disease classification and also limit their potential to benefit from CFTR modulators as these are mostly available for pwCF who carry *CFTR* variants for which a therapeutic benefit has been defined. Clinical trials that include pwCF with predefined *CFTR* variants has been the standard route to identify variants that are responsive to CFTR modulators [5]. Additionally, the drug manufacturer has used *in vitro* functional testing of rare CFTR variants that are introduced as cDNA in fisher rat thyroid (FRT) cells [6]. This *in vitro* approach has been instrumental to stimulate drug access for pwCF with predefined rare variants in the United States (US), but has not been accepted in the European Union and cannot be used for all pwCF [7]. The CF community continues to develop novel *in vitro* models to identify individuals who may benefit from CFTR modulators [8–11], since clinical trial designs are underpowered and thus unsuitable for rare *CFTR* variants due to the low number of individuals [12,13].

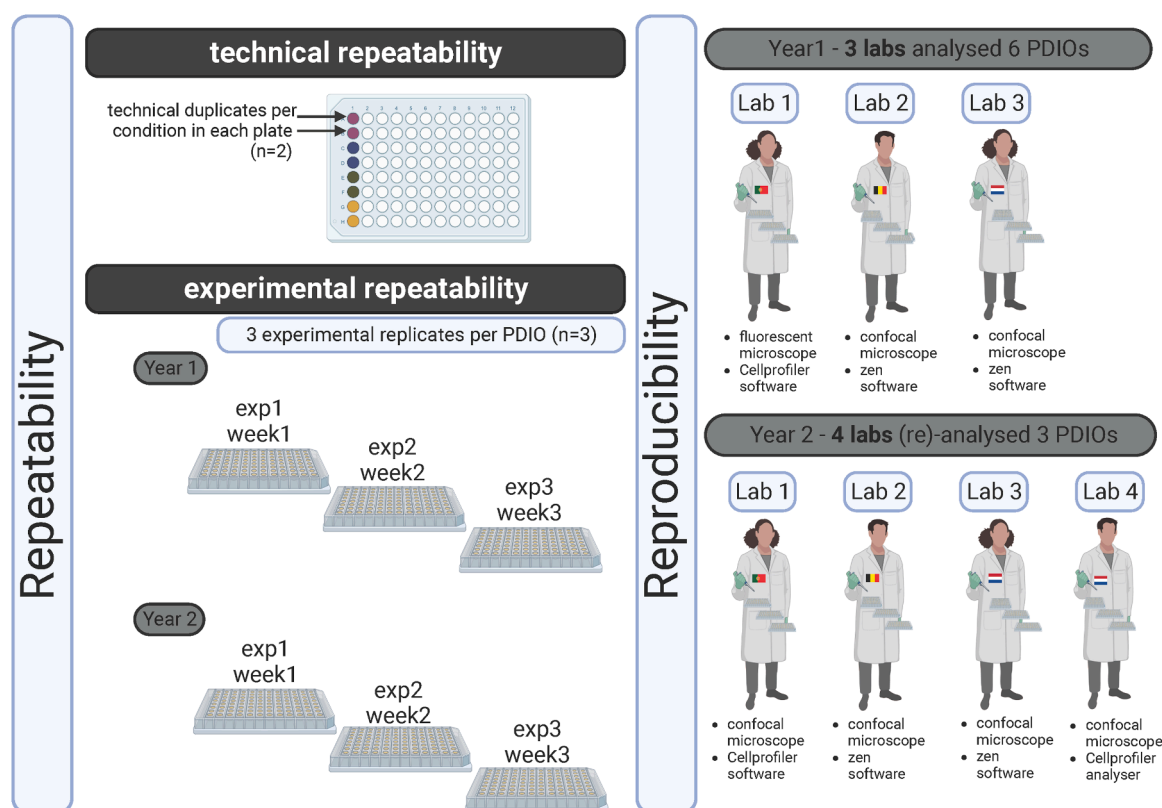
A widely used model in CF research that can identify any individual with CF who could benefit from CFTR modulators is the patient-derived intestinal organoid (PDIO) model [8]. Both residual CFTR function and CFTR function restoration after treatment with modulators in PDIOs can be quantified in the unique individual's genetic background with the forskolin-induced swelling (FIS) assay [14–16]. Forskolin induces CFTR-dependent fluid secretion into the organoid lumen that leads to a rapid increase of the whole organoid area that can be quantified by time-lapse live cell microscopy [15,17,18]. Recent studies show an inverse correlation between residual CFTR function measured by FIS and the individual's disease in the lungs, pancreas and liver [19]. Moreover, FIS measured after addition of CFTR modulators has already been shown

to correlate with clinical response on population and individual level, and multiple labs have successfully used the assay to inform on individual treatment decisions [20,21]. This is particularly important for pwCF who do not have access to treatment but carry *CFTR* variants that might responds to treatment. Currently, patient-derived organoids are being explored for precision medicine across many therapeutic domains, yet, as for CF, mostly in single centre studies. The European Medicines Agency (EMA) has expressed interest in the use of the organoid FIS assay as an *in vitro* assay for individual therapeutic selection, but a more precise quantification of the technical properties of the FIS assay such as repeatability (measurements in the same lab with the same equipment, conditions and operator) and reproducibility (measurements in a different lab with different equipment by different operators) is required for its approval by regulatory agencies [22,23]. Here, we quantify the repeatability and reproducibility of FIS across four laboratories using a European CF Society (ECFS)-endorsed standard operating protocol (SOP) [17] and PDIOs from six reference individuals with different *CFTR* genotypes and CFTR function levels upon various CFTR modulator treatments (Fig. 1).

