

1 THE CF QUANTUM[®] SWEAT TEST: NOT READY FOR CLINICAL USE

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9 Title: The CF Quantum[®] Sweat Test: Not Ready For Clinical Use

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12

13 **Abstract**

14 The CF Quantum Test (CFQT) showed promise in a previous pilot study, however there
15 was greater imprecision in one patch lot. Following the pilot study, the manufacturer changed
16 their fabricating procedures. Subjects with previously diagnosed CF (cystic fibrosis) or subjects
17 who required a sweat test for clinical reasons were invited to undergo the CFQT research test and
18 a conventional sweat test (Macroduct® collection and chloride analysis via the ChloroChek®
19 chloridometer). Previously diagnosed CF (n= 41) and CRMS (CFTR-related metabolic
20 syndrome)/CFSPID (cystic fibrosis screen positive inconclusive diagnosis) (n= 3) patients and
21 patients who required a sweat test for clinical indications (n=22) were recruited to have bilateral
22 CFQT along with the Macroduct® test performed on the same day. Pairs of data from each test
23 were plotted as a correlation graph, bias plot and Bland Altman plot. Coefficient of variation
24 (CV) between extremities and QNS rates for both tests were calculated.

25 The CV between left and right extremities was greater in the CFQT (9.5%) compared to
26 the Macroduct® (4.8%). The QNS (quantity not sufficient) rates of the two tests were
27 comparable (CFQT: 6.8%; Macroduct®: 6.0%). There was greater imprecision with the CFQT
28 results. The diagnostic agreement between the two tests was 100% positive percent agreement
29 (95% CI: 90 –100%), 100% negative percent agreement (95% CI: 80 –100%), 67% intermediate
30 percent agreement (95% CI: 30% –80%), and 92% overall percent agreement (95% CI: 80 –
31 100%).

32 This follow-up study demonstrated that the CFQT is not analytically nor diagnostically
33 reliable. (Clinicaltrials.gov identifier NCT01345617)

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35

36 MeSH Keywords: sweat glands; cystic fibrosis; diagnostic tests, routine; bias, statistical

37 Abbreviations:

38 CF: cystic fibrosis

39 CFF: Cystic Fibrosis Foundation

40 CFSPID: cystic fibrosis screen positive inconclusive diagnosis

41 CFTR: cystic fibrosis transmembrane regulator

42 CFQT: Cystic Fibrosis Quantum Test

43 CRMS: Cystic Fibrosis Related Metabolic Syndrome

44 CV: coefficient of variation

45 POCT: point of care test

46 SD: standard deviation

47 QNS: quantity not sufficient

48

49 **Introduction**

50 Cystic fibrosis (CF) is the most common, life-shortening autosomal recessive disease in
51 Caucasians, occurring with a frequency of approximately 1 case in every 3,300 live births. The
52 basic defect in CF is dysfunction of the cystic fibrosis transmembrane regulator (CFTR) protein.¹
53 CFTR is a chloride channel that is expressed in sweat ducts, respiratory epithelial cells,
54 pancreatic ductules and exocrine cells in the reproductive system (the vas deferens and cervix).
55 Common symptoms of CF are recurrent pulmonary infections leading to progressive loss of lung
56 function, infertility in the majority of males due to congenital bilateral absence of the vas
57 deferens and exocrine pancreatic insufficiency resulting in malabsorption of protein and fat with
58 subsequent failure to thrive in infants. The pulmonary infections accounts for the majority of
59 morbidity and mortality in CF.

60 Although the discovery of the CFTR gene in 1989¹ opened the door to a genetic
61 diagnosis, there are patients with CF in which two variants cannot be identified. Thus, sweat
62 chloride testing^{2,3} will always be necessary for diagnostic purposes and to assess the effect of
63 protein modifier drugs. The quantitative pilocarpine iontophoresis sweat test was first described
64 by Gibson and Cooke in 1959.⁴ Pilocarpine, a cholinergic agonist, is delivered to the sweat
65 glands by iontophoresis: a small electrical charge delivered for 5 minutes drives pilocarpine into
66 the skin. This is followed by a 30 minute collection period in which sweat is collected into gauze
67 or filter paper. After sweat collection, there are steps to elute sweat out of the gauze or filter
68 paper. Lastly, sweat chloride analysis occurs via quantitative analysis using a
69 coulometric/amperometric chloridometer. There are many steps in this process in which errors
70 can and do occur. An alternative collection method which is approved by the Cystic Fibrosis
71 Foundation (CFF) is the Macroduct® method.⁵ Similar to the Gibson-Cooke method, there is a 5

72 minute pilocarpine iontophoresis step. In the Macroduct® method, sweat is collected in
73 microbore tubing for up to 30 minutes. The pure sweat sample can be placed directly into a
74 chloridometer for sweat chloride analysis. Although there are fewer steps in this method
75 compared to the Gibson-Cooke method, technicians must be meticulous in all aspects of the
76 procedure: iontophoresis delivery of pilocarpine, sweat collection and sweat chloride analysis.⁵
77 Potential errors can also occur with the Macroduct® procedure.

