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1 **International survey on high- and low-dose synacthen test and assessment of**
2 **accuracy in preparing low-dose synacthen**

3

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7

8 **Short Title:** Synacthen: Survey and low-dose test inaccuracy

9

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29

30 **CONFLICT OF INTEREST STATEMENT**

31 R.J.R. is a Director of Diurnal Group Plc., and holds shares. C.J.E. and N.P.W. have a
32 patent application for nasal synacthen. All other authors declare there is no conflict of
33 interest that could be perceived as prejudicing the impartiality of the research reported.
34 A.S.C., E.H.K., A.W., L.W., S.M., P.S., and N.P.K. report no conflicts of interest in this
35 work.

36

37 **KEYWORDS**

38 dilution; low-dose synacthen; pituitary-adrenal function tests; questionnaires; surveys
39

40 **ABBREVIATIONS**

41 APEG, Australasian Paediatric Endocrine Group; CI, confidence interval; CV,
42 coefficient of variation; ESA, the Endocrine Society of Australia; ESE, European
43 Society of Endocrinology; ESPE, European Society for Paediatric Endocrinology; HDT,

44 high-dose test; LDT, low-dose test; PES, Pediatric Endocrine Society; SST, short
45 synacthen test; SfE, Society for Endocrinology.

46

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51 **Summary**

52 **Objective:** The short synacthen test (SST) is widely used to assess patients for adrenal
53 insufficiency but the frequency and protocols used across different centres for the low-
54 dose test (LDT) are unknown. This study aimed to survey centres and test the accuracy
55 of ten different synacthen preparation strategies used for the LDT.

56 **Methods:** Members of six international endocrine societies were surveyed regarding
57 diagnostic tests used for adrenal insufficiency, and in particular the SST. Synacthen was
58 diluted for the LDT and concentrations measured using a synacthen ELISA.

59 **Results:** Survey responses were received from 766 individuals across 60 countries (52%
60 adult, 45% paediatric endocrinologists). The SST is used by 98% of centres: 92% using
61 high-dose (250 µg), 43% low-dose, and 37% both. Ten low-dose dilution methods were
62 assessed and variation in synacthen concentration was demonstrated with intra-method
63 coefficients of variation (CV) ranging from 2.1% to 109%. The method using 5%
64 dextrose as a diluent was the least variable (CV of 2.1%). The variation in dilution
65 methods means that the dose of synacthen administered in a LDT may vary between
66 0.16 µg and 0.81 µg.

67 **Conclusions:** The high-dose SST is the most popular diagnostic test of adrenal
68 insufficiency but up to 72% of paediatric endocrinologists use a LDT. There is
69 considerable variation observed both within and between low-dose synacthen dilution
70 methods creating considerable risk of inaccurate dosing and thereby invalid results.

71 **INTRODUCTION**

72 The use of the ACTH-stimulation test, or short synacthen test (SST), has been growing
73 in popularity,^{1,2} and is the most widely used investigation of adrenocortical function in
74 some countries.³ It is being considered increasingly as the “standard” for the diagnosis
75 of adrenal insufficiency.⁴⁻⁶ The SST mimics the ACTH stimulus to the adrenal cortex
76 and involves administration of either high-dose supra-physiological 250 µg or low-dose
77 physiological, usually 1 µg, synacthen. Both the high and low-dose tests are used in
78 clinical practice and results of meta-analyses do not show significant superiority of one
79 test over the other.⁷⁻¹¹ Worldwide clinician preference for adrenal function testing and
80 the popularity of the high and low dose SST are unknown. We report the results of an
81 international survey, of both paediatric and adult endocrinologists, to assess current
82 practice.

83 One form of diagnostic-grade synacthen is commercially available,
84 manufactured in 250 µg/mL ampoules, necessitating large dilutions if administration of
85 a low-dose is required. A British survey of paediatric endocrinologists in 2012 reported
86 that, amongst the 82% of respondents who use the low-dose test, 14 different dilution
87 methods were used.³ These varied in the amount of synacthen utilised for the initial
88 dilution (0.1 mL to 1 mL), the volume of the diluent (10 mL to 1 litre), the diluent type
89 (5% dextrose and 0.9% saline), and the number of dilution steps (one, two, or three)
90 employed to prepare the required concentration.³

91 There is a paucity of literature on the accuracy or reproducibility of making up
92 low-dose synacthen. The majority of related work pertains to the analysis of adsorptive
93 losses on glass and plastic equipment during the dilution process, with losses
94 proportionate to the length of the plastic device used for administration.¹²⁻¹⁴ We
95 addressed this important clinical issue in an *in vitro* study and report the accuracy and

- 96 reliability of making up 1 μg doses of synacthen by ten of the different methods
- 97 currently in use.

98 MATERIALS AND METHODS

99 International survey

100 A thirteen-question online survey (Supporting Information) was distributed to the
101 members of six endocrine learned societies with a total of 6744 members: the USA
102 based Pediatric Endocrine Society (PES, $n = 1381$), the UK based Society for
103 Endocrinology (SfE, $n = 1188$), European Society of Endocrinology (ESE, $n = 1540$),
104 European Society for Paediatric Endocrinology (ESPE, $n = 1239$), The Endocrine
105 Society of Australia (ESA, $n = 1100$), and the Australasian Paediatric Endocrine Group
106 (APEG, $n = 296$). The survey sought to ascertain: the popularity of various diagnostic
107 tests for adrenal insufficiency; the indications for choosing the low-dose (LDT) or high-
108 dose (HDT) SST in preference to the other; LDT dose, administration route of
109 synacthen, cortisol sampling times and cortisol thresholds for test interpretation.

