Late night salivary cortisol

By : Dr. Amir Reza Goharian ,

Endocrinologist

Isfahan Endocrine Research Center – Sedigheh Tahereh

Salivary Cortisol

Salivary Cortisol


بسم الله الرحمن الرحيم

Saliva Analysis

- The SIS has been validated by Salimetrics for collecting saliva for the analysis of: Cortisol, Alpha-Amylase (sAA), Cotinine, Immunoglobulin G (IgG), Immunoglobulin M (IgM), Interleukin-1 Beta (IL-1β), Osteocalcin, Secretory IgA (SIgA), Testosterone, Uric Acid, and DNA.

Saliva measurements reportedly correlate with blood measurements for some hormones such as cortisol, progesterone, estradiol, and testosterone, but they do not correlate well for others (e.g., thyroid and pituitary hormones).[211–219] Multiple preanalytic vari-

IMPORTANCE OF SALIVARY CORTISOL (LNSC)

CLINICAL SIGNIFICANCE

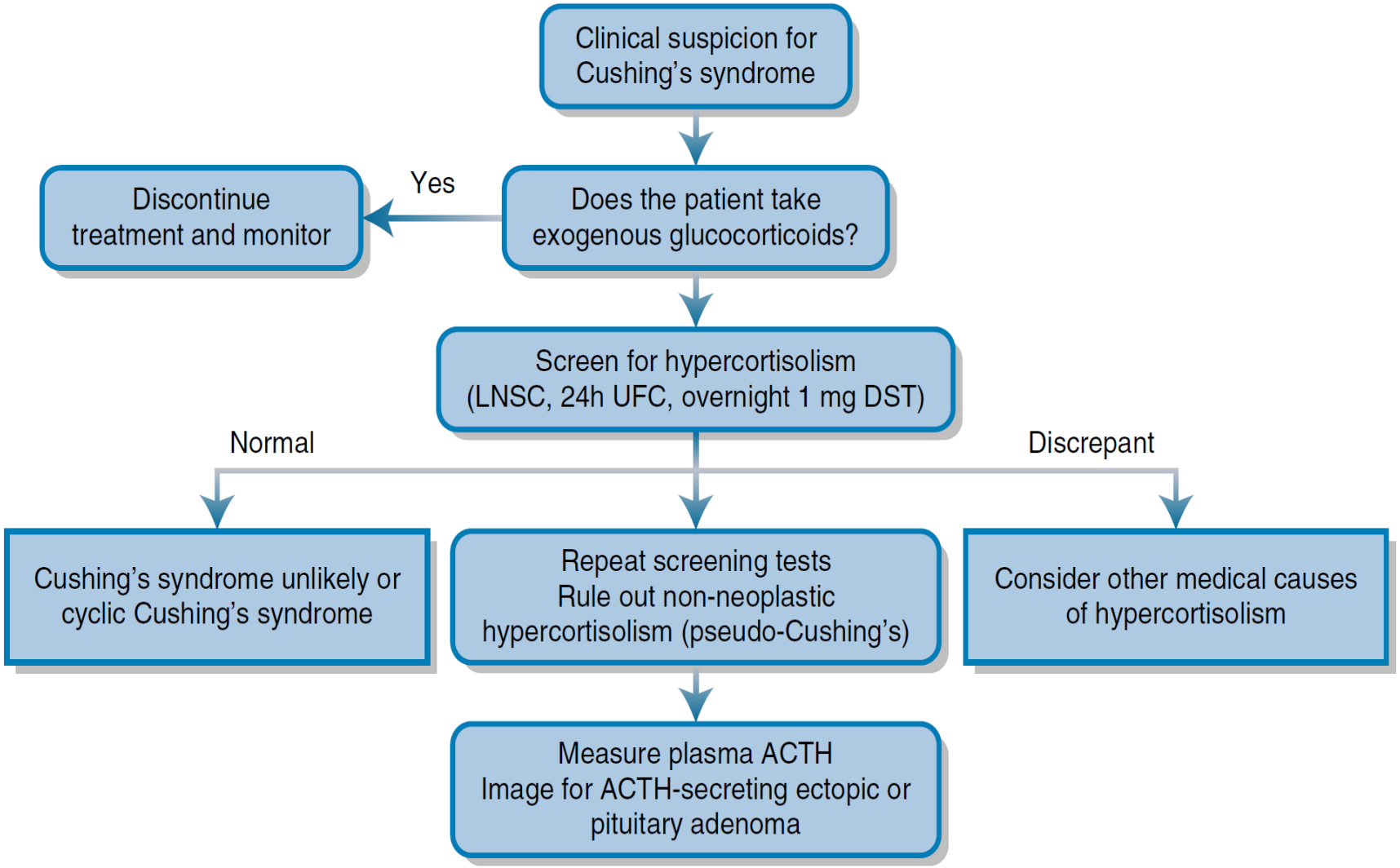
- The majority of cortisol in saliva is not bound and enters the saliva via intracellular mechanisms.
- Salivary cortisol levels are unaffected by salivary flow rate or salivary enzymes.
- It is a high correlations between serum and saliva cortisol levels.

