

# Current dilution methods cause large variations and inaccuracies when making up 1mcg Synacthen dose

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## Background

- The low-dose Short Synacthen Test (LDSST) is a popular diagnostic test for adrenal insufficiency in UK children
- Although various dosing strategies exist, 1mcg is most commonly employed, but not commercially available.
- A BSPED survey<sup>1</sup> in 2012 revealed 14 different methods for diluting the 250mcg/ml ampoules.
- We investigated whether differing dilution strategies; made up using ward equipment (reflecting current practice) rather than laboratory conditions, result in different Synacthen doses being administered.

## Methods

- The 10 most popular dilution methods were tested, varying in:
  - Diluents: 0.9% saline N=9, 5% dextrose N=1
  - Number of dilutions: Single N=6, Double N=4
  - Initial quantity of Synacthen: 0.1-1ml
- Each was made up five times by the same investigator with samples taken from the top, middle and bottom of the bag.
- Samples were frozen then batch-analysed on an hACTH radioimmunoassay validated for Synacthen detection.
- Samples were diluted with 0.9% saline by 1000-20,000 (under laboratory condition, using accurate, calibrated equipment) to achieve a final concentration of 250 pg/ml (most sensitive part of the assay measuring range).
- Mean concentrations and coefficient of variation (CV) were calculated for each dilution method.
- The inter-method, intra-method and intra-bag variability were calculated.

Method n°	Summary of method
1	1ml synacthen in 1L normal saline (NS)
2	1ml synacthen in 9ml NS syringe, 1ml of this in 4ml syringe
3	1ml synacthen in 250ml NS
4	1ml synacthen in 50ml NS, 1ml of this in 9ml NS syringe
5	1ml synacthen in 50ml NS, 0.2ml of this in 0.8ml NS syringe
6	1ml synacthen in 500ml 5% dextrose
7	0.2ml synacthen in 10ml NS syringe, 0.2ml of this in 0.8ml NS syringe
8	0.2ml synacthen in 50ml NS
9	0.1ml synacthen in 50ml NS
10	0.5ml synacthen in 500ml NS

## Results

- If no significant variation between or within methods exists, applying typical laboratory medicine standards, all samples should be 250 pg/ml  $\pm$ 10% (see dotted black lines *fig 1*).
- Mean Synacthen levels of the 10 methods ranged from 9.1 pg/ml to >490.48 pg/ml.

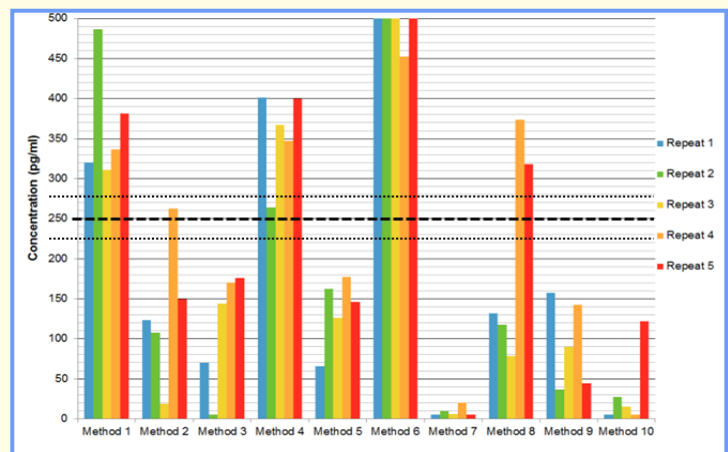


Fig 1. Mean Synacthen values of methods.

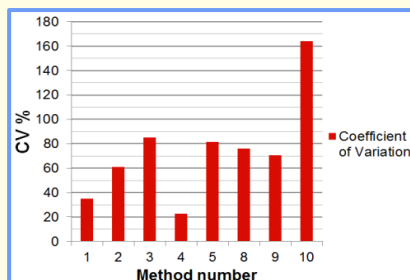


Fig 2. Inter-method variability. Methods 6-7 not included.

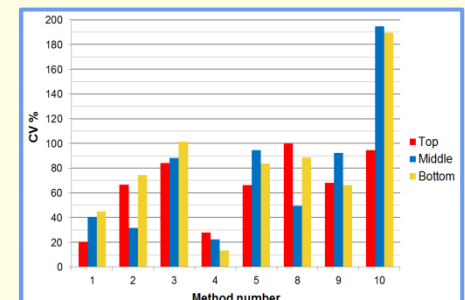


Fig 3. Intra-bag variability. Methods 6-7 not included.

- Large inter-method variability (CV range 22.9-163.8%), with method 4 showing the least and method 10 the greatest variability.
- Large intra-method variability (CV range 3.7- 149.7%), suggesting drawing up inaccuracies.
- Large intra-bag variability (CV range 13.4- 194.6%), suggest mixing issues.

## Discussion

Considerable variation was observed within and between methods.

There are many variables which may affect the actual dose of Synacthen:

- The use of inaccurate ward equipment causing drawing up inaccuracies and volume inconsistencies,
- Adsorption to plastic tubing is a particular issue with Synacthen,
- Further dilution of the samples to the most sensitive part of the assay range

## Recommendations

- A commercial preparation of 1mcg synacthen be made available.
- In the absence of a commercial preparation, low dose Synacthen should be made up under laboratory conditions to minimise inaccuracies.

## References

1. Elder C, Sachdev P, Wright N. The short synacthen test: a questionnaire survey of current usage. Archives of Diseases in Childhood. 2012;97:870-3.