## 2. Materials and methods

### 2.1. Organoid cultures

Intestinal organoids from six pwCF with varying *CFTR* genotypes (Table 1) were selected and prepared at the Foundation Hubrecht Organoid Biobank. These genotypes were chosen to span the entire dynamic range of the assay from no CFTR function to maximal CFTR function, which is associated with variable consequences on CF disease like R117H-7T. Preparation was done following the SOP [17] and transferred to the three academic laboratories and one commercial lab



**Fig. 1.** Experimental design. Illustration of the experimental design to test technical and experimental repeatability and reproducibility of the forskolin-induced swelling (FIS) assay in four different labs using patient-derived intestinal organoids (PDIOs) from six reference patients (figure created with BioRender.com).

**Table 1**

CFTR genotypes and variant classes of the six patient-derived intestinal organoids (PDIOs) analysed.

Reference PDIO	CFTR genotype	Class Variants
1	G542X /G542X	Class I /Class I
2	F508del/R1162X	Class II /Class I
3	F508del/F508del	Class II /Class II
4	F508del/G551D	Class II /Class III
5	F508del/S1251N	Class II /Class III
6	F508del/R117H-7T	Class II /Class IV

participating in the HIT-CF Europe project, namely, the CF Research Labs of UMCU (UMCU), University Leuven (KUL), University of Lisboa (ULIS) and the HUB organoid B.V. lab.

The six PDIO cultures were expanded and cultured according to the SOP [17]. Reagents were purchased by the UMCU and shipped to the three other sites or ordered by the sites using the same brand, supplier and catalogue number. Due to the limited stability of Wnt-3a, Wnt-3a conditioned media was produced at each site using identical reagents and Wnt-3a producing cell line [17]. FIS assays were performed for all six PDIOs in year 1 by the three academic labs and repeated for three PDIOs in year 2 by all four labs.

2.2. Forskolin-induced swelling (FIS) assay to test functional rescue by CFTR modulators

The protocol to assess CFTR function and rescue by CFTR modulators tezacaftor (VX-661) and ivacaftor (VX-770) in PDIOs was standardized among the four laboratories, as detailed in the SOP [17]. Briefly, three weeks after thawing, the PDIOs were tested for the following experimental conditions (in duplicate): 1) non-treated (forskolin alone); or treated with 2) VX-770 (3  $\mu$ M) alone; VX-661 (3  $\mu$ M, 24 h) alone; or VX-661 (24 h) plus VX-770 (3  $\mu$ M each). Due to different mode of actions the corrector VX-661 was incubated for 24 h and the potentiator VX-770 and forskolin were added acutely just before the FIS measurements. The swelling of the organoids was imaged by confocal microscopy (one picture per well each 10 min for 1 hour) during CFTR activation by forskolin (8 different concentrations, 0.008–5  $\mu$ M). In year 1 at ULIS a widefield microscopy was used instead. The total surface area of the organoids was measured using the Zen software (Zeiss) or the Cell Profiler Analysis Tool [24]. The area under the curve (AUC) of the time zero-normalized organoid swelling over one hour was calculated for each forskolin concentration. The experiments were repeated three times in three different weeks using the same thawed cultures [17].

2.3. Statistical analyses

The technical repeatability was assessed by the agreement between duplicate measurements 1 and 2 (combining all samples/experiments/conditions). The experimental repeatability was assessed within each lab by comparing the AUC values of experiment 1 versus 2 and 3, and 2 versus 3, for all samples/conditions, after averaging the results of the two duplicate measurements. Three independent experiments were performed with the identical cultures, upon weekly passaging and testing of the identical cultures. For comparisons among labs (reproducibility) or between different years in the same lab (repeatability over time), the mean of the three experimental replicate measurements was used (Fig. 1). Internal agreement was assessed, as no gold standard or true reference sample exists.

Agreement was measured as Lin’s Concordance Correlation Coefficient (CCC), and Pearson’s correlations are also provided (see figures), CCC values were chosen since they reflect on the agreement and not only on linearity. The 95 % limits of agreement were estimated using a Bland-Altman type analysis allowing for a non-constant mean and variance of the differences [25]. Trends over time measured in the UMCU lab were assessed using Spearman correlation between AUC and time, as well as

with a Mann-Kendall test for monotonic trend comparing AUCs in the same subject over different years. In an additional analysis reproducibility between labs as percentage of agreement for response to modulators was estimated for potential AUC cut-off values and forskolin concentrations, along with 95 % confidence intervals. No outliers’ analyses have been performed; all variation was seen as natural variation.

Analyses were performed using R version 4.2.2 [26], with use of the functions CCC(), binconf() and notrend\_test() from the packages DescTools (version 0.99.47) [27], Hmisc (version 5.1–0) [28], and funtimes (version 9.0) [29], respectively.

2.4. Study approval

The study was approved by the ethical committee at University Medical Center Utrecht (UMCU; TcBio #14–008) and each participant gave written informed consent at Wilhelmina Children’s Hospital (WKZ)-UMCU.

2.5. Data availability

All relevant data and reference PDIO lines are available on request from the corresponding author.

3. Results

3.1. Forskolin-Induced swelling assay repeatability within each lab

To assess FIS assay repeatability per lab, a comparison of technical duplicates (two wells of the same condition within the same plate) and between the experiment replicates (average values of duplicates of organoids from the same individual cultured and analyzed at three distinct weeks) was performed (Fig. 1).

