Micro-flowcell conductometric sweat analysis for cystic fibrosis diagnosis

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SUMMARY. This paper describes a device specifically designed to facilitate neonatal sweat testing. The components are sized appropriately for attachment to the limbs of newborns. Iontophoretic electrodes, with pilocarpine gel inserts, are latched into small holders attached by straps to the limb. The holder at the anodic site remains in place to receive and align the sensor cell, which uses a conical collecting surface to channel the sweat directly and anaerobically from the sweat ducts to the continuous flow-through conductivity cell within its body. A crib-side analysis unit incorporates an iontophoretic power supply and displays a continuous readout of sweat electrical conductivity. The average conductivity during a specific time interval and the initial sweating rate are automatically displayed. The method, which simplifies sweat tests, is currently being assessed in three neonatal clinical trials to test its ability to reduce test failures in the newborn due to insufficient sweat.

INTRODUCTION

Since the inception of the sweat test\(^1\) as a laboratory aid in the diagnosis of cystic fibrosis (CF) there has been much improvement in the critically important collection procedure and the analytical methods employed. The pad collection technique, first described by Gibson and Cooke,\(^2\) is still performed by some laboratories. About 50% of US hospital laboratories and a preponderance of international institutions are now using the Macroduct\(^\text{TM}\) collectors (Wescor Inc, Logan UT, USA), which, by collecting sweat anaerobically, avoid evaporation and condensation errors.\(^3\) Despite the much-improved state of confidence in the laboratory diagnosis of CF over the last two decades, the early diagnosis of CF in the newborn still remains a problem.

Effective treatment of CF patients requires identification of the disease as soon as possible after birth so that appropriate care can be given with minimum delay.\(^4,5\) Carrying out sweat tests on neonates is a daunting prospect especially when the baby is weak and sickly. The electrodes currently used, particularly those in the pad absorption procedure, are too large for satisfactory application to extremely small limbs. The problem is less severe with the Macroduct electrodes (which are approximately 2.5 cm diameter circles), but even these are regarded by most users as inappropriate for neonates. Furthermore, the aerobic nature of pad collection makes this procedure very prone to false elevation of sweat salt concentration, particularly with the low rate of sweating typical of the newborn.

Reducing the dimensions of the electrode and the collection area would appear to be an obvious first step to overcome the problems. However, this would reduce sweat volume. It should be emphasized in this context that by age 13 days most term babies are able to sweat\(^6\) and the concentration of electrolytes is typical of the mature level to be ultimately attained.\(^7\) Therefore, if sufficient sweat can be collected, the result will have diagnostic validity.

The apparatus described in this paper was designed to be used during the early neonatal period so as to reduce, or abolish, the incidence of test failures. Certain characteristics were considered to be highly desirable, if not mandatory. The method must involve a significant reduction in the size of the iontophoretic electrodes, and the dimensions of the collector must be correspondingly small, facilitating attachment to the infant’s limb. The resulting small sweat sample size will virtually rule out physical collection of sweat for analysis. Thus,
the analysis should be made in situ in a micro-
flow-through electrical conductivity cell con-
tained within a collecting device. This
combination, called a sensor cell, should be
disposable to avoid cross-contamination. The
sweat must be directly channelled into this cell in
a totally anaerobic manner to avoid errors due
to evaporation and condensation. In effect, the
collection and analysis stages would be com-
bined into a single operation. The continuous
record of electrical conductivity data should be
signalled to an induction/analysis instrument at
the crib-side. The induction/analysis unit should
provide an electronic averaging facility over a
pre-set time interval. A display of the initial
sweating rate in g/m²/min should be available.

In addition, the iontophoretic current density
duration, and the formula for the disposable
electrode gel, should be modified to provide
shorter and safer iontophoresis than hitherto
available.

The continuous display of changing sweat
electrolyte concentration so provided would
represent new information, previously unavail-
able in routine sweat test procedures. It poses
the interesting question of which value should be
selected for diagnostic purposes. This matter is
discussed below.

SUBJECTS, MATERIALS AND METHODS

All adult subjects who took part in this study
gave consent to be tested with the device
described after being fully informed of the
procedure. In all cases where children were
involved, the informed consent of a parent was
provided. All adults tested, and the parents of
the tested children, were advised that the test
made by the author in no way purported to have
the official recognition of the medical profession.