78 A novel point of care test (POCT), the CFQT (CF Quantum Test) (PolyChrome Medical
79 LLC, Eden Prairie, MN), was developed in an effort to simplify the determination of sweat
80 chloride.⁶ This utilizes an electrode and controller set that can be worn on the arm for the
81 delivery of pilocarpine. This differs from the iontophoresis device for the Gibson-Cooke and
82 Macroduct® devices in which the patient is tethered by wires to a box. After iontophoresis,
83 sweat is collected on a patch containing silver nitrate. An ion exchange reaction occurs between
84 the chloride in the sweat and the nitrate, resulting in silver chloride which is an insoluble white
85 precipitate in the center of the patch. In theory, the surface area of the white precipitate is
86 proportional to the sweat chloride value. After collection of sweat, the patch is placed into an
87 analyzer which consists of a camera and computer software. The sweat volume and chloride
88 level is derived by computer software in the analyzer. The CFQT does not involve the handling
89 of any liquids (including sweat) and is a simpler procedure compared to Gibson-Cooke and
90 Macroduct®. All methods for sweat collection and analysis require a minimum amount of sweat;
91 not because of the instrumentation involved, but because a valid sweat chloride result depends on
92 adequately stimulated sweat glands. Inadequately stimulated sweat glands will result in a
93 decreased volume of sweat collected and can lead to false negative results. These inadequate
94 sweat samples are referred to as “Quantity not sufficient” (QNS).⁷ A national survey of CFF

95 accredited care centers demonstrated that QNS rates could range as high as 40%.⁸ Thus, there is
96 a critical need for decreasing QNS rates with currently approved tests or development of a new
97 test that has lower QNS results.

98
99 Although the CFQT was feasible in the previous three site multicenter study, there was
100 a patch lot in one center that showed greater imprecision than the patch lots tested in the other
101 two centers.⁶ Following the results of the study, the manufacturer identified areas in the
102 processing of the patches that could account for the lot-to-lot variability in the results and made
103 changes to the patch manufacturing. After these modifications, this second multicenter study
104 was conducted. The aim of the study was to determine the analytic and diagnostic validity of the
105 CFQT and to compare the QNS rates of CFQT to collection of sweat in the Macroduct®.

106

107 **Materials and Methods**

108 A multicenter study (clinicaltrials.gov identifier NCT01345617) from 06 February 2017
109 to 27 September 2017, enrolled 44 subjects with previously diagnosed CF or CRMS⁹ (cystic
110 fibrosis related metabolic syndrome)/CFSPID¹⁰ (cystic fibrosis screen positive inconclusive
111 diagnosis) and 22 subjects who required a sweat test on clinical grounds (either as follow-up of
112 an abnormal CF newborn screening test, or their provider ordered a sweat test). Subjects were
113 invited to undergo a CFQT and sweat test via Macroduct® collection and analysis with the
114 ELITech ChloroChek® chloridometer Model 3400 (Logan, Utah). To assure that the reference
115 method for collection and analysis (Macroduct®/ChloroChek®) was correctly performed
116 according to CFF, CLSI (Clinical and Laboratory Standards Institute) and manufacturer's
117 guidelines, the sweat testing laboratories were visited by the principal author of the CLSI
118 guideline document on sweat testing and a written evaluation was provided. It was mandatory
119 that the suggestions for improvement were implemented prior to starting the study at each site.
120 The Institutional Review Board approved the study at each site and written informed consent was
121 obtained from parents/patients (and assent, if applicable) prior to commencing with any study
122 procedures.

123 Macroduct® sweat stimulation and collection was performed bilaterally (i.e. left and right
124 arm) per the CLSI guidelines.⁷ Pilocarpine iontophoresis occurred for 5 minutes and collection of
125 sweat into Macroduct® occurred for 30 minutes. If 15 µL of sweat was not collected within 30
126 minutes, then the test was deemed QNS. After collection and quantitation of sweat volume, the
127 sweat was titrated using the EliTech ChloroChek chloridometer according to the manufacturer's
128 protocol. The chloridometer contains a silver electrode which releases silver into an acid solution
129 containing the sweat sample and a timer is started. Chloride in the sweat sample combines with

130 the silver, forming insoluble silver chloride. When all of the chloride has been precipitated as
131 silver chloride, the measuring electrode detects the appearance again of free silver ions and the
132 timer is stopped. The amount of time that silver is generated is proportional to the chloride
133 concentration and is compared to an internal calibrator to convert the time to mmol/L. Three
134 levels of commercial control solutions (low, intermediate and elevated chloride concentration)
135 were assayed everyday of sample testing and had to be within the accepted range established by
136 the manufacturer before study samples could be analyzed.

