

1 Feasibility and normal values of an integrated conductivity (Nanoduct™) sweat 2 test system in healthy newborns

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17 4 Tables and 1 Figure

18 **Table 1:** Characteristics of the study population, and duration of the Nanoduct™ sweat

19 test in newborns at the age of four days and four weeks (N = 260)

20 **Table 2:** Proportion of successfully completed Nanoduct™ tests (success rate) in healthy infants at the
21 age of four days and four weeks

22 **Table 3:** Normal values of sweat conductivity (mmol/l) in healthy infants at the age of four days and
23 four weeks

24 **Table 4:** Results of studies looking at success rate and conductivity of the Nanoduct™ sweat test

25 **Figure 1:** Distribution of normal conductivity values (histogram) at the age of four days
26 and four weeks

27 **ABSTRACT**

28 **Background:** Nanoduct™ is a simple and practical sweat analysis system measuring conductivity in situ.
29 It requires only three microlitres of sweat, making it especially applicable to newborns.

30 **Methods:** We measured conductivity in 260 healthy term infants at the age of four days, and again at
31 four weeks to determine the proportion of successful tests, test duration, and normal values for sweat
32 conductivity in newborns.

33 **Results:** Sufficient sweat was collected in 159/260 of four-day olds (61%), and in 225/239 of four-week
34 olds (94%). Mean (sd) test duration was 27 (5) and 25 (5) min. Mean (sd, range) conductivity was 53
35 mmol/l (16, 8–114) at age four days, and 36 (9, 12–64) at four weeks.

36 **Conclusions:** Determination of sweat conductivity using Nanoduct™ cannot be recommended for
37 four-day old newborns. However, at the age of four weeks the success rate is high (94%), and
38 conductivity values at that age are comparable to older healthy children.

39 **INTRODUCTION**

40 The sweat test is a key component for establishing a diagnosis of cystic fibrosis (CF) in infants with a
41 positive result in newborn screening (NBS)^{1,2}. Collecting sufficient sweat for analysis is a challenge in
42 small infants and some guidelines recommend delaying the test until the infant is more than two
43 weeks of age or weighs more than 3 kg³⁻⁵. Although for infants below three months of age test failure
44 rates of up to 40% have been reported⁶⁻¹⁰, North American recommendations aim for a failure rate of
45 10% or less for sweat tests in NBS programs^{2,6}.

46 Since the invention of the sweat test based on the pad method by Gibson and Cooke 60 years ago^{11,12},
47 sweat testing has evolved. The nowadays widely accepted Macroduct™ collection system needs 15 µl
48 of sweat to analyze chloride concentration^{2,4}, while the sweat flow sensor of the Nanoduct™ sweat
49 test system requires only 3 µl. This makes it especially applicable to newborns. However, it does only
50 measure conductivity¹³.

51 Studies using Nanoduct™ discriminated well between children with and without CF¹³⁻¹⁷. This is also
52 true for other sweat tests that measure conductivity instead of chloride concentration, for instance
53 the Sweat-Check™¹⁸⁻²². Despite this, the European practice guidelines for neonatal screening and the
54 US guidelines for diagnosis of CF do not recommend measuring sweat conductivity to diagnose CF^{1,2}.
55 This study assessed the feasibility of sweat testing with the Nanoduct™ system in healthy newborns
56 at the age of four days and four weeks, by determining the duration of the tests and the proportion
57 of tests that were successful. We also wanted to determine the normal values of sweat conductivity
58 for Nanoduct™ in this age group.

59 **METHODS**

60 **Subject and study design**

61 We conducted this single-center study from June 2013 to December 2014 with 260 healthy term
62 infants born in the maternity unit of the Department of Gynaecology at the Cantonal Hospital St.
63 Gallen in Switzerland. Each infant was tested twice with the Nanoduct™ (Wescor, Utah, USA) sweat
64 system, at age 3–4 days and 3–4 weeks. We chose these age groups because in Switzerland NBS with
65 the Guthrie test is performed at the age of 3–4 days, before infants leave the birth clinic^{23,24}. When
66 they are 3–4 weeks old, children with a positive NBS result are recalled to a CF center for sweat testing.
67 We asked all parents of healthy infants born in the hospital during this period to participate, except
68 those whose infants were born prematurely (gestational age <37 weeks), had a birth weight below
69 3000 g, or were sick, presenting symptoms such as oedema, hyperbilirubinemia, signs of dysmaturity,
70 malnourishment, or a systemic disease. We also excluded children whose parents did not speak
71 German, and all those who were discharged on a weekend.