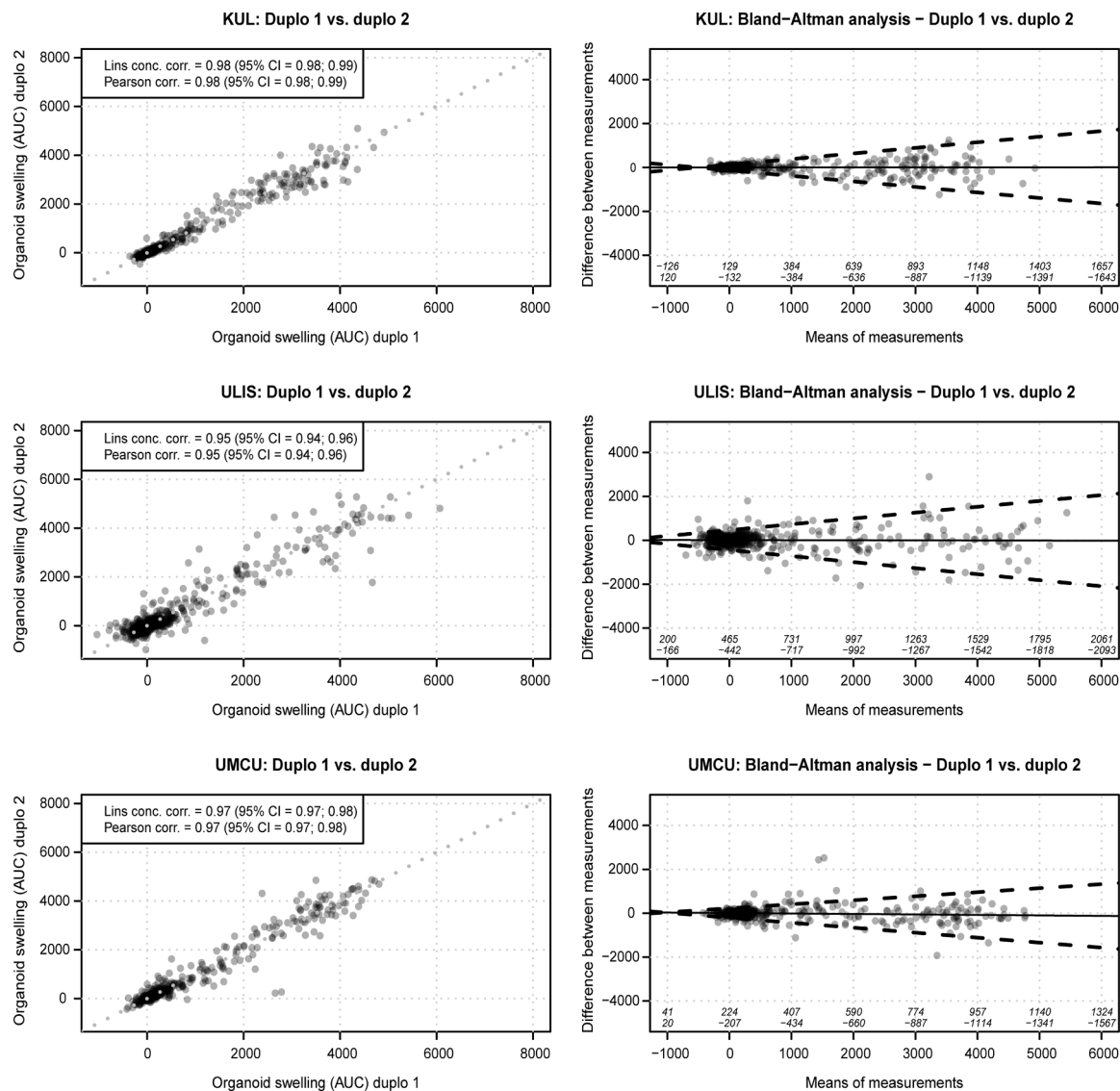
The Lin’s concordance correlation coefficient (CCC) between duplicates on the same plate, technical repeatability, ranged from 0.95 in the ULIS lab to 0.98 in the KUL lab (Fig. 2, left panels). The corresponding Bland-Altman plots showed larger measurement variability with higher mean AUC values (Fig. 2, right panels). The limits of agreement relative to the mean at a mean AUC of 1500 were 36 % (UMCU), 34 % (KUL) and 58 % (ULIS).

Repeatability among the three experiments showed that the CCCs ranged from 0.86 to 0.97. The CCCs ranges for each lab were: for KUL 0.86 to 0.94; for UMCU 0.95 to 0.97; and for ULIS 0.93 to 0.95 (Fig. 3). The same pattern as in the technical duplicates was observed for the limits of agreement in the Bland-Altman plots (Figure S1) with diverging limits when the mean AUC increases. The limits of agreement relative to the mean at a mean AUC of 1500 ranged from 44 to 71 % (UMCU), 60–85 % (ULIS) and 68–105 % (KUL).