110 Survey invitations were sent via the e-mailing list or communications bulletin of
111 the societies between March 2016 and January 2017. A follow-up reminder was sent
112 after the initial email. Respondents were given the choice of completing the survey using
113 an online surveying platform (<https://surveyplanet.com>) or an emailed Microsoft
114 Word™ document. Minor changes were made to the survey in order to meet the various
115 stipulations of the societies.

116

117 Low-dose synacthen dilution study

118 Results from the 2012 survey of British paediatric endocrinologists were used to
119 investigate precision and accuracy of the ten most commonly employed dilution
120 methods for making up 1 µg low-dose synacthen (Table 1).³ Each dilution protocol was
121 followed and the resultant solution made up five times in order to evaluate intra-method
122 variability. In the nine methods yielding a sufficient final solution, three 1 mL samples
123 were taken (from the top, middle and bottom of the bag of diluent or the syringe) to

124 assess any variation that may be caused by insufficient mixing. Samples were extracted
125 from the superior quarter of the sample bag/final mL of the syringe (top samples), the
126 vertical halfway point of the sample bag/middle mL of the syringe (middle samples), or
127 taken from the sample bag port/first mL ejected from the sample syringe (bottom
128 samples). All samples were prepared on a single day, by one of three investigators, with
129 each method made up by the same investigator.

130 Medical ward equipment (syringes, fluid bags, needles) was used in preference
131 to laboratory equipment to simulate clinical conditions. The 1 mL synacthen ampoules
132 containing 250 µg/mL (Mallinckrodt Pharmaceuticals, Dublin, Ireland) were all from
133 the same manufacturing batch. Synacthen is an inherently unstable drug, rapidly
134 degrading in natural light and at room temperature; therefore ampoules were refrigerated
135 until use.^{12,14} New needles were used for each dilution step to avoid cross contamination
136 with more concentrated samples. Syringes were re-flushed three times when injecting
137 into bags of diluent. Mixing was performed by slowly inverting the sample bag or the
138 syringe five times, replicating typical ward-based practice. All samples containing the
139 required final concentration of synacthen were frozen immediately at -80°C.

140

141 **Synacthen ELISA**

142 Synacthen concentrations were estimated using an ELISA format. Unless otherwise
143 stated, all reagents were from Sigma-Aldrich (Poole, UK). NUNC MaxiSorp™ high
144 protein-binding capacity 96-well ELISA plates (ThermoFisher Scientific Inc., Waltham,
145 MA, USA) were coated with anti-ACTH mouse monoclonal antibody A1A12 (which
146 recognises ACTH 1-24) at 2.5 µg/mL in coating buffer (103 mM sodium chloride; 41
147 mM di-potassium hydrogen phosphate; 8.75 mM potassium dihydrogen phosphate; pH
148 7.4). Standards were prepared in 0.9% saline at 0-10,000 pg/mL using solid synacthen
149 (Bachem, Bubendorf, Switzerland). Samples containing synacthen were diluted in 0.9%

150 saline to a concentration that was within the standard linear dynamic range (1000-7500
151 pg/mL) of the ELISA. To assess any variation or reduction in synacthen dose resulting
152 from the laboratory dilutions necessary for the ELISA quantification, two vials of
153 synacthen (250 µg/mL) were diluted as required and analysed in the ELISA.

154 A 100 µL aliquot of sample diluent (phosphate-buffered saline, pH 7.4; 4%
155 bovine serum albumin; 0.05% Tween 20) was added to each well followed by 100 µL
156 of synacthen standard or test sample in duplicate. Plates were incubated at room
157 temperature for 10 min, and then washed three times with washing buffer (150 mM
158 sodium chloride; 8.5 mM di-potassium hydrogen phosphate; 1.75 mM potassium
159 dihydrogen phosphate; 0.025% Tween 20; 0.0125% ProClin 300; pH 7.0). A 200 µL (1
160 µg/mL) aliquot of anti-ACTH (7-23) antibody conjugated to HRP (Bioss Antibodies,
161 Woburn, MA, USA) was applied to each well, and plates incubated for 30 minutes at
162 room temperature. Subsequent to washing three times, 200 µL of 3,3',5-5'-
163 tetramethylbenzidine substrate reagent (Europa Bioproducts Ltd., Cambridge, UK) were
164 added to each well. Following incubation at room temperature for 45 min the reaction
165 was stopped by the addition of 100 µL of 0.5 M hydrochloric acid. A Labtech LT4500
166 spectrophotometer (Labtech International Ltd., Uckfield, UK) was used to read
167 absorption of the wells at 450 nm. Synacthen concentrations (pg/mL) were estimated
168 from standard curves and corrected by the appropriate dilution factor (50-1000 times)
169 to give the expected concentration in the synacthen solution used to deliver a 1 µg dose

170 (Table 1). All samples were assayed four to six times and the mean synacthen
171 concentration determined.

172 The intra-assay coefficient of variation (CV) was 1.70% at 2500 pg/mL, 1.69%
173 at 5000 pg/mL, and 2.35% at 7500 pg/mL. The inter-assay CV was 4.54% at 5000
174 pg/mL.