Screening Tests

• BOX 13.5 Tests Used in the Diagnosis and Differential Diagnosis of Cushing Syndrome

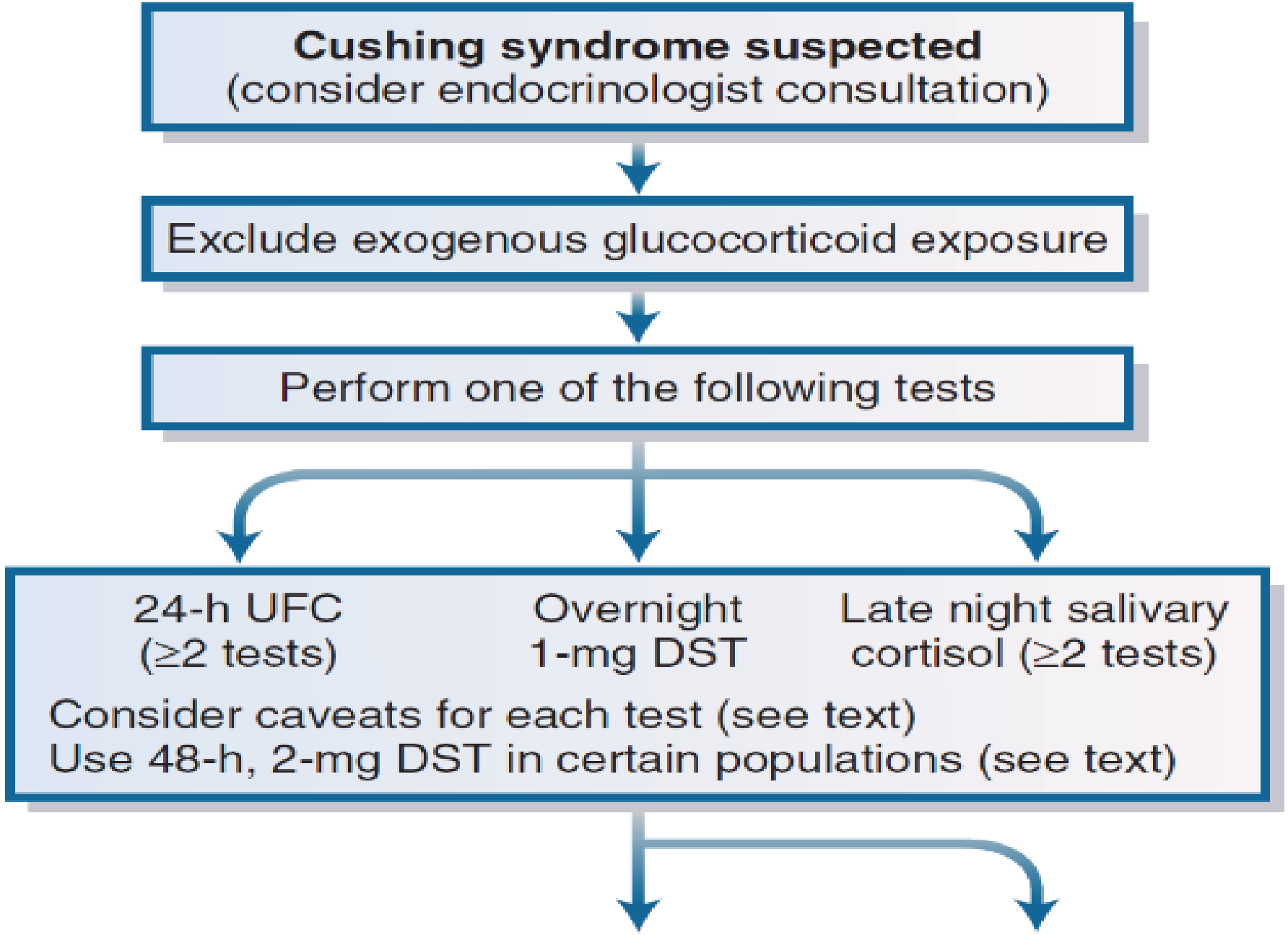
Diagnosis—Does the Patient Have Cushing Syndrome?