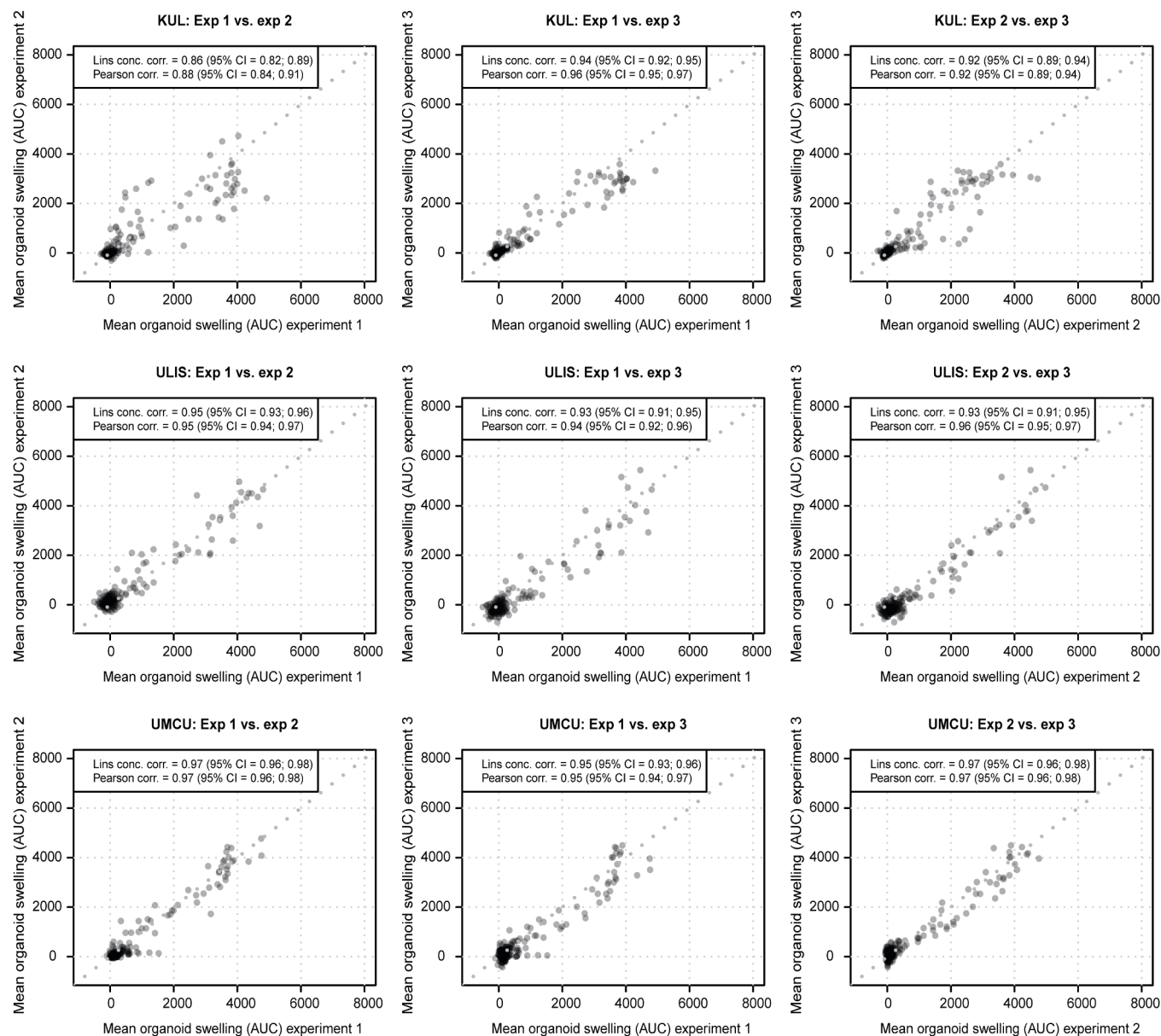
3.2. Forskolin-induced swelling assay reproducibility across three different labs

To assess the reproducibility of the FIS assay across different labs, the mean organoid swelling from three experiments per lab was assessed for the six reference PDIOs using the same conditions (Fig. 1).

Some differences in the magnitude of FIS values between labs were observed, but response patterns were identical for all six PDIOs in all three labs (Fig. 4A). Reference PDIO-1 (G542X/G542X) did not respond to any drug tested, and the same was observed for PDIO-2 (F508del/R1162X), with only minimal responses to the VX-661/VX-770 combination at the highest forskolin concentration. All three labs showed responses to the VX-661/VX-770 combination in reference PDIO-3 (F508del/F508del), with AUCs higher than 1000 at 0.8  $\mu$ M forskolin. For PDIO-4 (F508del/G551D) and PDIO-5 (F508del/S1251N), higher responses were seen for VX-770 alone (compared to responses observed in PDIO-3), further increased by the VX-661/VX-770 combination at higher forskolin concentrations ( $\geq 0.8$   $\mu$ M). PDIO-6 (F508del/R117H)