Agar (granulated) was supplied by Becton
Dickinson and Co, Cockneysville MD, USA.
Methyl and propyl parabens were supplied by
Penta Manufacturing, Livingston NJ, USA.
Pilocarpine nitrate (Merck KGaA, Darmstadt,
Germany) was of US Pharmacopea grade
standard. Trisodium citrate (Sigma Chemical
Co, St Louis MO, USA) was of analytical
reagent quality.

Temperature measurements were made with a
TH-65 digital thermocouple thermometer (Wescor Inc, Logan UT, USA).

The system and its operation

The first fittings to be applied to the patient’s
limb are the two holders, which are identical
plastic rings of external diameter 23 mm, fitted
with slits for strap attachment to limbs. They are
provided with overhanging rims on the inner
edge of the rings to allow latching retention of
flanged iontophoresis electrodes and, subse-
quently, the flanged sensor cell. The stainless
steel circular anode and cathode are 16 mm in
diameter and are housed in colour-coded
electrode assemblies (external diameter 17 mm)
provided with a finger-grip. Disposable pilo-
carpine-containing agar gel discs 15 mm in
diameter and 6 mm high fit into these electrode
assemblies (Fig. 1). These discs are chemically
similar to but not identical to the Pilogel® discs
(Wescor Inc) provided for the Macroduct
system. On insertion of the complete assembly
into the appropriate holder, the flanges can be
made to engage with the rims of the holder by
applying slight pressure while rotating the
assembly through 90° to a detent position. In
this way, the gel surface is automatically pressed
sufficiently firmly against the skin to provide an
even and anaerobic contact. Each electrode
leads via an iontophoresis cable to a common
plug for connection to a battery-powered circuit
that provides controlled current for sweat
induction and is housed in the induction/
analysis unit. A fail-safe, resistance-limited
circuit supplies direct current that automatically
rises to 0.5 mA in the first 30 s, a level that is
maintained for 2.0 min before rapidly switching
off. This reduction in the duration of ionto-
phoresis is made possible by the incorporation
of changes in the constitution of the gel
(described in the Results section).

After sweat stimulation, the electrode-gel
assembly at the anode is removed from its
holder. This holder remains strictly in place
because its most important function is to ensure

**Figure 1.** Cross-sectional diagram of electrode plus
gel disc, fitted to holder on patient’s limb.
subsequent perfect registration of the collection cone of the sensor cell with the iontophoresed area, an essential requirement to ensure maximum recovery of sweat. The cathode electrode-gel and its holder remain in place to provide an electrical contact with the skin, essential for the measurement of rate of sweating. The circular area of stimulated skin within the ring of the holder is washed free of salt with a cotton swab and deionized water, then dried thoroughly with fresh swabs. The disposable sensor cell (see Fig. 2) is immediately inserted into the holder in the same manner as for the anode, and rotated to the detent position to engage the flanges with the holder. As with the attachment of the iontophoretic electrode, the latching flanges provide a firm anaerobic contact between the conical collecting surface and the sweating skin. In both cases, this arrangement provides an ideal, reproducible contact pressure that is largely independent of the degree to which the holder strap is tightened on the limb. The conductivity cell electrodes are then connected by the sensor cell cable to the analysis port of the induction/analysis unit.

After a variable period of time with the sensor cell in position, usually 2–6 min depending on the initial sweating rate (during which the temperature of the cell has risen under the influence of the skin heat to an equilibrium level; see Results), the induction/analysis unit displays a sweat conductivity reading. This indicates that the sweat has filled the sensor cell between the conductivity electrodes and at this point the initial sweating rate indicator (see Results) registers a result. The conductivity value of the flowing sweat is now displayed continuously, and in most cases rapidly decreases for 2–4 min after readings commence, then continues to decrease at a slower but constant rate as the sweating rate also declines.

In order to standardize the conductivity result, the induction/analysis unit automatically displays an average sweat conductivity value over a 5-min time interval, commencing at 3 min after the time at which the first reading is displayed. This time frame was chosen on the basis of the data reported in the Results section.

Calibration of the system, with regard to both sensor cells and the induction/analysis unit, is discussed below.