175

176 **Statistical analyses**

177 Summary statistics of frequency (%) and mean were used to analyse survey data. Free
178 text responses detailing the clinical scenarios in which the HDT or LDT were used were
179 categorised into themes using content analysis. For each of the ten dilution methods
180 studied in the low-dose synacthen dilution analysis, intra-method and intra-bag/syringe
181 variance was calculated and expressed as mean, SD and CV. Method 7 was excluded
182 from intra-bag/syringe variance calculations due to an insufficient final volume.
183 Unpaired t-tests with Welch's correction were employed to compare components of the
184 different methods, including number of dilution steps, volume of diluent, and initial
185 volume of synacthen used. A threshold of $\pm 10\%$ (0.9 to 1.1 μg) was chosen as the
186 acceptable range for deliverable synacthen dose values to fall within, reflecting standard
187 laboratory practice.

188 **RESULTS**

189 **International survey**

190 Responses were received from 766 society members (11% overall response rate),
191 working in 60 countries (single response received from 19 countries). Response rates
192 varied between the societies: PES, 21% ($n = 290$), SfE, 19% ($n = 220$), ESE, 13% ($n =$
193 220), ESPE, 3% ($n = 36$), ESA, < 1% ($n = 7$), and APEG, 4% ($n = 13$). Responses were
194 received from clinicians working in the USA (36%), UK (29%), mainland Europe
195 (25%), North America (excluding the USA) (4%), Asia (3%), Australasia (3%), Africa
196 (< 1%), and South America (< 1%). Endocrinologists who worked mainly or entirely
197 with adults made up 52% of respondents and 45% worked mainly or entirely with
198 children and/or adolescents (97% of USA respondents). The remaining 3% of
199 respondents either did not indicate their patient base or were not clinicians.

200 The SST was the most popular test for assessing adrenal insufficiency (Table 2).
201 It was used by 98% overall with 92% using the HDT, 43% the LDT, and 37% both. The
202 LDT was considerably more popular amongst paediatric endocrinologists (72%)
203 compared with adult endocrinologists (17%). There was variation of LDT utility
204 amongst respondents from different geographical regions: 76% of all respondents
205 working in the USA used the LDT, 50% from the Middle East, 34% from mainland
206 European countries, 30% from Australasia and 6% from the UK (82% UK paediatric
207 endocrinologists in 2012 survey, not resurveyed). The most commonly utilised LDT
208 dose was 1 μg (86% of question respondents) and an intermediate dose (between 5 μg
209 and 15 μg) was used by 8%. Body surface area based doses (0.1 $\mu\text{g}/\text{m}^2$ to 1 $\mu\text{g}/\text{m}^2$) were
210 used by 5%, 2% used weight-based calculations.

211 Respondents stated their rationale for using the HDT or LDT: the most popular
212 reasons for using the HDT were diagnosis of primary adrenal insufficiency and
213 congenital adrenal hyperplasia, or because it was standard procedure. The LDT was

214 preferred to investigate secondary adrenal insufficiency. The majority administer the
215 HDT by the intravenous route (81%), with 37% and 5% using intramuscular and
216 subcutaneous routes, respectively.

217 Thirty different combinations of cortisol sampling times were specified for the
218 HDT and 37 for the LDT (Fig. 1). The most common times to sample were at 0, 30 and
219 60 minutes (HDT 46%, LDT 51%), while 17% of LDT respondents utilised a 20-minute
220 sample in their protocol. The most commonly used interpretive threshold for adequacy
221 of adrenal function (a “pass”) was > 500 nmol/L, used in 48% of HDT and 61% of LDT.
222 More HDT users (27%) than LDT users (11%) utilised the higher threshold of > 550
223 nmol/L. Similar proportions used thresholds below 500 nmol/L: HDT, 21% (range 374
224 to 475 nmol/L), and LDT, 25% (range 380 to 495 nmol/L).

225 Serum cortisol levels without stimulation were used in the diagnosis of adrenal
226 insufficiency by 76% (Table 2). When asked to specify further ($n = 290$), 92% used
227 morning serum cortisol and 19% random cortisol sampling. Paired ACTH and serum
228 cortisol sampling was used by 71% of all respondents. Less popular tests included the
229 insulin tolerance test (used by 36% of respondents: adult, 54%; paediatric 15%),
230 glucagon stimulation test (27%), metyrapone test (4%), clonidine stimulation test (3%),
231 corticotrophin releasing hormone test (2%), and depot (prolonged) synacthen test (1%).

232

233 **Low-dose synacthen dilution study**

234 For eight of the ten different dilution strategies, a marked intra-method variability of the
235 final synacthen concentration was observed, with CVs of over 10% (Table 1). The least
236 variable was method 6, with a CV of 2.1%; the most variable was method 10, with a CV
237 of 109%. Optimal dilution would have yielded synacthen concentrations able to deliver
238 a dose close to 1 μg (acceptable range, 0.9 to 1.1 μg). However, the method means
239 ranged from 0.16 μg (least accurate) to 0.81 μg (most accurate) (Table 1). The methods

240 bearing results closest to the range chosen as acceptable were 1, 4, and 6 (Fig. 2). Three
241 methods (7, 9 and 10) had a mean concentration of less than half the expected dose
242 ranging from 0.16 to 0.36 μg (Fig. 2), reflecting substantial losses of synacthen. To
243 assess any variation or reduction in synacthen dose resulting from the laboratory
244 dilutions necessary for the ELISA quantification, two vials of synacthen (250 $\mu\text{g}/\text{mL}$)
245 were diluted and samples run over 23 assays. This yielded results of $247 \pm 11 \mu\text{g}/\text{mL}$
246 and $223 \pm 12 \mu\text{g}/\text{mL}$, and indicated that the wide variation in deliverable dose detected
247 in samples was not due to inaccuracies in the required laboratory dilutions.

