WS10.2
Organoids as diagnostic test for cystic fibrosis

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Introduction: Organoids are promising for in vitro CFTR function assays, predicting response to CFTR modulators using patient derived cells. We explored whether organoid morphology can be used to assist in the diagnosis of cystic fibrosis (CF).
Methods: Rectal organoids from 33 subjects (26 CF and 7 non-CF) were plated (8 wells per subject), and stained one day later with calcein. Microscope images were acquired without forskolin stimulation of the organoids.
Based on morphological differences between CF and non-CF organoids, two parameters were calculated:
- Almost all non-CF organoids have a large and low fluorescent fluid-filled lumen, while most CF organoids have no lumen, and thus a cellular, high fluorescence center. The **intensity ratio (IR)** is the ratio between the mean fluorescence of the central area (obtained after "eroding" the outer zone of the organoid) and the mean fluorescence of the total area of each organoid. The larger the lumen, the lower the IR.
- Non-CF organoids have a more circular shape than CF organoids. The **circularity index (CI)** is a ratio between the area and the perimeter of each organoid ($4.\pi.\text{area}/\text{perimeter}^2$). The rounder the organoid, the higher its CI.
Automated image analysis was done with Nikon NIS elements software on 50–300 organoids from each individual.
Results: The **IR** was lower in non-CF (mean 0.81; range 0.76–0.92) than in CF organoids (1.12; 1.07–1.17), $p < 0.001$. The **CI** was higher in non-CF (mean 0.85; range 0.77–0.92) than in CF organoids (0.57; 0.44–0.68) ($p < 0.001$). Using a cut off of 1 for IR and of 0.7 for CI, both parameters discriminate between CF and non-CF organoids, with a sensitivity and specificity of 100%.
IR and CI were repeatable over time. Coefficients of variation calculated from 2–4 repeat experiments in 15 subjects were 3.6 ± 3.1% for IR and 4.4 ± 2.8% for CI.
Conclusion: IR and CI from organoids discriminate CF from non-CF. Organoid morphology could thus be used as diagnostic test for CF.

WS10.3
Chloride in saliva and sweat in age-matched individuals with and without CF

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Objective: To verify the performance of the chloride dosage in saliva by the equipment of gasometry for diagnosis of CF.
Methods: A cross-sectional study of individuals (CF = 123 and non-CF = 118). Samples of sweat and saliva were collected at the same time. SALIVA1 (collected in the first minute) and SALIVA 2 in the subsequent 03 minutes. The chloride was measured by the direct selective ion electrode technique (ABL Radiometer®, model 835, Denmark). In sweat the chloride was analysed by titration.
Results: As in sweat (CF:Mean = 120.6 mmol/L, Median = 122.5 mmol/L, SD = 29.1 mmol/L and control: Mean = 20.3 mmol/L, Median = 19.2 mmol/L, SD = 9.2 mmol/L), chloride concentration in saliva (Saliva1 for CF: Mean = 25.4 mmol/L, Median = 23 mmol/L, SD = 21 mmol/L and Control: Mean = 12.8 mmol/L, Median = 12 mmol/L, SD = 4.8 mmol/L in Saliva2 for CF: Mean = 23.97 mmol/L, Median = 21 mmol/L, SD = 15.29 mol/L and Control: Mean = 12.6 mmol/L, Median = 12 mmol/L, SD = 5.9 mmol/L) was higher in the CF group [Female = 68 (mean age = 11.7 years) and male = 55 (mean age = 8.8 years)] when compared to the control group [Female = 64 (mean age = 10.8 years) and Male = 54 (mean age = 9.2 years)] $p < 0.001$. We observed the chloride instability in SALIVA2 and lower variability of the values analyzed in the saliva collected in the first minute. Due to this, we established the cutoff point for salivary chloride only in the first minute (14.5 mmol/L), (sensitivity = 81.01%, specificity = 67.1%, PPV = 71.91%, NPV = 77.27%).

		Sweat (Gold Standard)	Saliva1 (Gasometry equipment)	Saliva2 (Gasometry equipment)
CF (n = 123)	Mean	120.6	25.4	23.97
	Median	122.5	23	21
	SD	29.1	13.9	15.29
Control (n = 118)	Mean	20.3	12.8	12.6
	Median	19.2	12	12
	SD	9.2	4.8	5.9
Value p*		<0.000	<0.000	<0.000

[Comparative analysis of chloride concentr]

Conclusion: The methods used in this study suggest a concordance between chloride concentrations in saliva and sweat, that is, both fluids have a physiologically similar behavior, evidencing the possibility of the use of the chloride dosage in the saliva by the technique of direct ion selective electrode for CF diagnosis and/or screening.

WS10.4
Performance of a four-step newborn screening strategy for CF in the Dutch screening program

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Since May 1, 2011, newborn screening for Cystic Fibrosis (NBSCF) is part of the Dutch NBS program
Objectives: To assess the validity of the four-step screening strategy when applied in a routine newborn screening program.
Methods: NBSCF was carried out as a 4-step strategy (Immunotrypsinogen (IRT)/Pancreatitis-associated Protein (PAP)/DNA-analysis for 35 *CFTR* mutations/extended gene analysis (EGA) (Thorax 2012;67: 289). As safety net EGA-analysis was performed in samples with IRT ≥ 100 µg/L but no CF mutations. In 2013 the strategy was adapted: besides newborns with two mutations, either disease-causing or of unknown clinical relevance, also newborns with one disease-causing mutation were referred to a designated CF centre for further diagnosis. Data analysis was carried out as if the screening strategy was adapted during the whole period of data collection (May 1, 2011 to January 1, 2016). CF centres reported results of sweat tests and CF patients not identified by NBSCF in the NBS-registry. Carriers and newborns with CFSPID were considered false positives. Before data-analysis a cross check was performed comparing data in the NBS- and the Dutch CF patient-registry.
Results: 819,518 newborns, 99.95% of all newborns, were screened for CF; in 8,131 IRT was ≥60 µg/L, in 1,079 DNA-analysis followed PAP analysis; 121 had 2 CF mutations; EGA analysis was performed in 85 samples with 1 CF mutation, in 474 as safety net. 193 were screen-positive. CF was confirmed in 122, we found 27 CFSPID, 37 carriers. CF was excluded in 7. A false negative screening test was reported for 16 (4 with meconium ileus (MI)), in 8 caused by PAP values < cut-off levels. We calculated a specificity of 99.99%, PPV 63%, CF/CFSPID ratio of 5/1 and a sensitivity of 91% (without MI).
Conclusion: PAP as additional marker in NBSCF appears to lead to an excellent true/false positive rate and the finding of a low number of carriers. Lower PAP cut-off from 1–7-2016 can improve sensitivity to standards of care.