Table 1: The main requirements to perform a good sweat test (ST) across Europe.

Institution and Sweat Test operators		
ST requirement	**Acceptable**	**Optimal**
Minimum number of ST performed in the centre or lab/year	≥80/year	>100/year
Operators	Experienced (see details below) and documented specific training. For new operators: at least 3 ST in infants (<6 months of age) with no QNS; at least 3 ST in children with no QNS and 2 ST in adults with no QNS. Start with bilateral testing if QNS For skilled operators: at least 3 ST in infants (<6 months of age) with no QNS/6 months; at least 3 ST in children and adults/6 months	Experienced (see details below) with regular (at least annual) and documented peer review For new operators: at least 5 sweat tests in infants (<6 months of age) with no QNS; at least 3 sweat tests in children with no QNS and 2 sweat tests in adults with no QNS. Start with bilateral testing if QNS For skilled operators: at least 5 ST in infants (<6 months of age) with no QNS/6 months; at least 5 ST in children and adults/6 months
Equipment	Approved for diagnostic use	Approved for diagnostic use with SOP for preventive maintenance
Information about ST to patient/parent/carer	Verbal or written leaflet (see **Appendix B**) in different languages whenever needed or use an interpreter	Verbal and written leaflet (see **Appendix B**) in different languages whenever needed or use interpreter
Informed consent	Verbal	Verbal or written according to local regulations

Subject conditions	Preterm infants (the test can be attempted) Age >48 hours (the test can be attempted) Weight ≥ 2kg (the test can be attempted) No oxygen by an open delivery system No pacemaker, no defibrillator Be cautious of conditions that can give false pos/neg results (see **Table 2**)	Full term infants Age >2 weeks Weight ≥ 2.5kg No oxygen by an open delivery system No pacemaker, no defibrillator Be cautious of conditions that can give false pos/neg results (see **Table 2**)
Sweat test procedure		
1. Sweat Stimulation		
ST requirement	**Acceptable**	**Optimal**
Site of stimulation	Flexor site of forearm or upper arm or thigh if forearm is not suitable No eczema, hair or wrinkles at the stimulation site Free of inflammation or visible abnormalities	
Cleaning the skin before stimulation	1/ Clean skin with alcohol and then dry 2/ Wash with distilled/deionized water and then dry	
Iontophoresis current source	Battery powered and safety cut-out	Battery powered with a low battery indicator and safety cut-out
Iontophoresis current application	Manually and gradually controlled (max 4mA)	Automatically controlled (max 4mA)
Duration of iontophoresis	Manually controlled: minimum 3 minutes - max 5 minutes	Automatically controlled (5 minutes)

Electrodes for iontophoresis	Free from irregularities and surface oxidation debris with a device that allows the attachment of straps; copper or stainless-steel	Commercially available electrodes. Free from irregularities and surface oxidation debris with a device that allows the attachment of straps; copper or stainless-steel
Electrode size	>1,5x1,5 inch or 3.75x3.75cm; suitable curvature	Commercially available electrodes for iontophoresis
Electrode straps	Suitable size	Rubber or velcro electrode straps of adjustable size
Placement of electrodes	Negative (black) and positive (red) electrode on the same limb. Positive electrode on the inner volar surface of the lower arm. Avoid bridging between the 2 electrodes: minimal distance between the 2 electrodes ≥ 1cm	
Analytical balance (in case of gauze or filter paper)	Sensitivity ≥ 0.0001 g	
Gauze or filter paper pads for iontophoresis	Chloride free gauze or filter paper pads Pads thickness = 1 cm Pads size ≥ 1cm larger than the electrode in all directions	Hydrophilic pocket pads instead of filter paper pads Pads thickness = 1 cm Pads size ≥ 1cm larger than the electrode in all directions
Pilocarpine for iontophoresis	If gauze or filter paper pads: pilocarpine nitrate solution (2-5g/L) at positive electrode (red), magnesium sulphate (0.05mmol/L) at negative electrode(black) or pilocarpine nitrate solution (2-5g/L) at both electrodes. Solutions can be prepared “in house” as pharmaceutical grade formulations. Do not use unbuffered solutions to reduce the risk of burns. If capillary tube system: not expired pilogel disks without crackles or any evidence of deterioration that fit perfectly the electrodes WARNING: do not combine different methods	

2. Sweat Collection		
ST requirement	**Acceptable**	**Optimal**
Sweat collection material	- Pre-weighed chloride free gauze or recommended filter paper fitting the size of the stimulated area (over the area stimulated by the positive electrode) or larger - Capillary tube system (over the area stimulated by the positive electrode)	
Fixing sweat collection material	For gauze or filter paper: seal with polyethylene or parafilm and waterproof tape For capillary tube system: fix with straps	
Sweat collection time	For gauze or filter paper: 30 min For capillary tube system: 30 min (or 20 min unless the tube is full)	
Sweat container	Airtight container. Use the same sweat container before and after sweat collection	Haematocrit tubes sealed with plasticine or PCR capped tubes. Use the same PCR tubes before and after sweat collection
Labelling of containers	Use permanent ink to label the container with patient identification and “left” or “right” in case of bilateral testing	Adhesive barcode labels on the container with patient identification and “left” or “right” in case of bilateral testing
Minimum sweat weight/volume	For gauze or filter paper: collect at least 75 mg to assure a sweat test rate = 1 g/m^2/min Nanoduct has an automatic control of sweat test rate For capillary tube system: collect >15 µl (also consider the minimum volume needed for analysis and duplicate readings) to assure a sweat test rate = 1 g/m^2/min	
3. Sweat Storage and Transport		
ST requirement	**Acceptable**	**Optimal**