248 Intra-bag/syringe variability was high but unpredictable, with no part of the
249 bag/syringe tending towards higher concentrated samples than another. Overall, top
250 samples ($n = 45$) had a mean \pm SD deliverable dose of $0.593 \pm 0.298 \mu\text{g}$ synacthen, CV
251 of 50.2%, middle samples ($n = 45$) $0.545 \pm 0.286 \mu\text{g}$, 52.5%, and bottom samples ($n =$
252 45) $0.573 \pm 0.293 \mu\text{g}$, 51.3%.

253 Method 6 was the only one to use 5% dextrose as a diluent and was the least
254 variable method (CV of 2.1%) and most accurate, with means closest to the desired 1
255 μg (0.79 to 0.84 μg). Six methods ($n = 90$ samples) involved a single dilution step, and
256 together had a mean synacthen deliverable dose of $0.547 \pm 0.319 \mu\text{g}$, whilst four
257 methods ($n = 50$) used double dilutions with an overall mean of $0.583 \pm 0.24 \mu\text{g}$ ($P =$
258 0.46 ; 95% confidence interval (CI): -0.058 to $0.131 \mu\text{g}$). When comparing the different
259 initial volumes of the 1 mL ampoule of 250 $\mu\text{g}/\text{mL}$ synacthen used for dilution, six
260 methods ($n = 90$) used all 1 mL and resulted in a mean synacthen deliverable dose of
261 $0.668 \pm 0.212 \mu\text{g}$. The remaining four methods ($n = 50$) used 0.5 mL or less and had a
262 mean synacthen deliverable dose of $0.365 \pm 0.318 \mu\text{g}$ ($P < 0.0001$; 95% CI: -0.404 to $-$
263 $0.204 \mu\text{g}$). A bag of diluent, rather than a syringe, was utilised in eight of the methods
264 ($n = 120$), four of which ($n = 60$) used a large volume of diluent, $\geq 250 \text{ mL}$, and had a
265 mean synacthen deliverable dose of $0.572 \pm 0.314 \mu\text{g}$, and four methods ($n = 60$) used

266 a small volume of diluent, 50 mL, yielding a mean synacthen deliverable dose of 0.584
267 $\pm 0.283 \mu\text{g}$ ($P = 0.837$; 95% CI: -0.097 to 0.119 μg).

268 **DISCUSSION**

269 This is the largest international survey of diagnostic tests for adrenal insufficiency to
270 date. Although the response rate of 11% was low, this was a survey of society members
271 some of whom are not in clinical practice and the response rate is in keeping with similar
272 internet surveys.¹⁵⁻¹⁶ There was geographical variations in responses. Not all endocrine
273 societies approached distributed the survey and this has contributed to the imbalance in
274 paediatric and adult endocrinologist responses from certain regions.

275 The SST was the most popular test for assessing HPA axis function and has been
276 growing in popularity amongst endocrinologists, increasing from 24% in 1988,¹ 69% in
277 1993,² 59% in 2005,¹⁷ to 98% in this survey and 100% of paediatric endocrinology
278 centres in the UK in 2012.³ It is regarded now as the “standard” test for adrenal
279 insufficiency.⁴ This is the first international survey to distinguish proponents of the HDT
280 from the LDT. Whilst the HDT is used by 92% of respondents, and is the test of choice
281 for diagnosing primary adrenal insufficiency, the LDT is used by 43%. Similar
282 proportions of survey respondents practised as adult and paediatric endocrinologists.
283 The LDT is popular amongst paediatric endocrinologists, 72% compared with 17% of
284 adult endocrinologists, resonating the results of the British Society for Paediatric
285 Endocrinology and Diabetes (BSPED) survey, where 82% used the LDT.³ This may
286 reflect respiratory guidelines, which recommend the LDT for assessment of adrenal
287 function in children on inhaled corticosteroids.^{18,19}

288 The sampling times and diagnostic cut-offs practised by the majority of
289 respondents were in keeping with Endocrine Society guidelines,⁴ which state a peak
290 cortisol less than 500 nmol/L at 30 or 60 min indicates adrenal insufficiency. Deviations
291 from these guidelines were seen in 52% of HDT and 39% LDT users for cut-off and <
292 1% HDT and 5% LDT users for timing. The tendency to employ lower diagnostic
293 thresholds for serum cortisol is likely reflect a change in practice to locally derived cut-

294 offs, dependent on the assay platform used. Additionally clinicians review the SST
295 results in the context of the clinical suspicion of adrenal insufficiency.^{20,21}

296 Responses were received from people working in 60 countries and six
297 continents, demonstrating a range of practises, resource settings and patient populations.
298 There was a preponderance of responses from endocrinologists working in Europe and
299 the USA; therefore the survey may not be truly representative of worldwide practice.
300 Additionally, national practice cannot be assumed in the 136 countries with no
301 respondent and 19 countries with a single respondent.