137 The CFQT was performed according to the manufacturer's protocol. Sweat stimulation
138 was performed bilaterally by pilocarpine iontophoresis for 8 minutes followed by the application
139 of a collection patch (figure 1). The maximum allowed time of sweat collection was 20 minutes.
140 The collection of sweat on the patch occurred until the sweat front (a red circle on the patch)
141 reached a stop test ring of 15 mm in diameter. The test was deemed QNS if the sweat front did
142 not reach the stop ring by 20 minutes. After an adequate quantity of sweat was obtained, the
143 patch was removed from the skin and allowed to dry for 15 minutes. The patch was then placed
144 in the analyzer (figure 2) and a sweat volume and chloride value were derived. The CFQT patch
145 detection is based on an ion exchange reaction between the chloride in the sweat and silver
146 nitrate in the test patch. When chloride ions in the sweat sample come into contact with the silver
147 chromate in the patch, silver chloride, an insoluble white precipitate, forms in the center of the
148 patch. An outer red ring, the sweat front, indicates the amount of sweat collected in the patch.
149 The surface area of the white precipitate in the middle of the patch compared to the total surface
150 area within the red ring is directly proportional to the sweat chloride value. The camera and
151 computer software in the analyzer were assessed everyday of sample testing using scanned

152 photographs of three levels of results representing low, intermediate and elevated chloride
153 concentrations and the values needed to be within the preset range.

154 If a subject was over 6 months of age, the Macroduct® collection and CFQT both
155 occurred on the forearm. For subjects under 6 months of age (n=10), there was inadequate space
156 to perform both tests on the forearm. Thus, the Macroduct® collection occurred on the forearm
157 and the CFQT occurred on the thigh. Areas of the skin only underwent sweat stimulation and
158 collection once. Pilocarpine iontophoresis for the Macroduct® and sweat collection occurred
159 first, and the CFQT was performed second. It was possible to perform the CFQT during the 30
160 minute Macroduct® collection time.

161 Agreement of sweat chloride values between left and right extremities were per the CLSI
162 guidelines⁷: for sweat chloride values ≤ 60 mmol/L, the extremities must be within 10 mmol/L;
163 and for sweat chloride values >60 mmol/L, the extremities must be within 15 mmol/L.
164 Exceeding these thresholds resulted in an invalid test.

165 The interpretation of sweat chloride values was per the updated guidelines from the
166 United States CF Foundation.² For all ages of subjects, a sweat chloride value of ≤ 29 mmol/L
167 was normal, 30-59 mmol/L was intermediate, and ≥ 60 mmol/L was abnormal and consistent
168 with CF. With bilateral sweat testing being performed, the interpretation of the results used the
169 higher of the two sweat chloride values.

170 *Sample size*

171 A sample size of 300 subjects, including n=150 subjects with previously diagnosed CF or
172 CRMS and n=150 subjects referred to the sweat test lab for clinical reasons, was proposed for
173 this study. It was estimated that of the 150 subjects referred to the sweat test lab on clinical
174 grounds, at least 120 would have sweat chloride values in the normal range. Hence, with a

175 sample size of at least 150 subjects with sweat chloride values in the non-normal range and 120
 176 subjects with sweat chloride values in the normal range and assuming a true
 177 sensitivity/specificity of 0.95, the sensitivity and specificity of the CFQT would be estimated
 178 with a standard error of less than 2% and the lower bound of the two-sided 95% confidence
 179 interval of the sensitivity and specificity would exceed 0.9.

180 *Statistical analysis*

181 Sweat chloride measurements obtained by Macroduct®/ChloroChek® and CFQT were
 182 considered 2 variables and were summarized in terms of number of observations, means,
 183 standard deviations and ranges. The coefficient of variation between paired extremities by test
 184 (Macroduct®/ChloroChek® and CFQT) were calculated. Bias assessment was conducted
 185 according to CLSI guidelines for method comparison and bias estimation.¹¹ A visual check for
 186 the relationship between the 2 variables was performed by evaluating (1) scatterplot of CFQT
 187 values versus Macroduct values, (2) bias plot of CFQT minus Macroduct®/ChloroChek® versus
 188 Macroduct®/ChloroChek® values, and (3) bias plot of individual results deltas versus the mean
 189 differences between the two tests (Bland Altman plot).¹² The proportion of QNS sweat tests were
 190 compared between Macroduct® collection and CFQT using Fisher's exact test. All p-values
 191 were two-sided and p-values <0.05 were considered statistically significant.