RESULTS

Conductivity–temperature considerations

Subsequent to the insertion of the sensor cell into the holder, its temperature rises to a level representing a balance between input of body heat via the skin versus loss of heat by radiation. In view of the known effect of temperature on electrical conductivity, experiments were carried out to establish the equilibrium temperature level and its degree of variation. For this purpose, a prototype of the sensor cell was modified by the placement of a fine thermocouple junction at a distance of 1 mm from its conductivity cell. This device was positioned on the skin surface of 26 subjects (both children and adults) immediately after the conclusion of iontophoresis and the temperature of the cell was recorded as a function of time. The temperature rapidly rose from ambient during the first 2 min and reached a steady level between 4 and 6 min. The mean final temperature was 31.6 °C, (range 30.6–32.8 °C). Figure 3 displays conductivity profiles corresponding to temperature changes in two cases in which the sensor cell was filled with standard NaCl solution and strapped to a non-stimulated site on the forearm, showing the good correlation between the two measurements. Assuming a temperature coefficient of 1.2 for the conductivity of salt solutions at this range of temperature, it follows that the observed range of 2 °C in equilibrium cell temperature represents an error of approximately ±2 mmol/L in sweat conductivity. Since this would not be regarded as clinically significant, it was concluded that temperature compensation of the analytical cell was not necessary.

Calibration

Assuming a constant temperature, the conductivity of a tubular cell filled with a salt solution is
directly proportional to the area of cross-section and inversely proportional to the length of the cell. In the mass production of disposable sensor cells, the geometrical dimensions of the conductivity micro-cells, particularly the radius, must be closely matched in order to maintain their conductivity characteristic within acceptable tolerances, thus making factory calibration of each sensor cell unnecessary. All induction/analysis units could then be pre-calibrated with a representative sensor cell containing a standard salt solution at 31·6 °C.

A number of sensor devices sampled from factory production were tested for uniformity by measuring, in a constant temperature environment, the conductivity displayed by their cells when filled with a standard solution of sodium chloride. The average reading in this subset of sensors (n=245) was 72 mmol/L, with a standard deviation of 1·8 mmol/L. This error of approximately ±2% in the conductivity value, due to sensor cell variation, is acceptable.

Although these statistics demonstrate a high level of reliability, a false sweat test result, however rare, has serious repercussions. It is therefore mandatory that mass-production of the sensor cells must be subject to stringent quality control procedures.

Automatic assessment of initial sweating rate

The induction/analysis unit incorporates electronic facilities to allow display of initial sweating rate in g per m² of skin surface per min. The volume of sweat required to occupy the conductivity cell within the sensor/collector device (that is, from electrode to electrode) is precisely known (1·56 μL), as is the area of the collecting surface (2·48 cm²). The time taken to fill this channel is sensed by a timing circuit, allowing an electronically applied algorithm to provide a readout of initial sweating rate. Such measurements were made on 19 of the subjects sweat-tested in this study. The lowest value was 0·54 g/m²/min (a female adult) and the highest was 14·7 g/m²/min (a 6-month-old infant). The initial sweating rate is not the maximum sweating rate, the latter being reached at approximately 10 min after the appearance of the first conductivity reading (unpublished observations).
Modifications to the iontophoretic procedure

Use of buffer as a safety feature

During iontophoresis, there is an accumulation of acid at the anode and alkali at the cathode due to electrochemical reactions. These pH changes may cause skin burns. Pilocarpine, as a weak base, exerts a buffering effect that is limited to resisting pH increase at the cathode. In the present study, the pilocarpine-containing agar gel (Pilogel) has been modified by the addition of citrate buffer (final concentration 10 mmol/L). The buffering effect under standard iontophoretic conditions was experimentally tested by comparing the pH change of unbuffered and citrated gel (both initially pH 6.0) adjacent to the anode and cathode electrode plates after electrophoresis. The results showed that the presence of citrate (pK1 3.14, pK2 4.77, pK3 6.39) increased the pH from 4.0 to 6.0 at the anode. At the cathode, the increase in alkalinity (pH 6.8) was more moderate and the presence of citrate had little additional effect.