302 This study has shown a high inter-method variability between different
303 commonly employed dilution strategies for the low-dose SST. The variation in dose was
304 from 0.16 µg to 0.81 µg when the dose should be 1 µg, thereby in all cases the dilution
305 methods used provide inadequate dosing, with doses up to seven-fold less than required.
306 There was variation when the same method was used to make up the 1 µg dose five
307 times (intra-method variability) and variation when individual samples from the same
308 final solution were compared (intra-bag/syringe variability), inferring inadequate
309 mixing. This inaccuracy in dosing and variability between and within dilution methods
310 may result in false positive synacthen tests with potentially important clinical sequelae.

311 When similar methods (e.g., volume of diluent, proportion of synacthen ampoule
312 used, number of dilution steps) were grouped and compared only the initial volume of
313 synacthen was shown to significantly affect the final concentration: dilution methods
314 using the full ampoule gave significantly higher concentrations and closer to the desired
315 concentration. The most accurate and least variable method was the only one to use 5%
316 dextrose, suggesting that dextrose may be the most suitable diluent for making up low

317 dose synacthen. However, this would require further investigation along with other
318 possible diluents for synacthen.

319 The plateau of the synacthen/cortisol dose response curve is thought to begin at
320 approximately 5 µg of synacthen.¹² The lowest dose of synacthen to maximally
321 stimulate the adrenal gland has been found to be between 0.5 µg and 1 µg.^{12,22-25} The
322 supra-physiological dose of 250 µg of synacthen employed by the HDT means that even
323 marked variation in the actual dose delivered to the patient is unlikely to manifest
324 clinically. However, the doses employed in the LDT are much closer to the amounts
325 needed to produce a maximal adrenal response and thus, small variations in the
326 administered dose, may have clinical ramifications, with the potential of false positive
327 diagnoses of adrenal insufficiency. Using the results of this study, a patient undergoing
328 a 1 µg LDT, using dilution methods 7, 9 or 10, may receive between 0.16 µg and 0.36
329 µg of synacthen. These three methods used half or less of the synacthen ampoule, with
330 methods 7 and 9 using 0.2 mL or less, a volume too small to draw up accurately using
331 1 mL ward syringes.

332 Intra-bag/syringe variability was high but similar between different parts (top,
333 middle, bottom), suggesting mixing inadequacy but no specific area the synacthen
334 settled in. In laboratory practice, mixing of constituents similar to those used in this
335 study may take place over many hours with the use of specialised equipment, to be
336 assured of uniform distribution throughout the diluent.

337 There is no “standard” way to make up the 1 µg synacthen dose. The method of
338 adding 250 µg/mL to 250 mL of 0.9% saline (method 3), described by Dickstein *et al*¹²
339 on introducing the 1 µg test in 1991, was later recommended by the meta-analysis of

340 Kazlauskaitė and colleagues,⁸ but was neither the most popular method in the 2012
341 British survey³ nor the most accurate method in the current study.

342 Other sources of variation have been considered. These include potential losses
343 caused by the adherence of synacthen to plastic, reported to be between 21.6 and 58.6%
344 and proportional to the length of the device.^{13,14} This study made up low-dose synacthen
345 under replicated ward conditions, using plastic syringes. Additional plastic laboratory
346 equipment was used in the dilutions prior to ELISA analysis, potentially adding to the
347 losses. However, the “control” samples diluted from a vial of synacthen with laboratory
348 equipment showed very little variation and only minimal losses. Pharmaceutical
349 industry standards require that an ampoule of 250 µg/ml synacthen contains between 95
350 and 105% of the declared content, 237.5 µg and 262.5 µg, respectively (Mallinckrodt
351 Pharmaceuticals, Dublin, Ireland) and this variation may be amplified when diluting the
352 synacthen to physiological doses.

353 Ward, rather than specialised, calibrated laboratory equipment was used for
354 simulation purposes, reflecting current clinical practice, but other variables were
355 controlled as far as possible. The synacthen was kept refrigerated until the point of use
356 and a single investigator performed all dilutions for each individual method. The
357 additional dilutions required to run the samples on the ELISA were performed under
358 strict laboratory conditions and by a single investigator. In the reality of a less controlled,
359 busy clinical environment ambient temperatures may vary, synacthen may degrade in
360 sunlight or if left out of the refrigerator and many different personnel may perform the
361 dilutions, all potentially increasing the inaccuracy of dilution and variability further. A
362 systematic review has shown pre-prepared syringes for intravenous medication can
363 reduce errors in the preparation and administration by 21%.²⁶

364 Our international survey showed the synacthen test is employed by 98% of
365 endocrinologists, with 43% using the LDT. Our dilution study demonstrated

366 considerable variation and inaccuracy when preparing the low-dose of synacthen. The
367 least variable methods were 1, 4 and 6 (Table 1). Although method 6 used 5% dextrose,
368 the effect of diluent needs to be investigated further before any recommendations can
369 be made. In addition, it would be expected that controlled laboratory/pharmacy
370 conditions would impact positively on the accuracy of the delivered dose.