Storage conditions for sweat in gauze or filter paper	At 2-8°C up to 72 hours in air-tight container	At 2-8°C in air-tight container up to analysis (as soon as possible or at least the same day of sample collection)
Storage conditions for liquid sweat (from capillary tube system)	At 2-8°C up to 72 hours in haematocrit tubes sealed with plasticine, ensuring an air gap or PCR tubes	At 2-8°C in sealed capillary tube system or capped PCR tubes up to analysis (as soon as possible or at least the same day of sample collection).
Transport	Minimize sample evaporation	In sealed plastic bag to minimize sample evaporation
4. Sweat Analysis		
ST requirement	**Acceptable**	**Optimal**
Getting sweat ready for analysis	<u>For sweat collected on gauze or filter paper</u>: elution step with deionized or distilled water (volume is method dependent). Gently invert the capped vial before analysis for more than 1 minute <u>For sweat collected with the capillary tube system</u>: expel slowly sweat out of the capillary system into a PCR tube with a syringe with adapted needle; cap the PCR tube and mix before analysis.	
Sweat analyte	Chloride Sweat electrolytes measured by conductivity (**chloride analysis if NaCl equivalents >50 mmol/L**)	Chloride
Sweat analyte units	Chloride concentration in mmol/L NaCl equivalents in mmol/L	Chloride concentration in mmol/L
Sweat analysis methods	Quantitative colorimetry, Ion Selective Electrodes (ISE) for chloride. Single reading. Follow regulations on collection and treatment of waste reagents with mercury Conductivity expressed in NaCl equivalents	Coulometry and other validated quantitative methods (e.g., mass or gas spectrometry) for chloride. Duplicate readings if sweat volume is enough

5. Quality Assurance (see **Appendix A**)		
ST requirement	**Acceptable**	**Optimal**
Responsibility for training, assessment of competence and certification for all staff undertaking sweat tests	A consultant (or equivalent) clinical chemist	
Responsibility for sweat testing (both collection and analysis)	A consultant (or equivalent) clinical chemist	
Internal Quality Control (IQC) materials	Aqueous simulated human sweat matrix samples prepared in house as-pharmaceutical grade formulations	Commercial aqueous simulated human sweat matrix samples
Internal Quality Control (IQC) samples concentrations	3 different concentrations: - normal level concentration: chloride <30 mmol/L - borderline level concentration: chloride 30-59 mmol/L - abnormal level concentration: chloride ≥60 mmol/L The Nanoduct system is equipped with the Nanoduct Patient Simulator	
Internal Quality Control (IQC) procedure	2 levels (including always the normal level)/sample batch; one level before patient's samples and one level after patient's samples	3 levels/sample batch; at least one level before patient's samples and one level after patient's samples

Internal Quality Control Coefficient of variation (CV%)	Between batch CV% between 7-10% at normal chloride concentration Between batch CV% between 5-7% at borderline and abnormal chloride concentrations Between batch CV% between 2-3% for NaCl equivalents at 50 mmol/L	Between batch CV% <7% at normal chloride concentration Between batch CV% <5% at borderline and abnormal chloride concentrations Between batch CV% ≤2% for NaCl equivalents at 50 mmol/L
Internal Quality Control (IQC) tools	Levey-Jennings charts	Levey-Jennings charts and Westgard rules
External Quality Assessment (EQA) scheme: criteria to make a choice	EQA scheme characteristics: Quality material: sweat like matrix N° of participants: low for the same method N° of analytical methods: only 1 Analytical and clinical ranges: covered Frequency of samples: fixed Reports: regular Education: at least 1 meeting/round Troubleshooting support	EQA scheme characteristics: Quality material: sweat like matrix; blind samples N° of participants: high for the same method N° of analytical methods: >1 Analytical and clinical ranges: covered Frequency of samples: flexible Reports: with a rapid turnaround time Education: > 1 meeting/round Troubleshooting support
EQA performance	Sufficient	High
EQA participation	Once a year	Each round of EQA scheme
Quantity Not Sufficient (QNS) samples	Between 10-15% in the total population Between 5-10% in children over 6 months of age	<10% in the total population <5% in children over 6 months of age

	Between 20-25% in children under 6 months of age	<20% in children under 6 months of age
6.Processing sweat test result		
ST requirement	**Acceptable**	**Optimal**
Reference values	See **Table 3**	
Sweat test report	See **Table 4**	
If SCC in the CF range	Confirm with a second sweat test Result reported by a CF specialist. Only 1 ST required if 2 CF-causing *CFTR* mutations *in trans.*	Confirm with a second sweat test and/or with population specific CFTR analysis. Result reported by a CF specialist. Only 1 ST required if 2 CF-causing *CFTR* mutations *in trans.*
If SCC in the intermediate range	Repeat sweat test. Result discussed by a CF specialist.	
If SCC in the normal range	Result reported by the lab technician or nurse. Result discussed by the doctor requesting the ST.	Result reported by a CF specialist
In case of QNS	Chloride results should not be reported; comment as “unreliable” chloride value; repeat the sweat test	
If non-physiological results (>150 mmol/L for chloride; >170 mmol/L NaCl)	Chloride results must not be reported; comment as “unreliable” chloride value; repeat the sweat test	