72

73 **Nanoduct™ sweat test analysis system**

74 The Nanoduct™ sweat test is a micro-flow conductometric device, which induces and analyzes the
75 conductivity of sweat in situ while attached to a patient. The procedure is described in detail
76 elsewhere^{13,20}. In brief, iontophoresis using small Pilogel™ iontophoretic discs and direct current
77 supplied by the Nanoduct™ inducer/analyzer is followed by a continuous-flow analysis of sweat
78 conductivity using a conductivity sensor. The continuous-flow principle allows display of the initial
79 sweat rate in grams per square meter of skin surface per minute, which is important in accepting sweat
80 test results (valid results: ≥ 1 g/m²/min). Its continuous sweat flow sensor requires only 3 μ l of sweat.
81 The value of conductivity is expressed as mmol/l eq NaCl. This is not equal to a quantitative chloride
82 measurement; its displayed equivalent is approximately 20 mmol/l higher than the sweat chloride
83 concentration because of additional anions such as lactate and bicarbonate^{18,19,21}.

84 The sweat tests at the age of four days were carried out in the Department of Gynaecology of the
85 Cantonal Hospital St. Gallen by two trained and experienced persons (Agnieszka Mazur and a research
86 nurse). The sweat tests at the age of four weeks were performed at infants' homes or in the Children's
87 Hospital in St. Gallen by the same two persons. The Nanoduct™ device was placed on a forearm or a
88 leg. The sweat test was considered valid (that is, a successful test) if the sweat rate was ≥ 1 g/m²/min,
89 and as not valid if the sweat rate was lower (< 1 g/m²/min) or zero (no sweat rate displayed on
90 Nanoduct™).

91 The parents were told by the technician that the test results needed to be interpreted by the doctor,
92 and were then informed by the investigator after the end of the two tests only if the result was
93 regarded as ambiguous or not normal. If the second sweat test at the age of 4 weeks was above an
94 upper limit of 60 mmol/l, we offered the parents another sweat test at the Children's Hospital,
95 followed by a chloride measurement using the Macroduct™ method, if the conductivity value was still
96 elevated.

97

98 **Statistical analysis**

99 We calculated the proportion of successful tests as the number of tests with valid results divided by
100 the total number of tests performed. We compared the proportion of successful tests across quintiles
101 of body weight at the day of the test, and across quintiles of weight loss between birth and the test
102 day, in percent of birth weight, using tests for trend to assess statistical significance. We checked the
103 distribution of quantitative data (duration of tests, conductivity) using histograms, Q–Q plots, and
104 Shapiro–Wilk and Shapiro–Francia tests for normality. Since data were normally distributed, we
105 described sweat conductivity (mmol/l) and test duration (minutes) as mean values and standard
106 deviations (sd), and determined 95% (99%) reference intervals for sweat conductivity as the mean ± 2
107 (± 3) standard deviations. For infants with paired data, we compared agreement with a Bland Altman
108 plot, displaying the mean of the two values on the x axis versus the difference between the two

109 measurements on the y axis. We analyzed the data using STATA version 13.1 (StataCorp. 2005. Stata
110 Statistical Software: Release 13.1 StataCorp LP, College Station, TX, USA).

111

112 **RESULTS**

113 Between July 1, 2013 and Dec 31, 2014, 2231 infants were born in the maternity unit in St. Gallen. Of
114 these, 366 were excluded because of preterm birth, 707 because of low birth weight and 898 for
115 different reasons (discharge at weekends, parent's insufficient knowledge of the German, clinical
116 symptoms such as hyperbilirubinemia, or no parental consent). None of the children born in this unit
117 had a positive CF-NBS result. Parents of 260 newborns agreed to participate (Table 1). In total, 239
118 infants had two sweat tests (Table 2). Twenty-one infants were lost to follow-up at four weeks, twelve
119 due to parental refusal, six due to no answer to repeated phone calls, and three because of
120 unavailability of the sweat test equipment on the test day. The time needed to perform the sweat test
121 was on average 27 min (5–40, sd 5 min) at the age of 4 days, and 25 min (14–40, sd 5 min) at the age
122 of 4 weeks.