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FIGURE LEGENDS

FIGURE 1 Chosen cortisol sampling times for respondents using high-dose and low-dose synacthen tests. Each bar represents the percentage of respondents (HDT, $n = 716$, and LDT, $n = 284$) who measure cortisol levels at the times provided. For clarity, not all combinations of timings have been included in the graph (HDT, $n = 30$ different combinations and, LDT, $n = 37$). HDT, high-dose test; LDT, low-dose test.

FIGURE 2 Accuracy and variability of 1 μg low-dose synacthen dilution methods. For each method tested, except method 7, each individual point indicates the mean deliverable amount of synacthen as calculated from three samples taken from the final bag/syringe dilution. For method 7, each individual point relates to a single sample measurement. Each method mean was calculated from five separate dilution experiments and is depicted by a short black line. The unbroken line at 1 μg represents the expected amount of synacthen administered if dilutions were optimal. The broken lines represent the upper (1.1 μg) and lower (0.9 μg) limits of the accepted range of dose variability of $\pm 10\%$.

TABLE 1 Dilution methods used to make up 1 µg synacthen dose and intra-method variability

Method number	Method summary	Dilution factor	Expected final concentration of synacthen	Observed final concentration of synacthen (mean ± SD; n = 5)	Intra-method variability (% CV)	Volume to deliver a 1 µg dose	Actual dose (µg) of synacthen deliverable in injected volume (mean ± SD; n = 5)
1	1 mL of synacthen ^a injected into a 1 litre bag of saline.	1000	250 ng/mL	195 ± 22 ng/mL	11.3	4 mL	0.78 ± 0.09
2	1 mL of synacthen ^a transferred to 10 mL syringe containing 9 mL of saline. 1 mL of resultant solution transferred to 10 mL syringe containing 4 mL of saline.	50	5 µg/mL	2.73 ± 0.79 µg/mL	28.9	0.2 mL	0.55 ± 0.16
3	1 mL of synacthen ^a injected into 250 mL bag of saline.	250	1000 ng/mL	522 ± 202 ng/mL	38.8	1 mL	0.52 ± 0.20
4	1 mL synacthen ^a injected into 50 mL bag of saline. 1 mL of resultant solution transferred to 10 mL syringe containing 9 mL of saline.	500	500 ng/mL	391 ± 36 ng/mL	9.06	2 mL	0.78 ± 0.07
5	1 mL of synacthen ^a injected into 50 mL bag of saline. 0.2 mL of resultant solution transferred to 2.5 mL syringe containing 0.8 mL of saline.	250	1000 ng/mL	559 ± 89 ng/mL	15.9	1 mL	0.56 ± 0.09
6	1 mL of synacthen ^a injected into 500 mL bag of 5% (w/v) dextrose.	500	500 ng/mL	407 ± 8 ng/mL	2.06	2 mL	0.81 ± 0.02
7	0.2 mL of synacthen ^a transferred into 10 mL syringe containing 10 mL saline. 0.2 mL of resultant solution transferred to 2.5 mL syringe containing 0.8 mL of saline.	250	1000 ng/mL	161 ± 39 ng/mL	24.7	1 mL	0.16 ± 0.04
8	0.2 mL of synacthen ^a injected into 50 mL bag of saline.	250	1000 ng/mL	632 ± 230 ng/mL	36.4	1 mL	0.63 ± 0.23
9	0.1 mL of synacthen ^a injected into 50 mL bag of saline.	500	500 ng/mL	181 ± 118 ng/mL	65.2	2 mL	0.36 ± 0.24
10	0.5 mL synacthen ^a of injected into 500 mL bag of saline.	1000	250 ng/mL	42 ± 47 ng/mL	109.6	4 mL	0.17 ± 0.19

^aSynacthen starting concentration was 250 µg/mL. Where the method states “saline”, a 0.9% sodium chloride solution was used.

TABLE 2 Percentage of adult and paediatric respondents using the different diagnostic tests for adrenal insufficiency

Diagnostic test for adrenal insufficiency	Percentage respondents using test		
	Total (<i>n</i> = 766)	Adult (<i>n</i> = 398)	Paediatric (<i>n</i> = 345)
Short cosyntropin test	97.8	97.7	98.8
High-dose test	92	95.7	88.4
Low-dose test	42.6	17.4	72.1
Paired ACTH and serum cortisol	71	73.3	66.9
Serum cortisol	76.4	67.3	87.5
Salivary cortisol	20.2	25.2	14.2
Insulin tolerance test	36	54.2	14.5
Glucagon stimulation test	26.9	25.4	29.1
Metyrapone test	4	5	2.9
Clonidine stimulation test	2.6	1.5	3.5
Corticotrophin releasing hormone test	1.9	1.8	2