Late-night salivary cortisol/circadian rhythm of plasma cortisol
Urinary free cortisol excretion[a]
Low-dose dexamethasone suppression test[a]



• **Fig. 7.52** Differential diagnosis of Cushing syndrome. *ACTH,* adrenocorticotrophic hormone; *DST,* dexamethasone suppression test; *LNSC,* late-night salivary cortisol; *UFC,* urinary free cortisol. (Modified from Fleseriu M, Auchus R, Bancos I, et al. Consensus on diagnosis and management of Cushing disease: a guideline update. *Lancet Diabetes Endocrinol*. 2021;9:847–875.)

Diagnostic Guidelines. The Endocrine Society, in collaboration with the European Society for Endocrinology, has issued evidence-based guidelines for the diagnosis of Cushing syndrome.[269] Recommendations are to proceed initially with one of four highly sensitive screening tests: urinary free cortisol, late-night salivary cortisol, overnight dexamethasone, or the 2-mg/48-hour dexamethasone suppression test. Abnormality detected by any of these tests in a patient with clinically suspected Cushing syndrome should be confirmed with one of the additional tests; if both test results are abnormal, patients should then undergo testing for the cause of the Cushing syndrome (Fig. 13.20).


Cushing syndrome suspected
(consider endocrinologist consultation)
Exclude exogenous glucocorticoid exposure
Perform one of the following tests
24-h UFC
(≥2 tests)
Overnight
1-mg DST
Late night salivary
cortisol (≥2 tests)
Consider caveats for each test (see text)
Use 48-h, 2-mg DST in certain populations (see text)