123 The proportion of successfully conducted sweat tests was 61% at age 4 days, and 94% in four-week
124 olds (Table 2). At age four days, 159 of 260 infants (61%) produced enough sweat ($\geq 3 \mu\text{l}$), while 23
125 (9%) had an insufficient quantity and 78 (30%) produced no sweat at all (Table 2). The proportion of
126 successful tests was positively associated with body weight, varying from 49% for those in the lowest
127 quintile (2.8–2.9 kg) to 69% in those in the highest (3.7–4.6 kg, $p = 0.024$). The proportion of successful
128 tests was also associated with weight loss: it was lowest (48%) in those infants who had lost most
129 weight between birth and the day of measurement ($p = 0.034$). At age four weeks, 225 of 239 infants
130 (94%) produced sufficient sweat. Five children (2%) had insufficient quantities and nine (4%) had no
131 sweat. At this age, the proportion of successful tests was $>90\%$ across all quintiles of body weight,
132 with no clear trend ($p = 0.221$).

133 At four weeks of age the sweat conductivity values were normally distributed (Table 3 and Fig. 1). The
134 mean value was 35 mmol/l (range 12–64) and the upper limit of the 95% reference interval (mean +

135 1.96 sd) was 53 mmol/l. Two children (2%) had a conductivity value above the threshold of 60 mmol/l,
136 and were invited for another sweat test at the Children's Hospital; one mother refused to come and
137 the other child had a normal sweat test. The Bland Altman plot illustrates that the 2nd measurements
138 were in average 18 mmol/l lower, with outliers at the two extremes, reflecting measurements that
139 were technically unsatisfactory at one of the two time points (online Fig. 1).

140

141 **DISCUSSION**

142 This is the first study to generate normal reference values for conductivity using the Nanoduct™ sweat
143 test in an unselected group of healthy newborns. Its main strength is the relatively large and
144 homogeneous population of healthy newborns and their young age. The study included only children
145 who cleared the CF-NBS program as healthy, non-CF children, but because we did no genetic testing
146 in these children we cannot exclude the possibility that some children were healthy CF-carriers or had
147 mild ('atypical') forms of CF²³. Based on an incidence of CF in Switzerland of about 1 in 3000^{23,24}, we
148 expect that our study population could include about five CF carriers. Yet even if conductivity were
149 slightly higher in carriers, this low number would not have strongly influenced our results. All sweat
150 tests were performed strictly according to guidelines, with the same equipment, by two trained testers
151 in a single center, which minimizes the probability of differences in test results caused by variations in
152 material or procedures. However, the very fact that this was a carefully conducted, single-center study
153 that excluded low birth weight and sick infants might reduce the generalizability of the results.
154 Although we included twice as many data points than generally requested as minimum number to
155 determine reference values (120)²⁵, a larger sample would have been even more informative.

156 We are aware of only six other studies that have investigated the performance of the Nanoduct™
157 sweat test system and reported values in the normal range for conductivity (Table 4). None of the
158 studies included children who could be considered totally healthy or normal: four analyzed data of
159 patients with chronic respiratory symptoms referred to outpatient clinics for exclusion of CF^{13-15,26},
160 and two reported on sweat tests in neonates with a positive NBS result^{16,17}. Still, our conductivity

161 results for healthy four-week old infants are comparable with these studies of older infants, children,
162 and adults, which all reported mean values between 30 and 42 mmol/l. When comparing our results
163 with reference values for sweat chloride in 5–6 week-old infants using the Macroduct™ collection
164 system (median 12 mmol/l (IQR 9–15) ²⁷, our equivalent conductivity values at four weeks (median 35
165 mmol/l, IQR 30–41) are on average 23 mmol/l higher (exactly the same value which was found by
166 Mastella et al. ²¹ in his healthy control group), but the distribution approximated a normal distribution
167 in both studies.

168 The proportion of successfully conducted sweat tests in our study was low (61%) at age 4 days, but
169 excellent (94%) in four-week old. This is comparable to our previous multicenterstudy, where it was
170 48% in newborns (55% in term, 31% in preterm), 90% in older infants, and 95% in children and adults
171 ¹⁴. Our results also confirm earlier studies focusing on the Macroduct™sweat test, which reported
172 lower proportions of successful tests in very young infants. For example, the Massachusetts NBS
173 program found that the percentage of sweat tests interpreted as “quantity not sufficient” (QNS) was
174 17% at the age of 2 weeks, 12% at 3 weeks, 8% at 4 weeks, and 5% at 5 weeks ¹⁰.