192 Categorization of diagnosis by test were summarized as follows:

193

		Macroduct/Chlorocek		
		>60 mmol/L	30-59 mmol/L	≤29 mmol/L
CFQT	>60 mmol/L	A	B	C
	30-59 mmol/L	D	E	F

	≤ 29 mmol/L	G	H	I
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195 Overall, Positive, Negative and Intermediate Percent Agreement was calculated as follows:

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197 Overall Percent Agreement = $100 \times (A+E+I) / (A+B+C+D+E+F+G+H+I)$

198 Positive Percent Agreement = $100 \times A/(A+D+G)$

199 Negative Percent Agreement = $100 \times I/(C+F+I)$

200 Intermediate Percent Agreement = $100 \times E/(B+E+H)$

201

202 Positive, Intermediate, and Negative Percent Agreement were reported along with the

203 corresponding 95% confidence intervals (CI).

204

205 **Results**

206 There were 66 subjects at 4 CF centers who completed the study. There were 22 subjects
207 at the University of Wisconsin (center 1), 27 subjects at the University of Minnesota (center 2),
208 13 subjects at the University of Michigan (center 3) and 4 subjects at the University of Alabama-
209 Birmingham (center 4). The characteristics of the subjects are in table 1.

210 For Macroduct®/ChloroChek®, of the potential 132 test results (66 subjects who had
211 bilateral tests performed), there was one technical problem with the chloridometer. (The stirring
212 bar in the ChloroChek® instrument stopped. The instrument was turned off and on, and although
213 a final result was obtained, this must be considered an invalid test.) For the CFQT, there were 13
214 technical problems in which no results were available: the analyzer generated an error code on 9
215 patches and there was a stimulator error bilaterally in two subjects. One CFQT result was invalid
216 due to a sweat chloride of >160 mmol/L. Means, standard deviations (SD), ranges, coefficient of
217 variation (CV) between extremities and QNS rates for the two methods are in table 2. Of the 66
218 subjects, 10 were infants with a positive newborn screen for CF. The Macroduct® QNS rate in
219 these infants was 20% and the CFQT QNS rate in these infants was 15%. (For sweat chloride
220 values of <10 mmol/L (the lower limit of detection for both methods), the value was rounded up
221 to 10 mmol/L.) Although there was no significant difference in the mean sweat chloride value
222 between the CFQT and Macroduct®/ChloroChek®, the mean sweat chloride value per CFQT
223 reflects the positive bias (discussed below for figure 4). Additionally, the higher CV between
224 extremities for CFQT reflects greater imprecision.

225 There were 9 infants who had the CFQT performed on the thigh. (One study site
226 performed Macroduct® collection and CFQT both on the forearms in one infant.) Although this
227 yields a potential 18 comparisons with bilateral testing, because of QNS tests with both methods

228 and stimulator errors with the CFQT, there were only 7 tests available for comparison of
229 Macroduct®/ChloroChek® versus CFQT. None of these infants had CF; the mean values for
230 Macroduct®/ChloroChek® was 15 mmol/L compared to a mean value of 19 mmol/L for CFQT
231 (again reflecting a positive bias of the CFQT, but the number of tests is too small for statistical
232 comparison).

233 The results of all bilateral Macroduct®/ChloroChek® were within the pre-stated
234 agreement of each other. For the CFQT, there were 6 subjects in which the results were invalid
235 due to exceeding the bilateral level of agreement.

236 The average sweat collection time for the CFQT was 10 minutes versus 30 minutes for
237 the Macroduct® ($p < 0.0001$).

238 The method comparison graph of CFQT results versus Macroduct®/ChloroChek® is in
239 Figure 3. The Pearson correlation coefficient = 0.97, y-intercept = -0.84, slope = 1.10 and $SD_{x/y}$
240 = 0.88. In a method comparison graph, the values obtained by the reference method
241 (Macroduct®/ChloroChek®) are plotted on the x axis and values obtained by the new method
242 (CFQT) are plotted on the y axis. If identical values were obtained with both methods, the
243 strength of the correlation would yield a correlation coefficient of 1.00, the y-intercept would be
244 0.00, the slope would be 1.00 and the $SD_{x/y}$ would be 0.00. In general, these values are
245 interpreted such that the slope indicates proportional error, the y-intercept indicates constant
246 error and the $SD_{x/y}$ indicates the imprecision of the values around the correlation line.¹³

247 Figure 4 is the bias plot of CFQT minus Macroduct®/ChloroChek® plotted against
248 Macroduct®/ChloroChek®. The circled symbols are results from the second patch lot in this
249 study. Only Center 2 had progressed in the study to the point of using the second patch lot. The

250 majority of the symbols on the bias plot are above zero, thus signifying a positive bias of the
251 CFQT results compared to Macroduct®/ChloroChek®.