Reduction in iontophoresis time

With the addition of sodium citrate to the gels, the ionic concentration is significantly increased. Sodium ions will therefore compete more strongly with pilocarpine ions as current transporters, hence lowering the stimulating effect of a given gel drug concentration. To provide extra competitive transport advantage for the pilocarpine, its concentration in the gel was raised threefold, to 15 g/L. Research with such gels showed that at constant current density, the increased drug concentration allowed the duration of iontophoresis to be reduced from 5 min to 2.5 min without compromising sweat yield. Furthermore, the increased pilocarpine level augments its buffering power at the cathode, thus further reducing the risk of cathodic burns from accumulation of alkali.

Sweat tests using the complete system

Profiles of sweat electrical conductivity versus time after the conclusion of iontophoresis were obtained from 35 sweat tests performed on a total of 22 subjects, comprising 17 healthy adults (14 women, 3 men), two healthy 5-month-old boys, two patients with CF, (a 41-year-old man and a 7-year-old girl) and a 6-day-old boy who was a sibling of the girl with CF. One of the adult healthy males was tested 14 times.

Figure 4 shows the conductivity/time profiles of five healthy adults, two infant controls, two CF subjects and the neonate. Throughout this paper, conductivity is expressed in mmol/L (equivalent sodium chloride), which is the concentration of aqueous sodium chloride solution that has the same conductivity as the sample at the sample temperature. The control data show profiles that were typical of non-CF subjects. The initial conductivity readout (representing the first 2–3 μL of secretion) usually decreased rapidly over the next 4 min, after which it decreased at a steady but slower rate. This early rapid fall in electrolyte concentration has been referred to in the literature as the ‘first sample phenomenon’. Occasionally, this phenomenon was not observed, and instead a relatively constant value was seen for a period before falling slowly. This was usually associated with a comparatively low rate of sweating. It is probable that this is due to diffuse mixing of the small, slow-moving amount of sweat during the relatively long period before the first reading is obtained.

In one CF case, the conductivity rose slightly to a plateau value over 4 min and then remained constant (see Fig. 4). It has been reported that the ‘first sample phenomenon’ is found significantly less often in the CF group. The time between start of collection and first appearance of a conductivity reading varies inversely with the initial rate of sweating, a delay that advantageously allows time for the sensor conductivity cell to reach an equilibrium temperature.

Changes in conductivity between 10 and 20 min after the beginning of sweat collection, which corresponds to the steady phase of decrease, were measured in a group of six adults and two children. The average fall was 15% (range 7–25%) during this 10-min period.

Figure 5 illustrates the conductivity data for all the sweat tests performed. Each result is the average value observed during the 5-min period commencing 3 min after the first appearance of a reading. These results were compared with the established diagnostic ranges for sweat conductivity in children, viz: normal 0–60 mmol/L, borderline 60–80 mmol/L and CF above 80 mmol/L. Of the 17 adult controls, six had values within the range regarded as borderline for children, confirming the well-known tendency toward increased sweat electrolyte concentration in adulthood. Both the control infants produced electrolyte values that were the lowest of the experimental group and well within the normal range. The two known CF subjects clearly produced abnormally high

Ann Clin Biochem 2000: 37
results and the 6-day-old premature boy born of obligate heterozygous parents provided a clearly positive test result that was later officially confirmed.

**Relationship of conductivity to rate of sweating**

This relationship was investigated with a prototype of the sensor cell that was modified so that the volume of sweat produced could be measured at the same time as the conductivity. As shown in Fig. 6 (adult control), the rate of sweating was maintained at a maximum level for about 15 min before showing a steady decrease. Sweating usually ceases at 1–1.5 h after stimulation. Repeat experiments showed that the time to onset of the decrease varied, but the rate of decline was similar in all cases. With conductivity, there was a brief period of about 3 min in which the values fell rapidly, followed by a uniform slow decrease.

The established diagnostic ranges for conductivity were based upon the conductivity values of a mixed sample from a 30-min collection. As the sweating rate falls significantly, the contribution of new sweat to the mixed sample decreases significantly. Thus, the mixed sample conductivity will be minimally affected by extension of collection time. The values for sweating rate and electrolyte concentration in the example were measured at times corresponding to constant sweat volume increments (each of 10 μL), allowing an estimate of the theoretical average conductivity of the mixed sweat secreted during 30 min. This value was

*Ann Clin Biochem* 2000: 37
47 mmol/L, and represents the result that would have been obtained, in this example, by the Hammond procedure using Macroduct. It may be compared with the continuous-flow cell result (46 mmol/L) that is an average over a 5-min period (C in Fig. 6). There is therefore no apparent need to review the established diagnostic ranges for sweat electrical conductivity, provided that the sensor cell continuous-flow data are averaged over the period pre-set for the instrument.