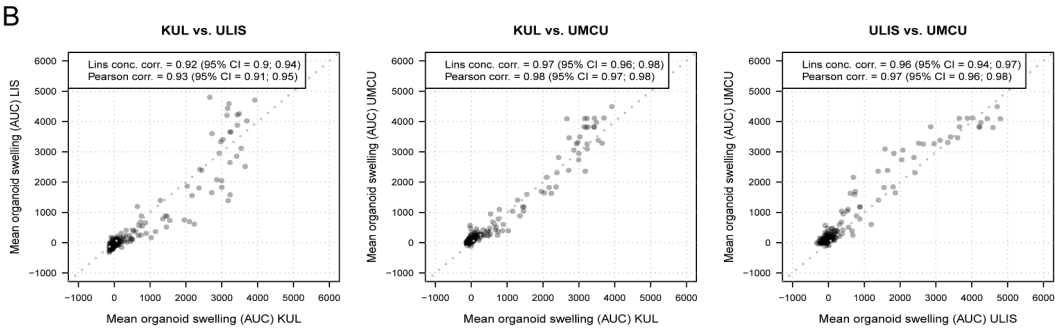
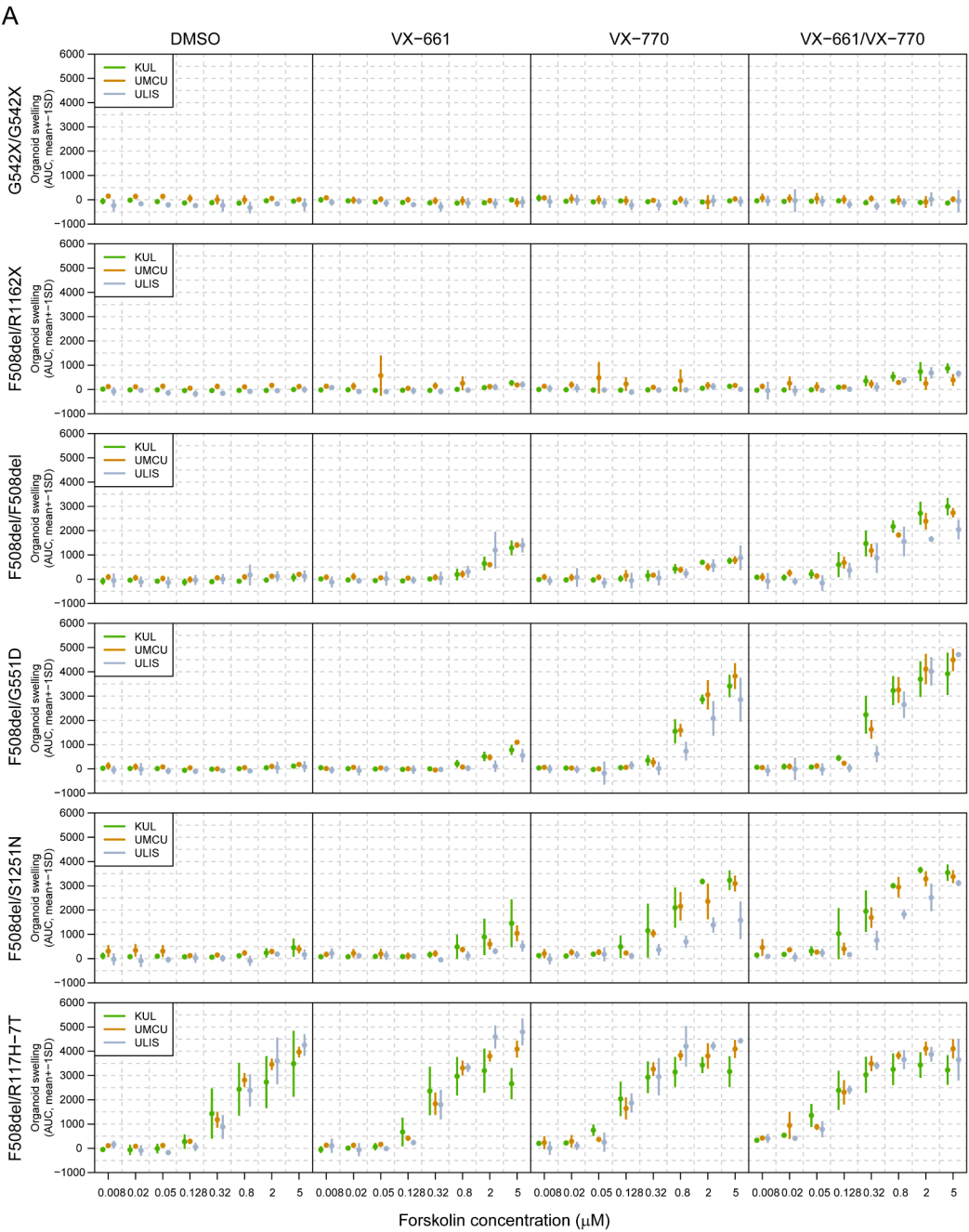


**Fig. 2.** Forskolin-induced swelling (FIS) assay technical repeatability per lab (Year 1). Left panels - Linear correlation X-Y plots (with Pearson correlation coefficient and Lin's Concordance Correlation Coefficient, CCC) for each individual lab of all FIS responses comparing the two technical duplicates performed in all experiments. The dotted line represents the line of equality. Right panels - Bland-Altman plot for each individual lab of all FIS responses comparing the two technical duplicates performed in all experiments. The plain line is the mean difference in area under the curve (AUC), the dashed lines represent the 95 % limits of agreement. Organoids swelling is expressed as the AUC of the normalized organoid surface area over time (baseline = 100 %,  $t = 60$  min).



**Fig. 3.** Forskolin-induced swelling (FIS) assay experimental repeatability per lab (Year 1). Linear correlation X-Y plots (with Pearson correlation coefficient and Lin's Concordance Correlation Coefficient) for comparison of biological replicates ( $n = 3$ , mean of two technical replicates per experiment) performed in all experimental conditions in each individual lab. The dotted line represents the line of equality. Organoids swelling is expressed as the area under the curve (AUC) of normalized organoid surface area over time (baseline = 100 %,  $t = 60$  min).





(caption on next page)

**Fig. 4.** Forskolin-induced swelling (FIS) assay reproducibility between labs (Year 1). A) Comparison of FIS responses from six reference patient-derived intestinal organoids (PDIOs) bearing different CFTR genotypes measured independently in three labs. The genotypes are indicated in each panel. Organoids swelling is expressed as the area under the curve (AUC) of organoid surface area over time (baseline = 100 %,  $t = 60$  min), for each modulator combination at forskolin (Fsk) concentrations of 0.008, 0.02, 0.05, 0.128, 0.32, 0.8, 2 and 5  $\mu$ M. Different colours define the different labs. Each column of the panel represents a modulator combination (DMSO alone, VX-661 alone, VX-770 alone and VX-661/VX-770 in combination). Each dot represents the mean of 3 experiments and the error bars represent SD values. B) Linear correlation X-Y plots (with Pearson correlation coefficient and Lin's Concordance Correlation Coefficient, CCC) for paired comparison of all organoids swelling data performed in all experimental conditions in each individual lab. The dotted line represents the line of equality. Organoids swelling is expressed as the area under the curve (AUC) of organoid surface area over time (baseline = 100 %,  $t = 60$  min).

