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

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

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CONFLICT OF INTEREST STATEMENT

R.J.R. is a Director of Diurnal Group Plc., and holds shares. C.J.E. and N.P.W. have a patent application for nasal synacthen. All other authors declare there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. 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 work.

KEYWORDS

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

ABBREVIATIONS

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

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

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Summary

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

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

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

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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

International survey

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

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

Low-dose synacthen dilution study

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

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

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

Synacthen ELISA

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

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

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

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

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

Statistical analyses

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

RESULTS

International survey

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

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

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

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

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

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

Low-dose synacthen dilution study

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

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

Intra-bag/syringe variability was high but unpredictable, with no part of the bag/syringe tending towards higher concentrated samples than another. Overall, top samples (n = 45) had a mean ± SD deliverable dose of 0.593 ± 0.298 µg synacthen, CV of 50.2%, middle samples (n = 45) 0.545 ± 0.286 µg, 52.5%, and bottom samples (n = 45) 0.573 ± 0.293 µg, 51.3%.

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

266 a small volume of diluent, 50 mL, yielding a mean synacthen deliverable dose of 0.584

267 ± 0.283 µg (P = 0.837; 95% CI: -0.097 to 0.119 µg).

DISCUSSION

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

The SST was the most popular test for assessing HPA axis function and has been growing in popularity amongst endocrinologists, increasing from 24% in 1988,[1] 69% in 1993,[2] 59% in 2005,[17] to 98% in this survey and 100% of paediatric endocrinology centres in the UK in 2012.[3] It is regarded now as the "standard" test for adrenal insufficiency.[4] This is the first international survey to distinguish proponents of the HDT from the LDT. Whilst the HDT is used by 92% of respondents, and is the test of choice for diagnosing primary adrenal insufficiency, the LDT is used by 43%. Similar proportions of survey respondents practised as adult and paediatric endocrinologists. The LDT is popular amongst paediatric endocrinologists, 72% compared with 17% of adult endocrinologists, resonating the results of the British Society for Paediatric Endocrinology and Diabetes (BSPED) survey, where 82% used the LDT.[3] This may reflect respiratory guidelines, which recommend the LDT for assessment of adrenal function in children on inhaled corticosteroids.[18,19]

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

offs, dependent on the assay platform used. Additionally clinicians review the SST results in the context of the clinical suspicion of adrenal insufficiency.[20,21]

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

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

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

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

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

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

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

Kazlauskaite and colleagues,[8] but was neither the most popular method in the 2012 British survey[3] nor the most accurate method in the current study.

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

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

Our international survey showed the synacthen test is employed by 98% of 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.
371

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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 ± 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

[a]Synacthen 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 (n = 766)	**Adult (n = 398)**	**Paediatric (n = 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

