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

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| **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 synacthena 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 synacthena 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 synacthena 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 synacthena 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 synacthena 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 synacthena 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 synacthena 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 synacthena 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 synacthena 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 synacthena 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.