Figure 1

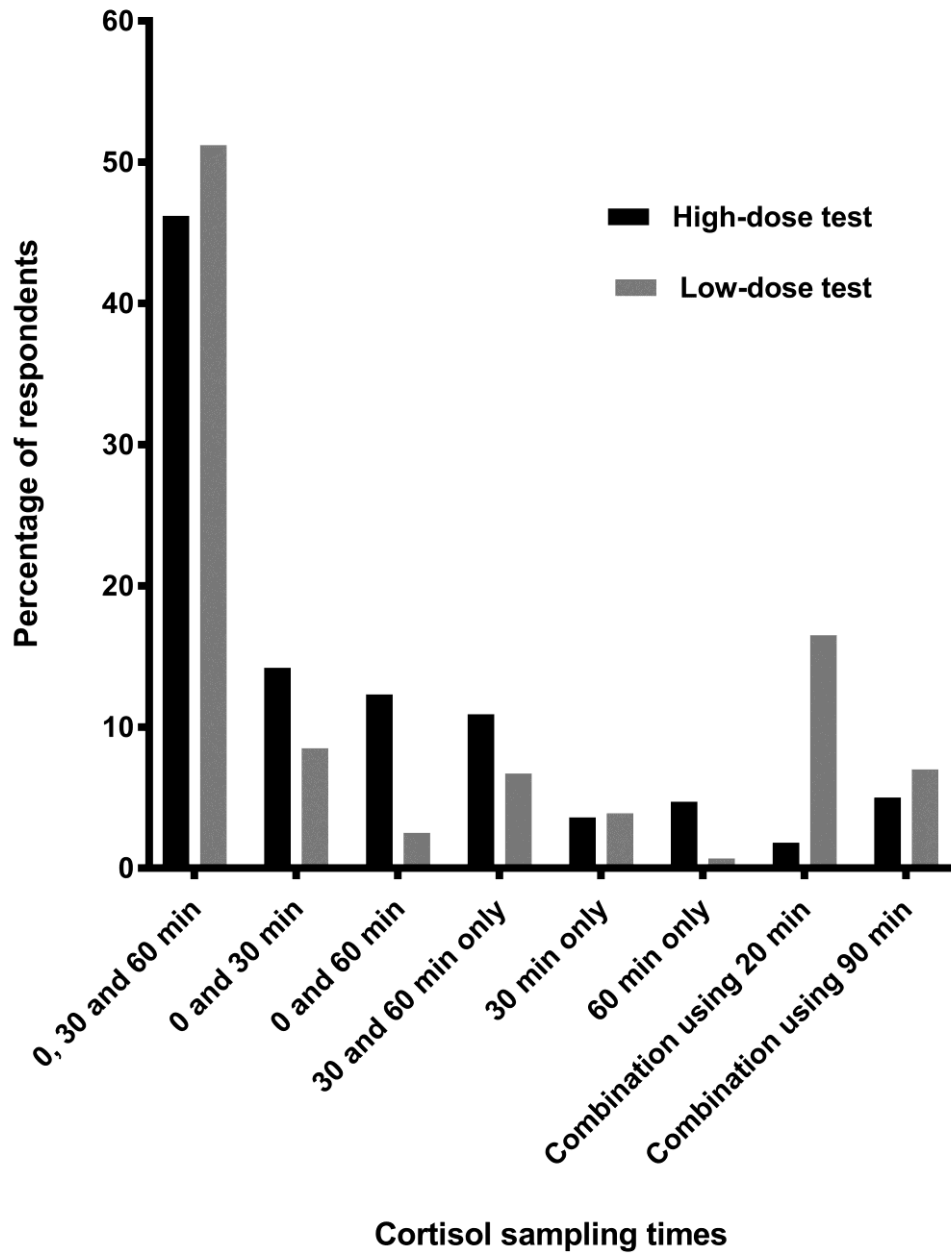
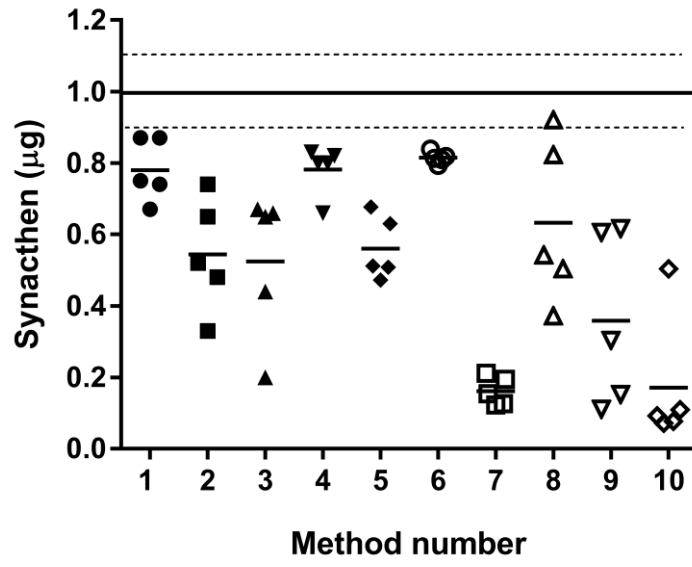


Figure 2



Supporting Information

Diagnostic Tests for Adrenal Insufficiency Survey

Dear Endocrinologist/Endocrine Specialist Nurse/ Paediatric Endocrine Society/ The Endocrine Society/ ESA/ ESE/SfE/ESPE member,

We are surveying clinical approaches to diagnosing adrenal insufficiency, in particular the (short) Synacthen test (Cosyntropin, Cortrosyn, ACTH test, tetracosactide), within endocrinology departments (adult and paediatric) via the membership of Paediatric Endocrine Society, The Endocrine Society, Endocrine Society of Australia, European Society of Endocrinology, Society for Endocrinology and European Society of Paediatric Endocrinology.

The different testing strategies for the HPA-axis, in particular the use of different doses of Synacthen, are controversial and this is the first survey to gather such information worldwide.

We would be grateful if you would take a few minutes to complete this very short questionnaire.

Thank you for your time.