175 In studies that have compared the Macroduct™directly with the Nanoduct™, Nanoduct™ usually
176 performed better. For example, the Vernooij-van Langen et al. study reported a difference in QNS
177 rates of 7% for Nanoduct™ tests, and 22% for Macroduct™ tests ($p = 0.002$) ¹⁷, while the Barben et al.
178 study reported QNS results of 3% for Nanoduct™ tests and 15% for Macroduct tests ($p = 0.003$) ¹³.

179 What do these results mean? The current guidelines for CF-NBS in Europe and the guidelines for CF
180 diagnosis in all age groups do not recommend conductivity measurements for the diagnosis of CF in
181 newborns ^{1,2}. Our data suggest that at an age of four weeks, if not before, the Nanoduct™ sweat test
182 method could be a useful tool for diagnosing CF in NBS programs, using 50 mmol/l as an upper limit,
183 the threshold recommended by the CFF for performing confirmatory sweat chloride measurements ⁴.
184 Twelve of the 225 children in our study (5%) had a conductivity above the threshold of 50 mmol/l, and
185 2 (2%) above 60 mmol/l, the suggested upper normal value by the manufacturer ¹³.

186 In CF-NBS programs, a definite diagnosis should be made as quickly as possible to reduce parental
187 anxiety²⁸. We believe that Nanoduct™ is a good tool for excluding CF at the age of 3–4 weeks, as the
188 success rate is high and the results are available within half an hour. In contrast, chloride
189 measurements need hours to get a result, and the collection in newborns is challenging due to the
190 higher quantity of sweat (15 µl), and thus high failure rates infants⁶⁻⁹. In Switzerland, children with a
191 positive CF-NBS result are recalled for a sweat test at the age of 3–4 weeks. The failure rate of the
192 Nanoduct™ at this age (6%) is acceptable according to the current recommendations, which aim for
193 less than 10%³⁻⁶. Within the Swiss CF-NBS program, we use the Nanoduct™ and the Macroduct™ test
194 in parallel since 2011, to reduce delays for parents of infants where the Macroduct™ is not successful
195^{13,14,17}. The Nanoduct™ test system has the potential disadvantage that parents can spot the results,
196 because conductivity is displayed on the screen of the apparatus attached to the child. Our lab
197 technicians do not report any result. Parents who ask the lab technician are told that the data quality
198 needs to be checked by the physician before results can be interpreted. In clinical routine in the Swiss
199 neonatal screening, a CF specialist is called as soon as the Nanoduct™ result is available and
200 informs the parents.

201 This study is the first to provide normal ranges for sweat conductivity using the Nanoduct™ sweat test
202 system for healthy newborns. Determination of sweat conductivity using Nanoduct™ cannot be
203 recommended for four-day old newborns. However, at the age of four weeks the success rate is high
204 (94%), and conductivity values at that age are comparable to those reported for older healthy children.
205 The generated reference range improves the available evidence on the validity of this method. This
206 suggests that Nanoduct™ might be a suitable sweat test for NBS programs in which children are seen
207 in the first months of life.

208 Part of this work has been presented at the European Cystic Fibrosis Conference in Brussels, Belgium,
209 June 10–13, 2015.

210

211

212 **CONFLICT OF INTEREST**

213 There is no conflict of interest. The study was sponsored by the Lung League of Canton St. Gallen,
214 Switzerland, and the pulmonology funds of the Children's Hospital of Eastern Switzerland.

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292 **TABLES**293 **Table 1**

294 Characteristics of the study population, and duration of the Nanoduct™ sweat test in newborns at the
 295 age of four days and four weeks (N = 260). ^aMeasurement available for 259 out of 260 infants.

	Mean	sd	Range
Baseline (n = 260)			
Birth weight (g)	3519	381	3000–4845
Gestational age (weeks)	40.9	1.1	36.3–42.1
First measurement (at age 4 days, n = 260)			
Weight (g) ^a	3346	363	2780–4600
Age (days) ^a	4	2	2–8
Test duration (min)	27	5	15–40
Second measurement (at age 4 weeks, n = 239)			
Weight (g)	4184	492	3200–5500
Age (days)	27	5	19–64
Test duration (min)	25	5	14–40

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298 **Table 2**

299 Proportion of successfully completed Nanoduct™ tests (success rate) in healthy infants at the age of four days and four weeks. Abbreviations: CI, confidence
 300 interval; n, number of children; n/a, not applicable. ^a Values in quintiles overlap due to rounding.