252 Figure 5 is the Bland Altman plot of the data. The solid line is the mean of the differences
253 (-5.8 mmol/L) and the dotted lines are ± 1.96 SD of the differences (13.5 and -24.8 mmol/L).
254 Similar to the bias plot, the Bland Altman plot shows significant scatter between the two
255 techniques and that there are 4 paired tests in which there are extreme outliers (more than 2 SD
256 of the differences.)

257 In assigning diagnostic categories, the positive percent agreement was 100% (95% CI: 90
258 -100%), the negative percent agreement was 100% (95% CI: 80 -100%), the intermediate
259 percent agreement was 67% (95% CI: 30% -80%) and the overall percent agreement was 92%
260 (95% CI: 80 -100%). (Table 3)

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262

263 Discussion

264 This study examined 66 subjects undergoing comparative sweat chloride tests to evaluate
265 a redesigned point of care testing device (POCT), the CFQT, and the reference method of
266 Macroduct®/ChloroChek®. The potential advantages of such a POCT device could be
267 decreased testing time providing results faster to the physician with less operator intervention. In
268 addition, a method with a low QNS rate would be highly desirable. A previous pilot study with
269 170 subjects showed promise, but noted concern about greater imprecision with differing lot
270 numbers of patches, thus prompting this study which was designed for 300 subjects using a
271 reformulated patch design. However, early results in this study demonstrated unacceptable
272 positive bias with the CFQT and the project was terminated after 66 subjects.

273 A visual review of the comparison plot in Figure 3 shows a reasonable range of data and
274 establishes a relationship between the pairs of sweat chloride values with slight proportional
275 error when compared to the perfect correlation line. This proportional error is supported by an
276 examination of both the bias plot (figure 4) and the Bland Altman plot (figure 5) which shows
277 that the results of the CFQT may be anywhere from 14 mmol/L lower to 25 mmol/L greater than
278 the reference method.

279 In evaluating the implications of the bias upon clinical decisions, the positive and
280 negative percent agreement were 100% but the intermediate percent agreement was only 67%.
281 Thus, if one were to rely on the CFQT as a diagnostic tool, the categorization of patients with the
282 CRMS/CFSPID diagnosis would be incorrect 33% of the time. (CRMS/CFSPID is a
283 consequence of newborn screening. These patients do not fit the full diagnostic criteria for CF
284 and their sweat chloride values are either normal or intermediate.) The cause of the lack of
285 agreement in the intermediate range and the overall proportional bias is unknown but may be

286 related to the manufacturing process and composition of the test patches. In the manufacturing
287 process of the patches, silver chromate is added to the patch and excess reagent is removed by a
288 roller apparatus. Prior to this study, the manufacturer of the CFQT obtained a new roller
289 apparatus in an attempt to eliminate the variability of the results. Unfortunately, the new roller
290 apparatus did not solve this issue. Post hoc analysis by the manufacturer utilizing scanning
291 electron microscopy revealed that the silver chromate was variably impregnated into the
292 chromatography paper due to the fibrous structure of the paper, random stacking and orientation
293 of fibers and variability in the thickness of the paper which may account for the observed
294 imprecision.

295 The bilateral agreement between the left and right extremities on the same subject were
296 greater for the CFQT (CV=9.5%) vs. the Macroduct/ChloroChek (CV=4.9%), with 6 subjects
297 having invalid results with the CFQT due to greater differences between the extremities
298 exceeding accepted concentrations, suggesting greater imprecision with the CFQT.
299 Unfortunately, it was not possible to further evaluate precision with the CFQT given the design
300 of the device. Traditionally, as with the ChloroChek® and other clinical laboratory based
301 analyzers, precision is determined by repeated measurements of the same level of control and
302 calculation of SD and CV for within run and across run precision which assesses the entire
303 testing process. The CLSI has suggested that the CV for low controls be less than 7% and for
304 high controls, less than 5%.⁷ The CFQT controls were limited to assessing only the camera
305 scanning and software portions of the test and did not assess the total testing process to include
306 variation in patch manufacturing and design. Because of the potential variation in single use
307 devices, the College of American Pathologists requires that if a laboratory limits quality control
308 to an internal device such as electronic check instead of also running external (liquid) controls,

309 then the laboratory must develop an individualized quality control plan to evaluate risk and
310 assess the effectiveness of the internal quality control and quality assurance processes.¹⁴

311 A limitation of this study is that there may have been differences in analytical variation
312 amongst the 4 participating sites. However, such differences for the Macroduct®/ChloroChek®
313 procedures should have been minimal because each site needed to demonstrate that they could
314 perform the procedure exactly as recommended by the CLSI. For the CFQT procedure, each site
315 received an in-person instruction of how to perform this test at site initiation.