**Table 2**  
Percentage of agreement based on DMSO corrected response ( $n = 18$ ). PoA = Percentage of Agreement, est = estimation, ci.l = lower limit confidence interval, ci.u = upper limit confidence interval and FSK = forskolin.

Cut off	FSK	Lab1	Lab2	PoA est (ci.l - ci.u)
1000	0.32	KUL	ULIS	0.78 (0.55–0.91)
1000	0.32	KUL	UMCU	0.94 (0.74–1)
1000	0.32	ULIS	UMCU	0.83 (0.61–0.94)
1000	0.80	KUL	ULIS	0.78 (0.55–0.91)
1000	0.80	KUL	UMCU	0.89 (0.67–0.97)
1000	0.80	ULIS	UMCU	0.89 (0.67–0.97)
1500	0.32	KUL	ULIS	0.78 (0.55–0.91)
1500	0.32	KUL	UMCU	0.89 (0.67–0.97)
1500	0.32	ULIS	UMCU	0.89 (0.67–0.97)
1500	0.80	KUL	ULIS	0.78 (0.55–0.91)
1500	0.80	KUL	UMCU	1 (0.82–1)
1500	0.80	ULIS	UMCU	0.78 (0.55–0.91)

showed high residual CFTR function, illustrated by a substantial increase in the AUC under forskolin alone. This was expected since R117H is a residual function variant (class IV) associated with variable consequences in terms of disease penetrance. The FIS response profiles shown here are in line with the clinical benefit observed in clinical trials [20].

The magnitude of the responses differed among labs in some of the PDIOs and conditions. Paired comparison of the average FIS responses measured in the three labs showed agreement comparable to that of repeatability within a single lab, with CCCs of 0.92 for KUL vs. ULIS, 0.96 for ULIS vs. UMCU and 0.97 for KUL vs. UMCU (Fig. 4B and S2). The reproducibility decreased with higher mean AUC values (Figure S2). The limits of agreement relative to the mean at an AUC of 1500 ranged from 48 % to 76 %.

In an exploratory analysis, we looked at the agreement between labs in classifying subjects as responders or non-responders to a given treatment. To this end, predefined threshold values (of 1000 and 1500) and forskolin concentrations (0.32 and 0.8  $\mu$ M) were used as expected to be of clinical significance. The percentage of agreement between labs (PoA), estimated for the different AUC thresholds and forskolin concentrations, ranged from 78 to 100 % (Table 2).

3.3. Forskolin-Induced swelling assay repeatability over time

To assess the reproducibility of the FIS assay over time, the responses of PDIOs from one F508del/F508del subject were reassessed twice a year during six years in the UMCU lab (see Figure S3). Mann-Kendall and Spearman correlation tests showed no significant time trends of responses per forskolin concentration (all  $p$ -values > 0.1).

When the FIS assays of PDIOs (3, 5 and 6) from three reference patients were repeated in each lab after one year, consistent response patterns to the drug combinations were observed (Figs. 4A, 5A and S4). The CCC values for technical (Figure S5) and experimental (Figure S6 and S7) repeatability in the second year ranged from 0.97 to 0.98 and 0.9 to 0.98, respectively. In the UMCU and KUL labs, responses were higher in the second year, with an average AUC increase of 600 and 553, respectively (paired  $t$ -test  $p$ -values both <0.001), especially for PDIO reference 5 (Figs. 5A and S4). In the ULIS lab no significant mean difference was observed (average increase: -37, paired  $t$ -test  $p$ -value=0.46) (Fig. 5A), with responses in PDIO reference 6 being slightly lower

in the repeat measurement (Figure S4). The agreement over time per lab showed CCCs of 0.76 (UMCU), 0.85 (KUL) and 0.92 (ULIS) (Fig. 5A).

3.4. Comparison between the three academic labs and one central lab (reproducibility year 2)

In the second year, a fourth (central) lab joined (HUB) and also performed FIS assays on reference PDIOs 3, 5 and 6. The reproducibility among the four labs was lower this second year, especially in the comparisons with ULIS data. Indeed, CCC values were: ULIS vs. UMCU - 0.67, ULIS vs. KUL - 0.75 and for ULIS vs. HUB - 0.81 (Fig. 5B and S8). The data from the other three labs showed CCCs comparable to those from one year earlier (UMCU vs. HUB - 0.94, KUL vs. UMCU - 0.95 and KUL vs HUB - 0.95) (Fig. 5B and S8).