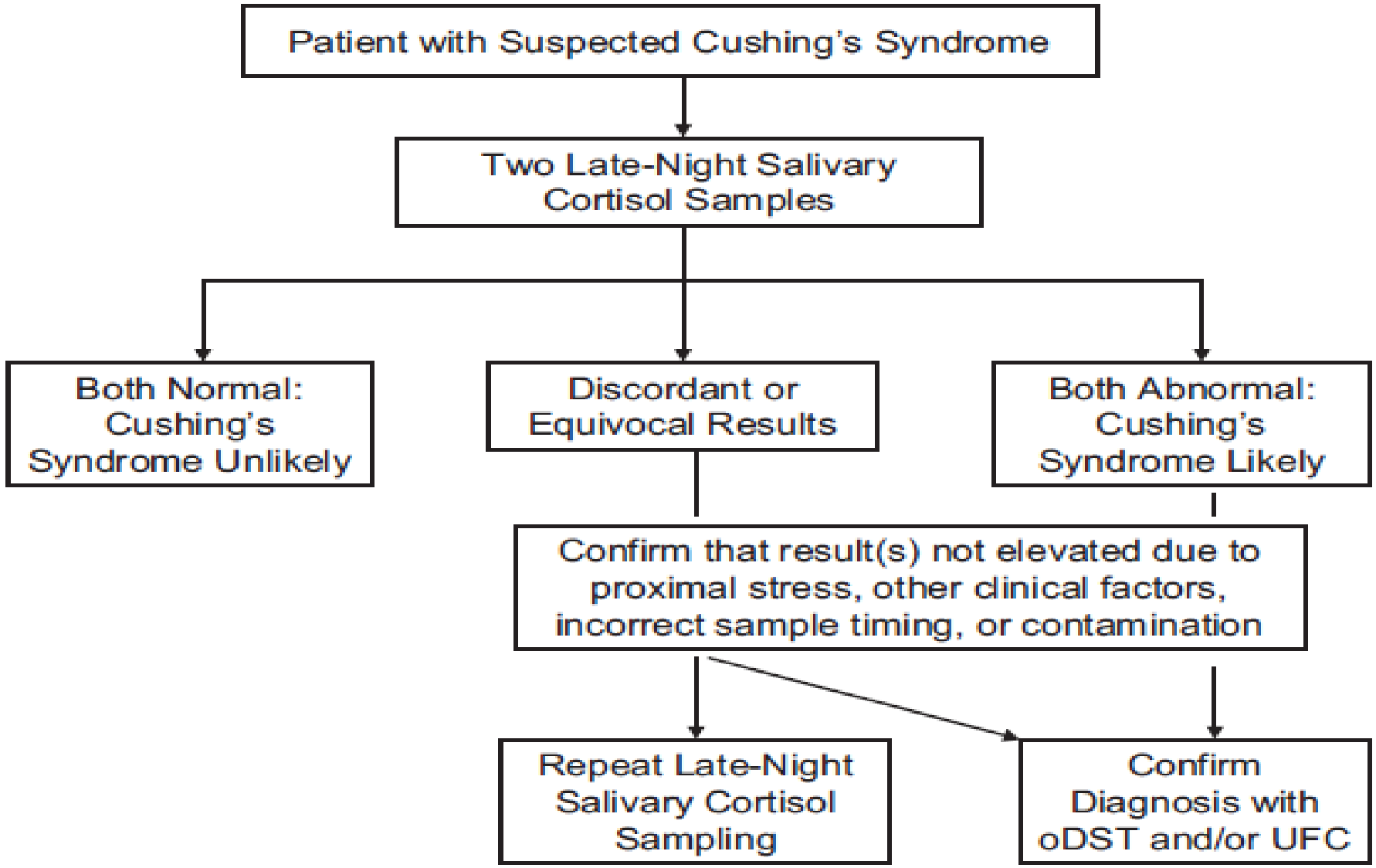


FIG. 1. A paradigm using salivary cortisol as the initial test to evaluate patients with suspected Cushing's syndrome (adapted from Ref. 4). oDST, Overnight 1 mg dexamethasone suppression test; UFC, 24-h urine free cortisol.

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Late-Night Salivary Cortisol as a Screening Test for Cushing's Syndrome*