316 In conclusion, the CFQT device using chromatography paper-based patches did not yield
317 results that were comparable to sweat collection with the Macroduct® and chloride analysis with
318 the ELITech ChloroChek® chloridometer. In order for a new method of sweat chloride analysis
319 to be accepted by the clinical laboratory and CF communities, the method must yield results as
320 accurate as the established method of coulometric titration determination of chloride
321 concentration.

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330

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370 August 21, 2017
371

372 **Table 1:** Characteristics of study subjects

373		<u>Known CF/CRMS</u>	<u>Referred for sweat test</u>
374	Total number	44	22
375	Center 1	16	6
376	Center 2	20	7
377	Center 3	8	5
378	Center 4	0	4
379	Female gender---no. (%)	25 (57)	14 (64)
380	Age- years		
381	Mean \pm SD	16.1 \pm 10.1	19.2 \pm 24.1
382	Age range	31 days –50 yrs	18 days –69 yrs
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408 **Table 2:** Means, SD, ranges, CV and QNS rates

409		CFQT	Macroduct®/ChloroChek®
410	Number of tests	119	130
411	Mean sweat chloride (mmol/L)	70.0	62.7
412	SD	39.5	34.9
413	Ranges (mmol/L)	10 ->160	10 -118
414	QNS rate*	6.8%	6.0%
415	CV between extremities†	9.5%	4.8%

416 * p = 0.8

417

418 † p = 0.15

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420

421 **Table 3:** Categorization of diagnosis

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		Macroduct/Chlorocek		
		>60 mmol/L	30-59 mmol/L	≤29 mmol/L
CFQT	>60 mmol/L	28	4	0
	30-59 mmol/L	0	10	0
	≤29 mmol/L	0	1	16

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436 **Figure legends**

437 Figure 1: CFQT collection patch

438 Figure 2: CFQT analyzer

439 Figure 3: Method comparison graph of CFQT results versus Macroduct®/ChloroChek®

440 Figure 4: Bias plot

441 Figure 5: Bland Altman plot

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460 Figure 1: CFQT collection patch

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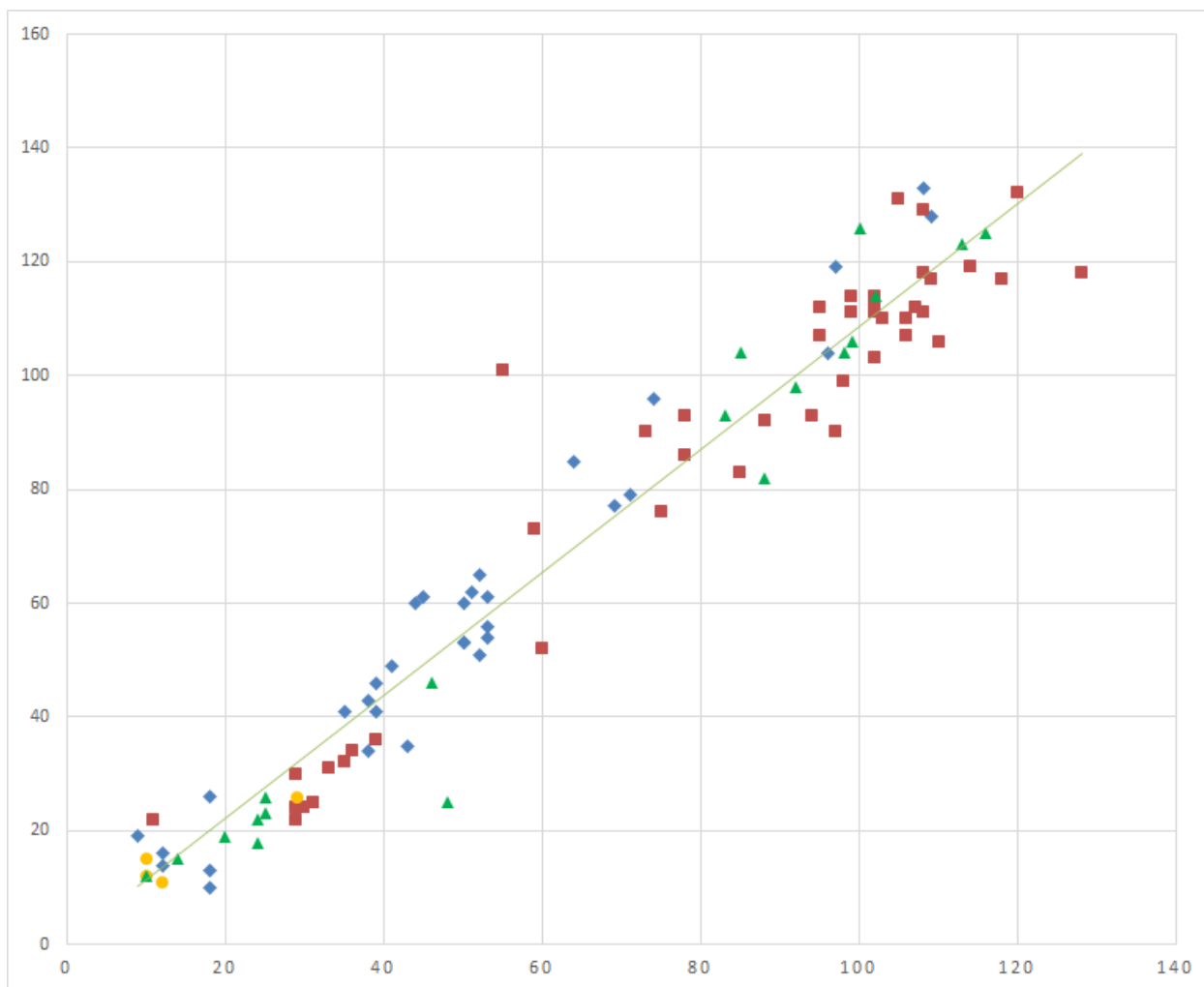