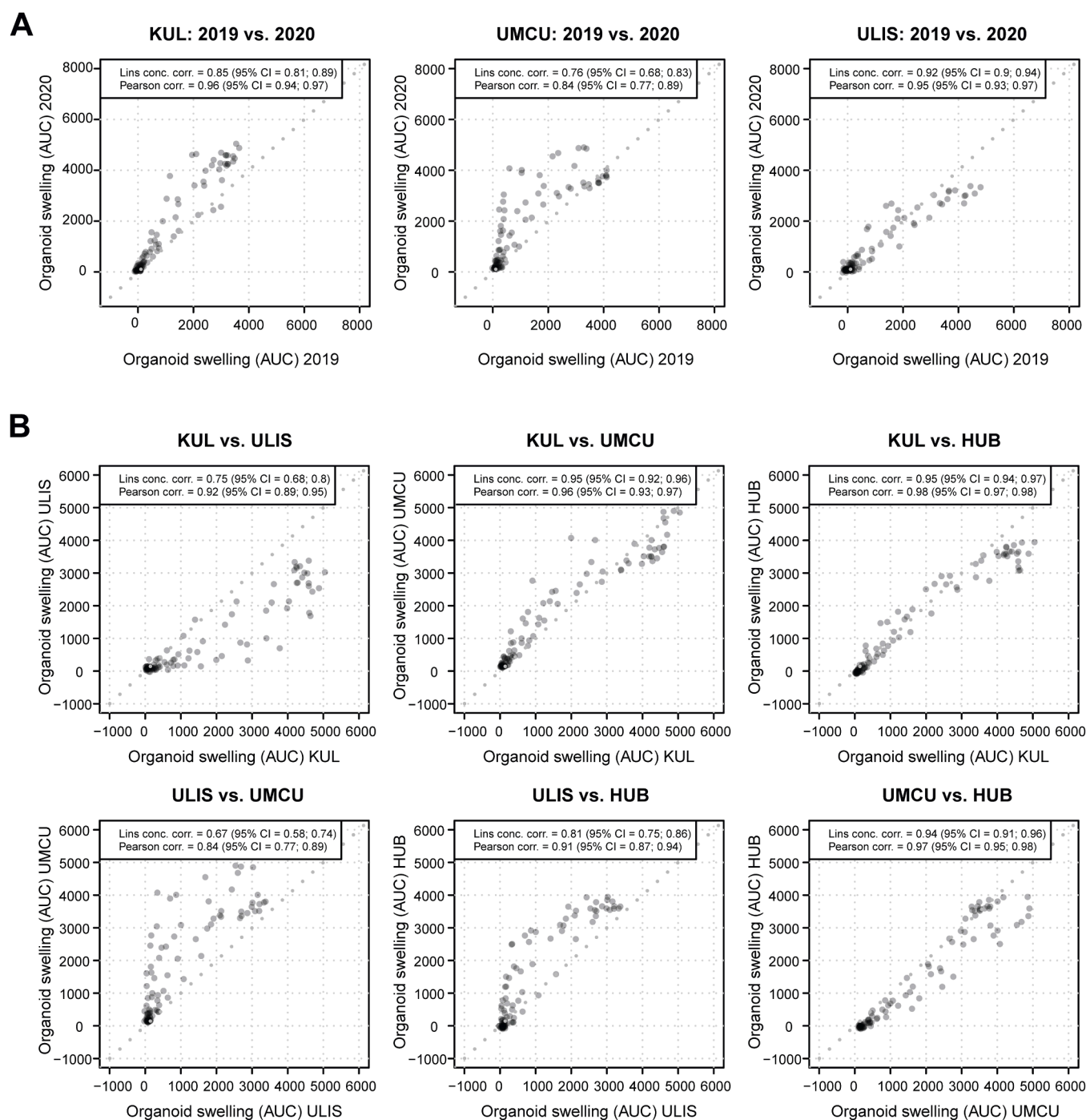
4. Discussion

Functional tests in patient-derived stem cell cultures such as 3D organoids are increasingly being explored for precision medicine applications, but data on repeatability and especially reproducibility are lacking for these living cell technologies. We here evaluated the repeatability and reproducibility of the FIS assay on PDIOs. The intra-assay repeatability of the duplicate measurements was the highest with CCCs ranging from 0.95 to 0.98. Experimental repeatability within labs showed CCCs above 0.90, indicating that the  $n = 3$  suggested by the STAR protocol [17] used in this study is adequate.

Reproducibility between labs was also high in the first year (CCCs 0.92 to 0.97), but somewhat reduced in the second year (CCCs ranged from 0.67 to 0.95) when a smaller sample collection was analyzed. However, response patterns remained consistent throughout all experiments. Besides the smaller sample size, factors such as media preparations, operators or changes in technical equipment might have led to higher variations across labs in the second year (lower values were only seen in the comparison with a single lab). These results indicate that under controlled conditions, the FIS is a technically repeatable and reproducible assay that can be used as a biomarker to estimate individual CFTR function in the presence or absence of CFTR modulators.

One key advantage of patient-derived stem cell cultures such as intestinal organoids is the ability to generate biobanks with large collections of cells that can be shared across sites. In this study, we shared six reference PDIOs so that assays could be executed according to an ECFS-endorsed SOP [17]. These specific lines were chosen to span a large range of CFTR function. Through sharing these references lines and using the established SOP for the FIS assay, individuals and labs can be trained and qualified for measurement of CFTR function in PDIOs. The data presented here can serve as a benchmark. We have previously showed repeatability of PDIO FIS across six months of culture (26 passages) and a single freeze-thaw step [30]. We here extend data by long-term observations over a five-year period with an identical PDIO line, never exceeding 26 passages, that did not show altered trends in responses, confirming the re-usability and expandability of PDIOs of long periods of time using biobanked materials [31,32]. This supports that PDIOs stored in a biobank could be used for a precision medicine approach and reused over extended periods of time for quality control purposes or retesting of drugs.