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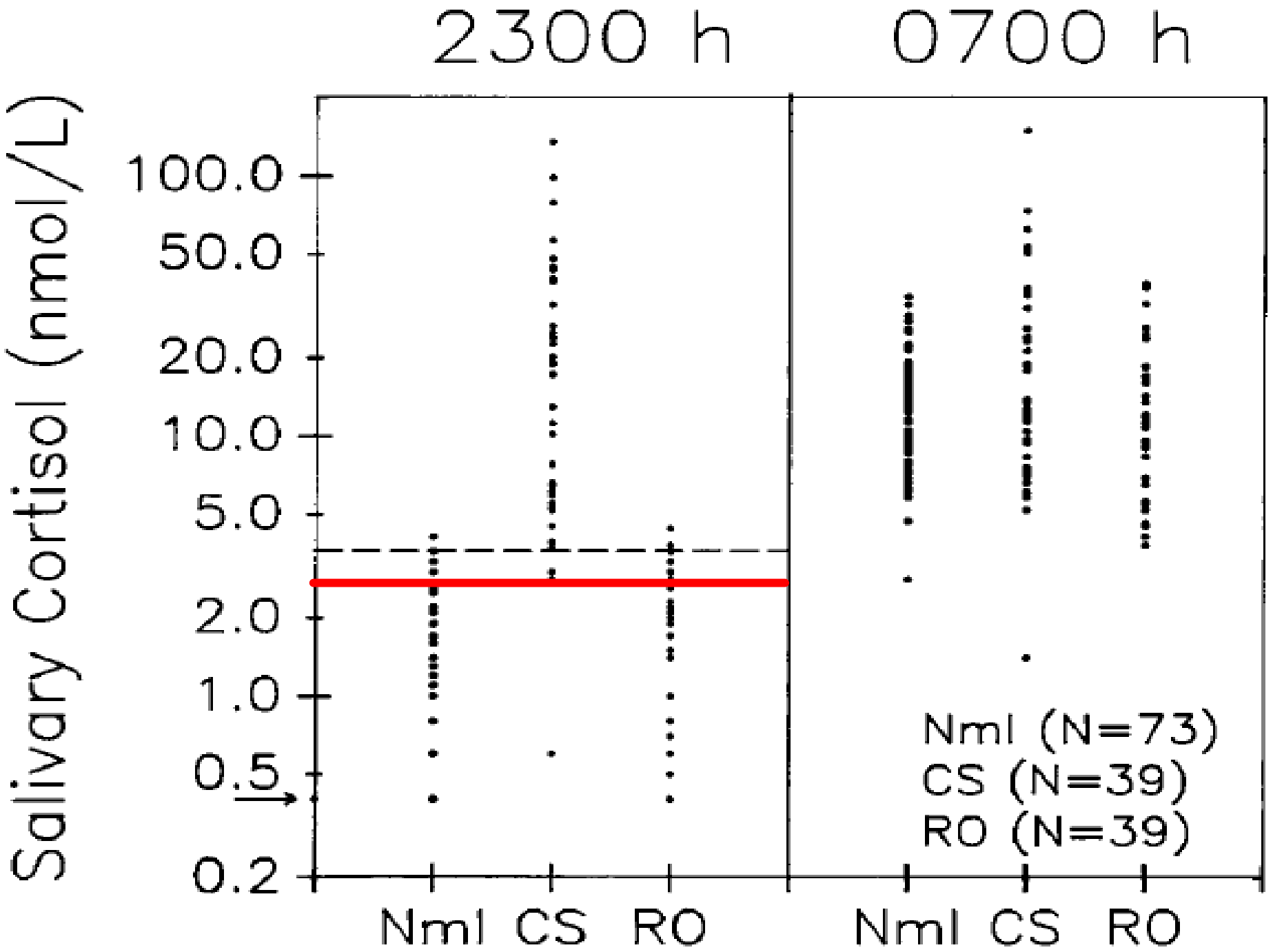
1998

TABLE 1. Mean ± SE for 2300-h and 0700-h salivary cortisol and the ratio of 0700-h to 2300-h salivary cortisol

	Salivary cortisol		
	nmol/L		0700-h:2300-h ratio
	2300 h	0700 h	
Normal subjects (N = 73)	1.2 ± 0.1	14.5 ± 0.8[a]	18.5 ± 1.9
Male (N = 35)	1.2 ± 0.1	15.6 ± 1.3[a]	
Female (N = 38)	1.2 ± 0.1	13.6 ± 1.0[a]	
Cushing's syndrome (N = 39)	24.0 ± 4.5[b]	23.0 ± 4.2[c]	1.8 ± 0.4[b]
R/O Cushing's (N = 39)	1.6 ± 0.2	15.3 ± 1.5[a]	14.7 ± 2.3

[a] 0700 h greater than 2300 h ($P < 0.001$); Cushing's syndrome different from other groups ([b] $P < 0.001$, [c] $P = 0.013$). R/O Cushing's was group in whom Cushing's syndrome was excluded or not firmly established.


