Reference	**Kalaria 2020**[3]
Study type	Cross sectional diagnostic study
Study methodology	Data source: Outpatient assessments Recruitment: Between March 2016 and January 2019, consecutive patients attending the endocrine dynamic function clinic for a SST to evaluate HPA recovery were invited to participate
Number of patients	n = 47 (56 SSTs - seven patients underwent two SSTs each and one patient three SSTs)
Patient characteristics	Age, median (IQR): 60 (48.3–69.5) years Gender (male to female ratio): 16:31 Ethnicity: Not reported Setting: Single centre, department of endocrinology Country: UK Inclusion criteria: consecutive patients attending the endocrine dynamic function clinic for a SST to evaluate HPA recovery were invited to participate. All patients had previously been on prolonged supraphysiological therapeutic doses of oral glucocorticoids. Exclusion criteria: Those with periodontal disease were excluded. Reason for referral: To assess hypothalamic–pituitary–adrenal axis recovery in patients previously treated with prolonged supraphysiological therapeutic doses of oral glucocorticoids.
Target condition(s)	Adrenal insufficiency
Index test(s) and reference standard	**Index tests:** Basal serum cortisol, basal salivary cortisol and basal salivary cortisone SSTs were performed between 09:00 and 10:30 after oral and inhaled glucocorticoid withdrawal for at least 24 h. Baseline salivary sample was collected immediately before the baseline serum sample and then 0.25 mg of tetracosactide acetate (Synacthen) was injected either intravenously or intramuscularly and a further blood sample collected 30 min later. Saliva was collected in

<table>
<tr><td>Reference</td><td>Kalaria 2020[3]</td></tr>
<tr><td></td><td>SalivetteVR tubes (plain cotton swab; Sarstedt, Germany) according to manufacturer’s instructions. Blood was collected in S-MonovetteVR 4.7 mL Z-gel tubes (Sarstedt, Aktiengesellschaft & Co, Germany).

<u>Threshold cut-off value</u>: HPA suppression: Baseline serum cortisol: 170(nmol/L), baseline salivary cortisol: 1.92(nmol/L), baseline salivary cortisone: 9.42 (nmol/L).
HPA recovery: Baseline serum cortisol: 365(nmol/L), baseline salivary cortisol: 25.4(nmol/L), baseline salivary cortisone: 37.2 (nmol/L)

<u>Assay:</u> Salivary samples were centrifuged at 1500 g for 5 min, the cotton was discarded, and the extracted saliva frozen at 80C until analysed for cortisol and cortisone using liquid chromatography with tandem massspectrometry (LC-MS/MS) on a Shimadzu Prominence HPLC system coupled to an AB Sciex 3200 mass spectrometer based on previously described method. The LC-MS/ MS salivary cortisol and salivary cortisone assays both had a quantitation limit of 0.83 nmol/L. The intra-assay CVs were 4.9% at 9.5 nmol/L for both salivary cortisol and salivary cortisone and the inter-assay CVs were 10.8% at 9.7 nmol/L and 6.0% at 10 nmol/L, respectively.

<u>Reference standard</u> Short Synacthen test
SSTs were performed between 09:00 and 10:30 after oral and inhaled glucocorticoid withdrawal for at least 24 h. Baseline salivary sample was collected immediately before the baseline serum sample and then 0.25 mg of tetracosactide acetate (Synacthen) was injected either intravenously or intramuscularly and a further blood sample collected 30 min later.
Blood was collected in S-MonovetteVR 4.7 mL Z-gel tubes (Sarstedt, Aktiengesellschaft & Co, Germany).

<u>Threshold</u>: A SST was labelled as ‘pass’ (adequate response) if the 30-min serum cortisol was >/=450 nmol/L.

Assay: Blood was separated, and serum cortisol measured by a chemiluminescence microparticle immunoassay on an Abbott Architect i2000 (Abbott Laboratories, USA). The Abbott Architect cortisol assay has a quantitation limit of 22 nmol/L with an intra-assay and inter-assay coefficients of variation (CV) of 2.5% at 118 nmol/L and 3.5% at 110 nmol/L, respectively.