In this study, we aimed to mitigate source for variation through sharing culture media and assay reagents where possible. All labs



**Fig. 5.** Forskolin-induced swelling (FIS) assay reproducibility impact of time and lab. Comparison of FIS from three reference patient-derived intestinal organoids (PDIOs) bearing different CFTR genotypes measured at two timepoints (one year apart) in three labs and comparison to results generated in a fourth lab in the same time period. A) Linear comparison of the results obtained in year 1 and year 2 per lab. B) Paired comparison of organoids swelling among the four labs after one year. Comparison of all organoids swelling data performed in all experimental conditions in each individual lab. The dotted line represents the line of equality. Organoids swelling is expressed as the Area Under the Curve (AUC) of organoid surface area over time (baseline = 100 %,  $t = 60$  min).

obtained reagents with identical lot numbers and preferably matching batches to ensure optimal standardization, including R-spondin-1 and Matrigel. The conditioned media production for Noggin was centralized in the UMCU lab and distributed to the other sites. This factor can be produced at bulk and can be shared including a freeze-thaw step. Due to limited stability of Wnt-3a and sensitivity to a freeze-thaw cycle, conditioned media with this factor was produced locally using the same cell line and a common protocol [17]. As such, the production of Wnt-3a remains an important factor that could contribute to variation in

reproducibility between labs, as differences in Wnt-3a can affect the differentiation status of PDIOs and CFTR function measurements [33]. Synthetic alternatives for biological products such as Matrigel and conditioned Wnt-3a media are currently being developed [34,35], and might further improve the overall quality and standardization of the culture and assay reagents over time.

Another important current limitation is quality control at the level of the individual PDIO cultures. In current procedures, one identical reference line is cultured and assayed across experiments.



This reference line informs on general media and assay conditions, but misses experimental variation of individual PDIO conditions. Currently, quality control criteria for individual PDIO conditions (e.g. the percentage of viable organoids, minimal/maximal number of organoids) are individually assessed by technical operators. A further automation of procedures and integration of recently developed image analysis strategies during growth and assaying may further help to standardized procedures and further increase experimental reproducibility [36].

The variability as reported here for FIS assay should be compared to other biomarker tests of CFTR function, such as the sweat test, nasal potential difference measurement or intestinal current measurement (ICM). Unfortunately, few studies report on these tests' repeatability and even less on reproducibility to measure CFTR function in the context of disease liability. Moreover, the repeatability and reproducibility of these tests in measuring responses to modulators remains undefined. As an example, diagnostic variability is reported for the sweat test (SCC), the most used CF diagnosis assay and regulatory approved biomarker for CFTR function. In a retrospective study, the between test-variability was large, with differences between  $-18.2$  mmol/L (p5) and  $+14.1$  mmol/L (p95) [37]. In addition, a survey of real-life sweat test practice across Europe showed considerable variance in practice [38]. Even with full standardization of material and methods between centers and with central analysis of sweat samples in the context of a clinical trial, SCC variability remains large with limits of repeatability for repeat measurements ranging from  $-19.7$  to  $+21.6$  mmol/L [39]. Similar variability has been reported by others [40]. Previous work that directly assessed organoid FIS, SCC and ICM from identical subjects also indicated FIS as a more precise indicator of CFTR function, enabling better discrimination between subgroups and individuals with respect to different severity indicators or therapeutic response indicators by FIS as compared to ICM and SCC [41–43].

Various contexts of uses can be identified for FIS, and currently PDIO FIS is being explored as an individual predictor of long term disease expression and progression, or as an individual predictor of potential therapeutic benefit. Our exploratory analysis demonstrated that, from a technical perspective, there was a 78–100 % agreement between labs when applying a predefined threshold and forskolin concentrations. This suggests that it is possible to define and compare absolute thresholds across different labs. Alternatively, a “floating cut-off” approach could be used to compare results from PDIOs directly to a reference PDIO (or collection of PDIOs) within a single lab. This approach is accepted when residual variability cannot be further reduced by adaptations of the test procedure [44]. The decision to use continuous FIS values for estimating individual probabilities of clinical outcomes, such as the likelihood of benefiting from CFTR modulators or developing CF-related diabetes, or to establish thresholds for these probabilities, requires further clinical research.

In conclusion, we here demonstrate that FIS in PDIOs is a repeatable and reproducible *in vitro* biomarker of CFTR function, both within and among different labs. These data support further studies into the use of FIS as a tool to help to better interpret to probability on individual disease expression, progression and potential benefit of CFTR modulators.

#### CRedit authorship contribution statement

**Marlou C. Bierlaagh:** Data curation, Formal analysis, Validation, Visualization, Project administration, Writing – original draft. **Anabela S. Ramalho:** Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Validation, Visualization, Writing – original draft. **Iris A.L. Silva:** Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Validation, Visualization, Writing – original draft. **Annelotte M. Vonk:** Conceptualization, Investigation, Methodology, Resources, Project administration, Data curation, Formal analysis. **Rutger M. van den Bor:** Formal analysis, Software. **Peter van**

**Mourik:** Conceptualization, Writing – review & editing. **Johanna Pott:** Resources, Methodology, Data curation. **Sylvia W.F. Suen:** Resources, Methodology, Data curation. **Sylvia F. Boj:** Conceptualization, Writing – review & editing. **Robert G.J. Vries:** Writing – review & editing. **Elise Lammertyn:** Writing – review & editing. **François Vermeulen:** Formal analysis, Supervision, Writing – review & editing. **Margarida D. Amaral:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing. **Kris de Boeck:** Conceptualization, Funding acquisition, Validation, Writing – review & editing. **Cornelis K. van der Ent:** Conceptualization, Funding acquisition, Validation, Writing – review & editing. **Jeffrey M. Beekman:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

We would like to thank all people with CF who provided samples for this study and to thank the teams at the CF labs at KUL, HUB, UMCU and ULIS for all the support given to this research.

#### Funding

This work was supported by funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 755021 - H2020-SC1-2017-755021.

Work in MDA lab is also supported by UIDB/04046/2020 and UIDP/04046/2020 centre grants (to BioISI), from FCT/MCTES Portugal.

The confocal microscope used at KUL CF research lab was financed by the Belgian CF patient association 'Mucovereniging'.

#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2024.04.014.

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