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468 Figure 2: CFQT analyzer

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472 Figure 3: Method comparison graph of CFQT results versus Macroduct®/ChloroChek®

473 y-axis: CFQT (mmol/L)

474 x-axis: Macroduct®/ChloroChek® (mmol/L)

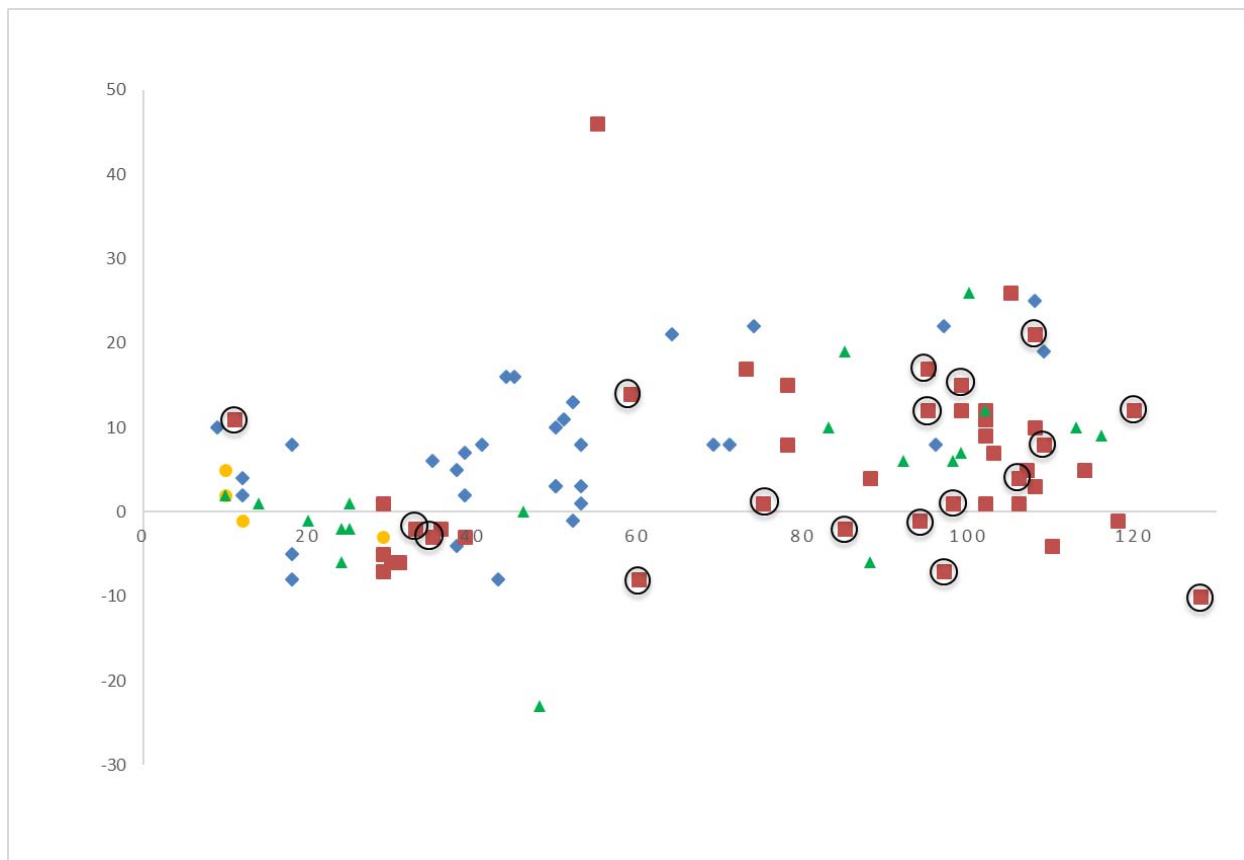
475 ◆ Center 1

476 ■ Center 2

477 ● Center 3

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481 Figure 4: Bias plot of CFQT minus Macroduct®/ChloroChek® plotted against