Alex Cross, Charlotte Elder, Neil Wright, Nils Krone, Richard Ross.
University of Sheffield/Sheffield Children's Hospital, UK.

If you have any problems completing this document, please contact Alex Cross on
ascross1@sheffield.ac.uk

1. In which country do you work?
2. Which endocrine patient group do you work with?

Drop down box: *Children/adolescents only*

Mainly children/adolescents but some adults

Both children/adolescents AND adults

Mainly adults but some children/adolescents

Adults ONLY

Other (specify/add comments in the text box provided)

3. Which tests do you use to assess hypothalamic-pituitary-adrenal (HPA) axis hypofunction?
Please select all that apply.

- Early morning serum cortisol
- Random serum cortisol
- Paired ACTH and serum cortisol
- Salivary cortisol
- Insulin tolerance test
- Standard-dose (short) Synacthen (Cosyntropin, Cortrosyn) test (250 mcg)
- Low-dose (short) Synacthen (Cosyntropin, Cortrosyn) test (e.g. 1 mcg)
- Glucagon stimulation test
- Metyrapone test
- Clonidine stimulation test
- Other (please specify)

4. We want to know what makes people choose between the standard-dose and low-dose (short) Synacthen (Cosyntropin, Cortrosyn) test.

If you have indicated that you use the Synacthen test (standard-dose AND/ OR low-dose), please detail in which clinical scenarios/situations you would use each test, in preference to the other test.

Standard-dose (short) Synacthen (Cosyntropin, Cortrosyn) test (250 mcg)

Low-dose (short) Synacthen (Cosyntropin, Cortrosyn) test (e.g. 1 mcg)

Any other comments

5. In the assessment of adrenal insufficiency, if you use the STANDARD-DOSE (250 mcg) form of the (short) Synacthen (Cosyntropin, Cortrosyn) test, which route(s) do you most commonly administer it? Please select all that apply.

- Intravenous (IV)
 Intramuscular (IM)
 Subcutaneous (SC)

6. In the assessment of adrenal insufficiency, if you use the LOW-DOSE form of the (short) Synacthen (Cosyntropin, Cortrosyn) test, what DOSE do you use?

7. If you use the **STANDARD-DOSE** (short) Synacthen (Cosyntropin, Cortrosyn) test (**250 mcg**), at what times do you take your cortisol samples?

Please select all that apply

- I do not use the **STANDARD-DOSE** Synacthen test
 0 minutes
 10 minutes
 20 minutes
 30 minutes
 60 minutes
 90 minutes
 Other time(s)
(please specify)

8. If you use the **LOW-DOSE** (short) Synacthen (Cosyntropin, Cortrosyn) test (**e.g. 1 mcg**), at what times do you take your cortisol samples?

Please select all that apply

- I do not use the **LOW-DOSE** Synacthen test
 0 minutes
 10 minutes
 20 minutes
 30 minutes
 60 minutes
 90 minutes
 Other time (s)
(please specify)

9. Which assay(s) do you use to analyse your cortisol samples? If you do not know, please write "don't know" in the text box provided.

10. How have your diagnostic cut offs for adrenal insufficiency been set?

Drop down box: *Locally according to your specific assay*

Locally- other (please specify below)

From textbook definitions (please specify below)

From another source (please specify below)

Don't know

Please add further information here

11. If/when interpreting the results of a (short) Synacthen (Cosyntropin, Cortrosyn) test (standard or low-dose), which of the following diagnostic criteria do you use?

Drop down box: *I do not use the Synacthen (Cosyntropin, Cortrosyn) test*

Peak cortisol ONLY

Rise from baseline (absolute or fold increase) ONLY

Both peak cortisol and rise from baseline

12. If you use the **STANDARD-DOSE** (short) Synacthen (Cosyntropin, Cortrosyn) test, what cut off for normal do you use?

Please select **all** that apply, e.g. peak threshold AND rise from baseline increment.

- I do not use the **STANDARD**-dose Synacthen test
- Peak cortisol >400 nmol/l
(>**14.5 µg/dL**)
- Peak cortisol >450 nmol/l
(>**16.3 µg/dL**)
- Peak cortisol >500 nmol/l
(>**18 µg/dL**)
- Peak cortisol >550 nmol/l
(>**20 µg/dL**)
- Peak cortisol >580 nmol/l
(>**21 µg/dL**)
- Rise from baseline
>150 nmol/l (>**5.4 µg/dL**)
- Rise from baseline
> 200 nmol/l (>**7.2 µg/dL**)
- Other concentration
(please specify)

13. If you use the **LOW DOSE** (short) Synacthen (Cosyntropin, Cortrosyn) test, what cut off for normal do you use?

Please select **all** that apply, e.g. peak threshold AND rise from baseline increment.

- I do not use the **LOW**-dose Synacthen test
- Peak cortisol >400 nmol/l
(>**14.5 µg/dL**)
- Peak cortisol >450 nmol/l
(>**16.3 µg/dL**)
- Peak cortisol >500 nmol/l
(>**18 µg/dL**)
- Peak cortisol >550 nmol/l
(>**20 µg/dL**)
- Peak cortisol >580 nmol/l
(>**21 µg/dL**)
- Rise from baseline
>150 nmol/l (>**5.4 µg/dL**)
- Rise from baseline
> 200 nmol/l (>**7.2 µg/dL**)
- Other concentration
(please specify)