2300 h
0700 h
Salivary Cortisol (nmol/L)
100.0
50.0
20.0
10.0
5.0
2.0
1.0
0.5
0.2
Nml (N=73)
CS (N=39)
RO (N=39)
Nml CS RO
Nml CS RO

Endocrine (2013) 44:346–349
DOI 10.1007/s12020-013-0013-0

ENDOCRINE METHODS AND TECHNIQUES

Update on late-night salivary cortisol for the diagnosis of Cushing's syndrome: methodological considerations

Hershel Raff

When we first started using LNSC in the 1990s, we proposed sampling two nights in a row [5].

5. H. Raff, Salivary cortisol: a useful measurement in the diagnosis of Cushing's syndrome and the evaluation of the hypothalamic pituitary adrenal axis. Endocrinologist 10, 9–17 (2000)

- The measurement of late-night salivary cortisol (LNSC) is now accepted as the best approach to screen patients for suspected Cushing's syndrome [1–7].

- Most reference laboratories routinely perform this test, and so there should be no barrier to its widespread use in clinical practice.

- **LNSC** is very useful in screening for Cushing's syndrome in women with increased corticosteroid-binding globulin resulting from estrogen therapy or pregnancy.

- Two **LNSCs** from each patient is recommended for routine screening, although one adequate saliva sample seems to perform well.

LNSC in women

- A recent study demonstrated 95 % sensitivity and 95 % specificity for Cushing's syndrome in women on OCPs [18], in agreement with studies in nonpregnant Cushing's patients [19].

- The sensitivity and specificity were less (85 %) for pregnant women, but still quite useful.
- **LNSC seems to be a useful approach to rule out Cushing's syndrome in pregnancy and in women taking OCPs.**

Sample contamination

- **The problem with these materials is that they contain authentic cortisol that is indistinguishable from endogenous cortisol, even by LC/MS–MS.**

Table 1 Scheme for screening patients suspected of Cushing's syndrome [2]

4. If both LNSCs are >20 times the upper limit of the reference range and the patient's symptoms are mild, then suspect contamination of the samples with authentic cortisol (e.g., topical hydrocortisone) and/or synthetic steroids that cross-react in many immunoassays (e.g., prednisolone). Evaluate one of the samples for cortisol and cortisone by LC/MS–MS

a. If the salivary cortisol and cortisone concentrations are increased and the cortisol to cortisone ratio is <1 (normal), then Cushing's syndrome is established

b. If salivary cortisol concentration is very low, then evaluate the sample for synthetic steroids that may cross-react in the immunoassay (e.g., prednisolone)

syndrome is established

c. If the cortisol to cortisone ratio is high and salivary cortisone concentration is normal, then contamination with topical hydrocortisone is proven and Cushing's syndrome is extremely unlikely