482 Macroduct®/ChloroChek®. Circled symbols are from the second patch lot.

483 y-axis: difference (CFQT - Macroduct®/ChloroChek®) (mmol/L)

484 x-axis: Macroduct®/ChloroChek® (mmol/L)

485 ◆ Center 1

486 ■ Center 2

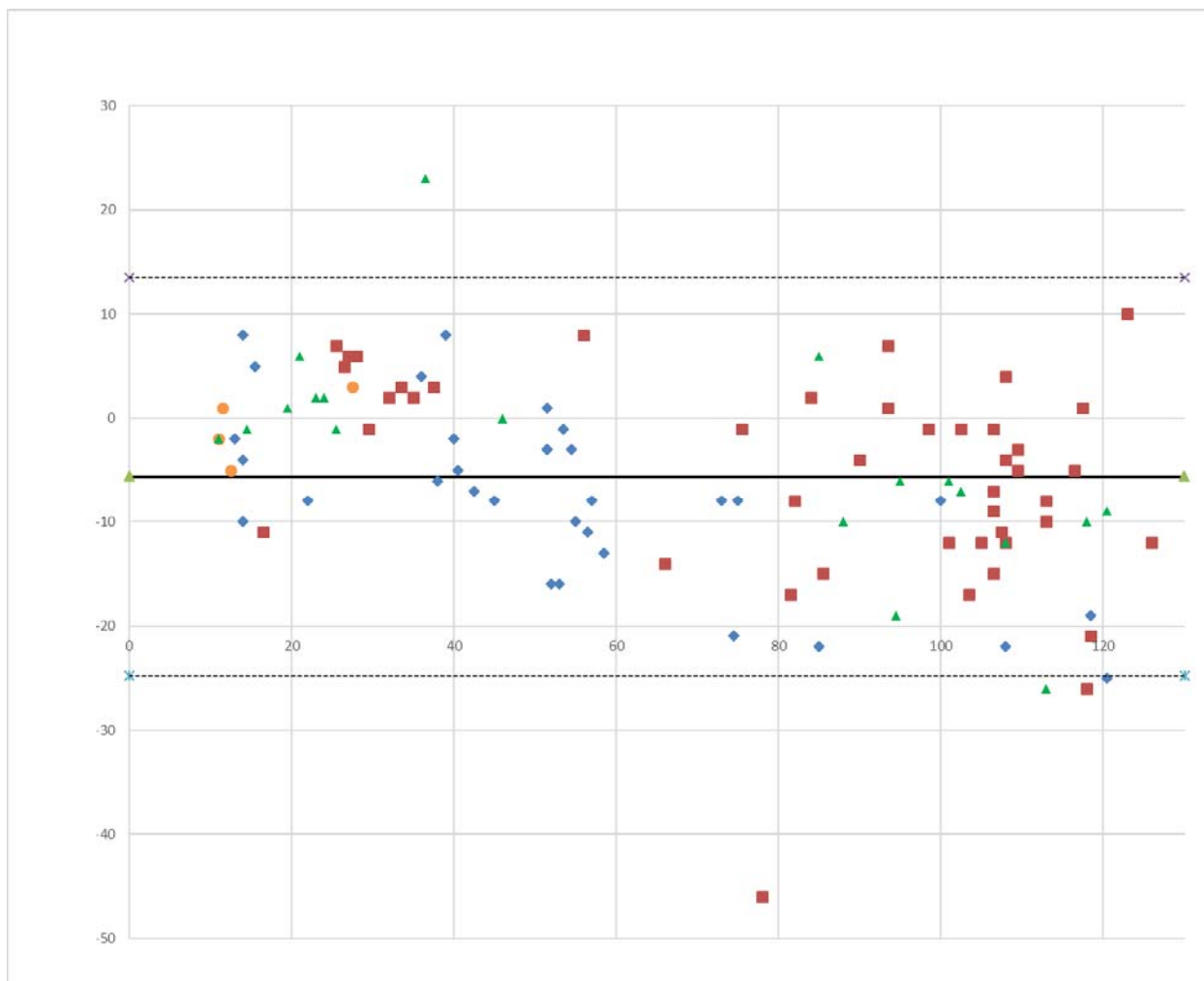
487 ● Center 3

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493 Figure 5: Bland Altman plot. Solid line = mean of differences. Dotted lines = ± 1.96 SD of the
 494 differences

495 y-axis: Sweat chloride difference (Macroduct®/ChloroChek® - CFQT)(mmol/L)

496 x-axis: Sweat chloride average (mmol/L)

497 ◆ Center 1

498 ■ Center 2

499 ● Center 3

500 ▲ Center 4

501