**DISCUSSION**

The continuous-flow micro-cell within the sensor cell body provides constant monitoring of sweat electrolyte concentration that is changing as a function of time after stimulation. A decision must therefore be made about which time interval is selected to produce the test ‘result’. The selection should avoid the very early period, representing the ‘first sample phenomenon’. It should also reflect the sweat electrolyte concentration when sweating rate is near maximal. The time/conductivity profiles showed that the average over a period of 5 min, commencing at 3 min after the appearance of the first reading, is best.

Data on the initial sweating rate are provided by the present apparatus because sweating rate has been considered by many to be diagnostically important in the interpretation of sweat test results. However, there is little published evidence on sweating rate and its relationship to solute concentration. Gibson proposed a sweat production rate of 1 g/m²/min as minimal for acceptance of the sweat test result in diagnosis. Gibson and di Sant’Agnese measured sweat conductivity soon after pilocarpine stimulation (when sweating rate is greatest) and later when the rate had fallen to a minimum level. The decrease in conductivity observed was not diagnostically significant. Emrich et al. analysed sweat from single sweat glands stimulated to a different extent by total body heating, by local heating or by pilocarpine iontophoresis. Their results indicated a dependence of solute concentration on sweating rate, and this varied with the nature of the solute. Even so, their data did not suggest that the effect is in any way large enough to compromise a diagnostic sodium, chloride or

**Figure 5. Distribution of conductivity results within the diagnostic ranges. CF = cystic fibrosis.**
osmolality [and therefore a total electrolyte (conductivity)] result, either in controls or CF subjects.

This micro-conductivity sweat test is fundamentally different from previous methods of sweat testing and therefore forces a review of the question of quality control. External quality assurance (QA) programmes for sweat testing only provide an appraisal of the laboratory’s ability to analyse the samples satisfactorily. Other aspects of the test, such as stimulation and collection, cannot be quality controlled. The laboratory may perform the test using a sample of the disposable sensor cells by filling the cell with either standard salt solution or a QA sample, attaching it to non-sweating skin for 3 min to attain proper temperature, and recording the displayed value. Verification of the accuracy of the analysis unit is accomplished by circuitry which, when switch-selected, interposes fixed-value resistors (representing the resistance of sensor cells containing known standard salt solutions, at proper temperature, as calibrated in the factory). Should the conductivity measuring circuitry fail to perform properly, the operator will not obtain the appropriate reading for the resistors, alerting to the need for re-calibration of the analyser, either in the laboratory or at the factory.

Three clinical trials in neonates up to 2 weeks old, both term and premature, have been commenced in major hospitals in the USA and the UK in order to determine ease and speed of operation, diagnostic accuracy and failure rate. Although the premature 6-day-old CF infant was successfully tested, it must be noted that the sweating capacity is not fully realized until 13 days after birth in full-term and premature babies. These authors measured sweating rate by observing the water vapour gradient close to the skin surface, a technique vastly different from, and much more complex than, the method described here.

The continuous flow analyser may be useful, not only for very early diagnosis of CF, but also for physiological studies of sweat gland

Figure 6. Decrease of conductivity and sweating rate after stimulation of an adult non-cystic fibrosis subject. Sweating rate = ----●----; conductivity = ----△----; A = variable delay before the first conductivity reading; B = 3-min automatic delay before beginning the averaging process; C = 5-min automatic averaging period.
maturation, since it provides both sweating rate and salt concentration.

**CONCLUSION**

The described technique promises marked reduction, if not virtual elimination, of the failure rate in neonatal sweat tests. The significant reduction in the size of the stimulated area, made feasible by in situ analysis in a flow-through micro-cell, greatly facilitates the performance of the test on very young patients. Since only about 3 μL of sweat are needed to obtain a reading, the operator can be confident that although the appearance of a readout may be delayed somewhat in cases of very low sweating rates, it will eventually register and provide reliable diagnosis. The proposed system could be used at the bedside and would complement DNA analysis and immunoreactive trypsin assay.

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