Time between measurement of index test and reference standard: reference standard approx 30 mins later</td></tr>
<tr><td>2×2 table (calculated from reported sensitivity, specificity and true positive and true negative rates)</td><td>HPA suppression - baseline serum cortisol: 170(nmol/L)
<table>
<tr><td></td><td>Reference standard +</td><td>Reference standard −</td><td>Total</td></tr>
<tr><td>Index test +</td><td>15</td><td>17</td><td>32</td></tr>
<tr><td>Index test −</td><td>0</td><td>24</td><td>24</td></tr>
<tr><td>Total</td><td>15</td><td>41</td><td>56</td></tr>
</table></td></tr>
</table>

Reference	Kalaria 2020[3]			
(calculated from reported sensitivity, specificity and true positive and true negative rates)	HPA suppression - baseline salivary cortisol: 1.92(nmol/L)			
		Reference standard +	Reference standard −	Total
	Index test +	15	20	35
	Index test −	0	21	21
	Total	15	41	56
(calculated from reported sensitivity, specificity and true positive and true negative rates)	HPA suppression - baseline salivary cortisone: 9.42(nmol/L)			
		Reference standard +	Reference standard −	Total
	Index test +	15	19	34
	Index test −	0	22	22
	Total	15	41	56
(calculated from reported sensitivity, specificity and true positive and true negative rates)	HPA recovery - baseline serum cortisol: 365(nmol/L)			
		Reference standard +	Reference standard −	Total
	Index test +	4	0	4
	Index test −	11	41	52
	Total	15	41	56
(calculated from reported sensitivity, specificity and true positive and true negative rates)	HPA recovery - baseline salivary cortisol: 25.4(nmol/L)			
		Reference standard +	Reference standard −	Total
	Index test +	0	0	0
	Index test −	15	41	56
	Total	15	41	56
(calculated from reported sensitivity,	HPA recovery - baseline salivary cortisone: 37.2(nmol/L)			
		Reference standard +	Reference standard −	Total
	Index test +	4	0	4

Reference	**Kalaria 2020**[3]				
specificity and true positive and true negative rates)	Index test −	11	41	52	
	Total	15	41	56	
Statistical measures	**HPA suppression** Index text – HPA suppression - baseline serum cortisol: 170(nmol/L) Sensitivity 100 Specificity 58.5 PPV 0.47h NPV 1 PLR 2.41 NLR 0 AUC 0.772 Index text – HPA suppression - baseline salivary cortisol: 1.92(nmol/L) Sensitivity 100 Specificity 51.2 PPV 0.43 NPV 1 PLR 2.0408 NLR 0 AUC 0.770 Index text – HPA suppression - baseline salivary cortisone: 9.42(nmol/L) Sensitivity 100 Specificity 53.7 PPV 0.44 NPV 1 PLR 2.1598 NLR 0 AUC 0.785 **HPA recovery**				

Reference	**Kalaria 2020**[3]
	Index text – HPA recovery - baseline serum cortisol: 365(nmol/L) Sensitivity 26.7 Specificity 100 PPV 1 NPV 0.79 PLR 0 NLR 0.73 AUC Index text – HPA recovery - baseline salivary cortisol: 25.4(nmol/L) Sensitivity 0 Specificity 100 PPV 0 NPV 0.73 PLR 0 NLR 1 AU Index text – HPA recovery - baseline salivary cortisone: 37.2(nmol/L) Sensitivity 26.7 Specificity 100 PPV 1 NPV 0.789 PLR 0 NLR 0.73 AUC
Source of funding	The author(s) received no financial support for the research, authorship, and/ or publication of this article.
Limitations	Risk of bias: serious (due to patient flow) Indirectness: none
Comments	Unclear if it was 47 patients or 56 SSTs included in analysis. The cut-offs derived in this study may not be applicable to the evaluation of HPA function in cohorts without HPA suppression or in females on oral oestrogen or premenopausal.