	Age 4 days (N = 260)					Age 4 weeks (N = 239)				
	Weight	n	%	95% CI	p-Value	Weight	n	%	95% CI	p-Value
All children		159	61	55–67			225	94	91–97	
By quintiles of weight ^a					0.024					0.221
	2.8–2.9	26	49	36–62		3.2–3.7	45	94	82–98	
	3.0–3.2	30	57	44–70		3.8–4.0	50	91	78–96	
	3.2–3.4	33	66	52–78		4.0–4.3	39	91	77–97	
	3.4–3.6	34	65	51–77		4.3–4.6	47	100	n/a	
	3.7–4.6	36	69	55–80		4.6–5.5	44	96	84–99	
By weight loss (in % birth weight)					0.034					
	–7.9–2.9	34	65	51–77						
	3.0–4.5	37	73	59–83						
	4.6–5.8	31	60	46–72						
	5.9–7.1	32	62	48–74						
	7.2–13	25	48	35–62						

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302

303 **Table 3**

304 Normal values of sweat conductivity (mmol/l) in healthy infants at the age of four days and four weeks

Age	Mean	sd	Range	95% reference interval	99% reference interval	Percentiles								
						1%	5%	10%	25%	50%	75%	90%	95%	99%
4 days	53	16	8–114	22–84	7–100	17	31	36	44	53	62	72	81	98
4 weeks	35	9	12–64	19–53	10–61	17	22	26	30	35	41	46	51	59

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307 **Table 4**

308 Results of studies looking at success rate and conductivity of the Nanoduct™ sweat test

Authors, journal, year of publication	Ref.	N	Age	Country	Inclusion criteria	Success rate	Mean conductivity for participants without CF mmol/l (sd, range)
<i>Studies including children with respiratory symptoms</i>							
Barben et al., J Pediatr, 2005	[13]	111	3 weeks–60 yrs. (median: 1.3 yrs.)	Switzerland	90 outpatients with pulmonary symptoms, 21 with CF	97%	36 (9, 17–59)
Losty et al., Ann Clin Biochem, 2006	[15]	100	14 days–56 yrs. (median in healthy: 1.1 yrs.)	Wales	Patients requiring sweat tests (58 healthy, 36 CF, 6 nonclassic CF)	97%	39 (median) (range 15–62)
Desax et al., Eur J Pediatr, 2008	[14]	1041	1 day–60 yrs.	Switzerland	Patients requiring sweat test, including 66 newborns (term and preterm), 237 infants, 690 children, 48 adults >16 yrs.	94% (overall) 48% (newborns) 90% (infants) 95% (children)	Overall: 37 (2–108) Infants: 51 (35–76)
Sezer et al., J Clin Med Res, 2013	[26]	2664	7 days–17 yrs. (median: 17 months)	Turkey	Patients requiring sweat test, including 366 infants <6 months	NA	Infants <6 months: 35 (14, 12–131)
<i>Studies including children with a positive NBS result</i>							
Sands et al., Folia Histochem Cytobiol, 2010	[16]	528	4–8 weeks	Poland	Infants with a positive NBS result (480 healthy, 42 CF, 6 inconclusive)	NA	30 (8, 17–57)
Vernooij-van Langen et al., Eur J Pediatr, 2015	[17]	108	12–90 days (mean: 30 days)	Netherlands	Infants with a positive NBS result (84 healthy, 17 CF, 7 inconclusive)	93%	42 (11, 26–83)
<i>Studies including healthy newborns</i>							
Kuehni et al. (this study)	NA	260	4 days	Switzerland	Healthy infants from maternity clinic	61%	53 (16, 8–114)
		239	4 weeks			94%	35 (9, 12–64)

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311 **FIGURES**

312 **Figure 1**

313 Distribution of normal conductivity values (histogram) at the age of four days and four weeks.

314 a. Sweat test four days after birth: normal values (mean \pm 2 sd) indicated by red bar b. Sweat test four

315 weeks after birth: normal values (mean \pm 2 sd) indicated by red bar