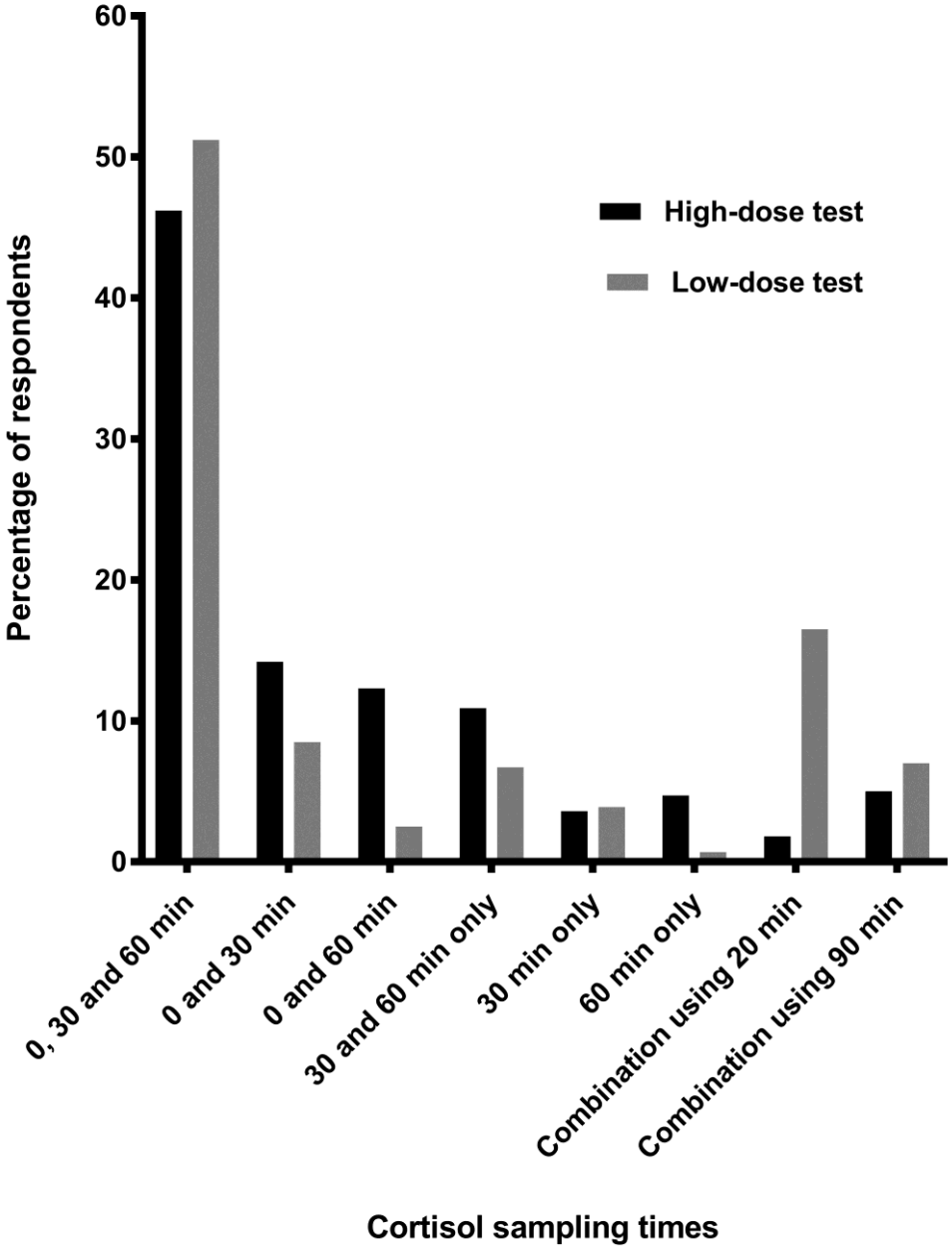
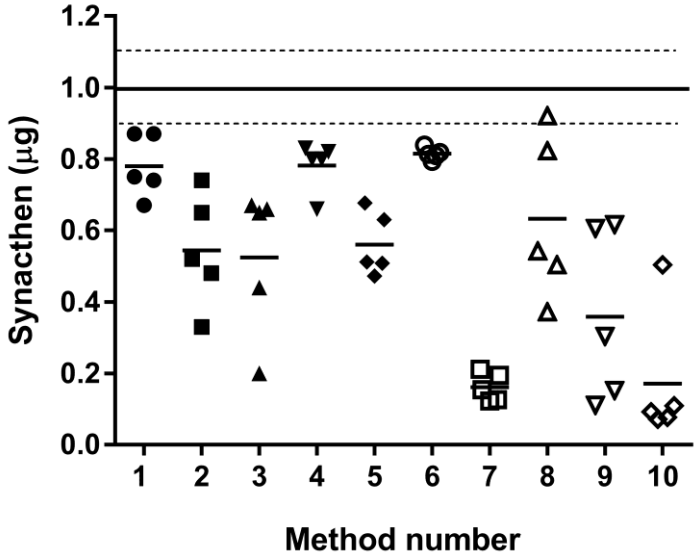

60
50
40
30
20
10
0
Percentage of respondents
High-dose test
Low-dose test
0, 30 and 60 min
0 and 30 min
0 and 60 min
30 and 60 min only
30 min only
60 min only
Combination using 20 min
Combination using 90 min
Cortisol sampling times

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.

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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)