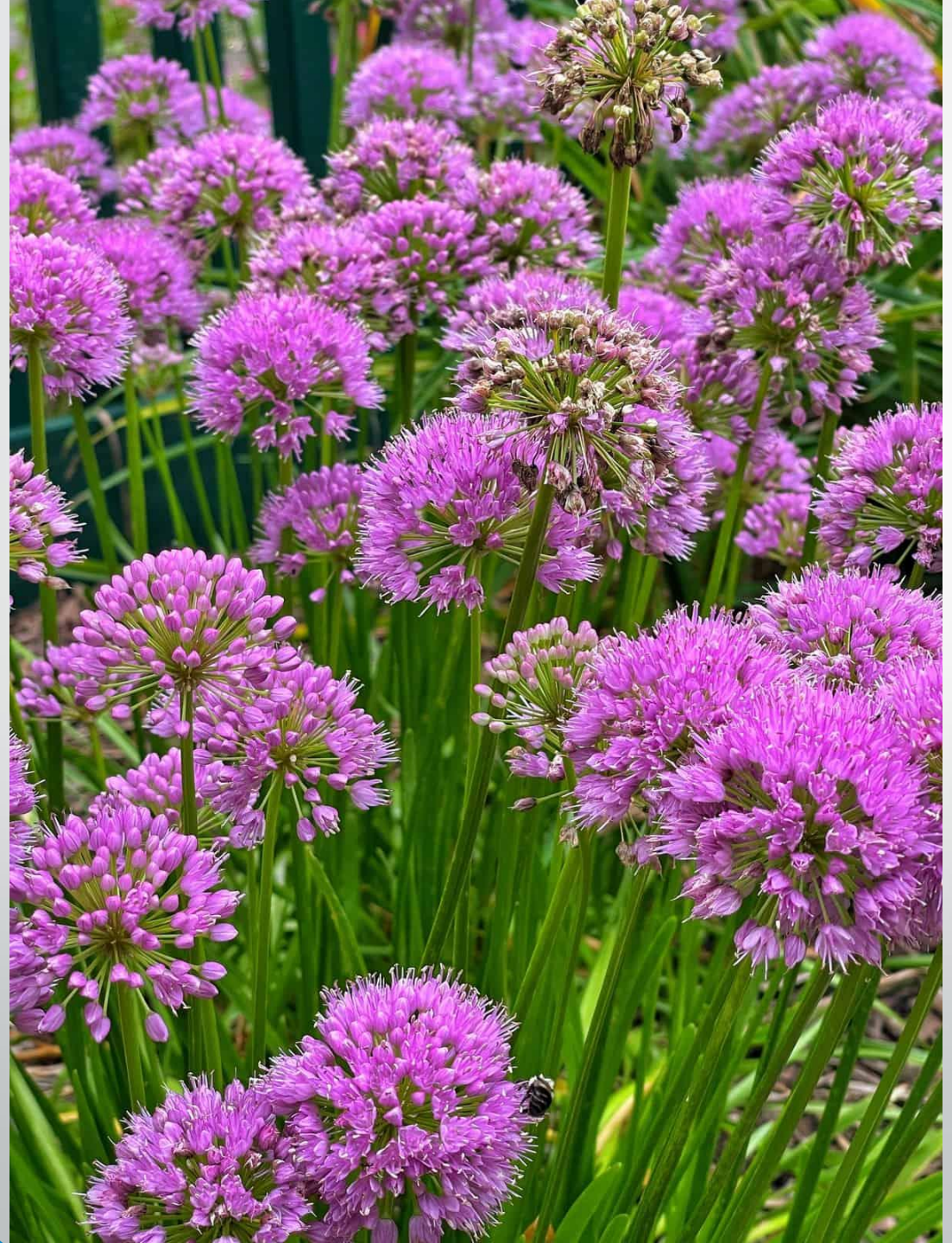
LNSC late-night salivary cortisol, *oDST* overnight dexamethasone suppression test, *UFC* urine free cortisol, *LC/MS–MS* liquid chromatography/tandem mass spectrometry

Caveats and restrictions for tests used to screen for hypercortisolism

Bedtime salivary cortisol	May be falsely abnormal in older men and women, and in hypertensive or diabetic patients (115)	If used in these populations, consider accepting only normal results.
	May be falsely abnormal in individuals with variable sleeping times (eg, shift workers) (116)	If used in this population, consider accepting only normal results.

SURVEILLANCE of TSS

- Periodic assessment of LNSC is extremely useful in monitoring patients for recurrence of Cushing's disease after pituitary surgery.

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Endocrine CONNECTIONS

R S Mohamed *et al.* | 11:7 | e220050

RESEARCH

Late-night salivary cortisol and cortisone should be the initial screening test for Cushing's syndrome

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- **Saliva collection is non-invasive and can be carried out at home.**

- Despite the fact that LNSC has been shown to have high diagnostic sensitivity and specificity and that it has been shown to be cost-effective, it still remains the least widely used biochemical screening tool for CS (5, 6).

- Salivary glands express 11-β-hydroxysteroid dehydrogenase (11B-HSD2) which converts salivary cortisol to cortisone.

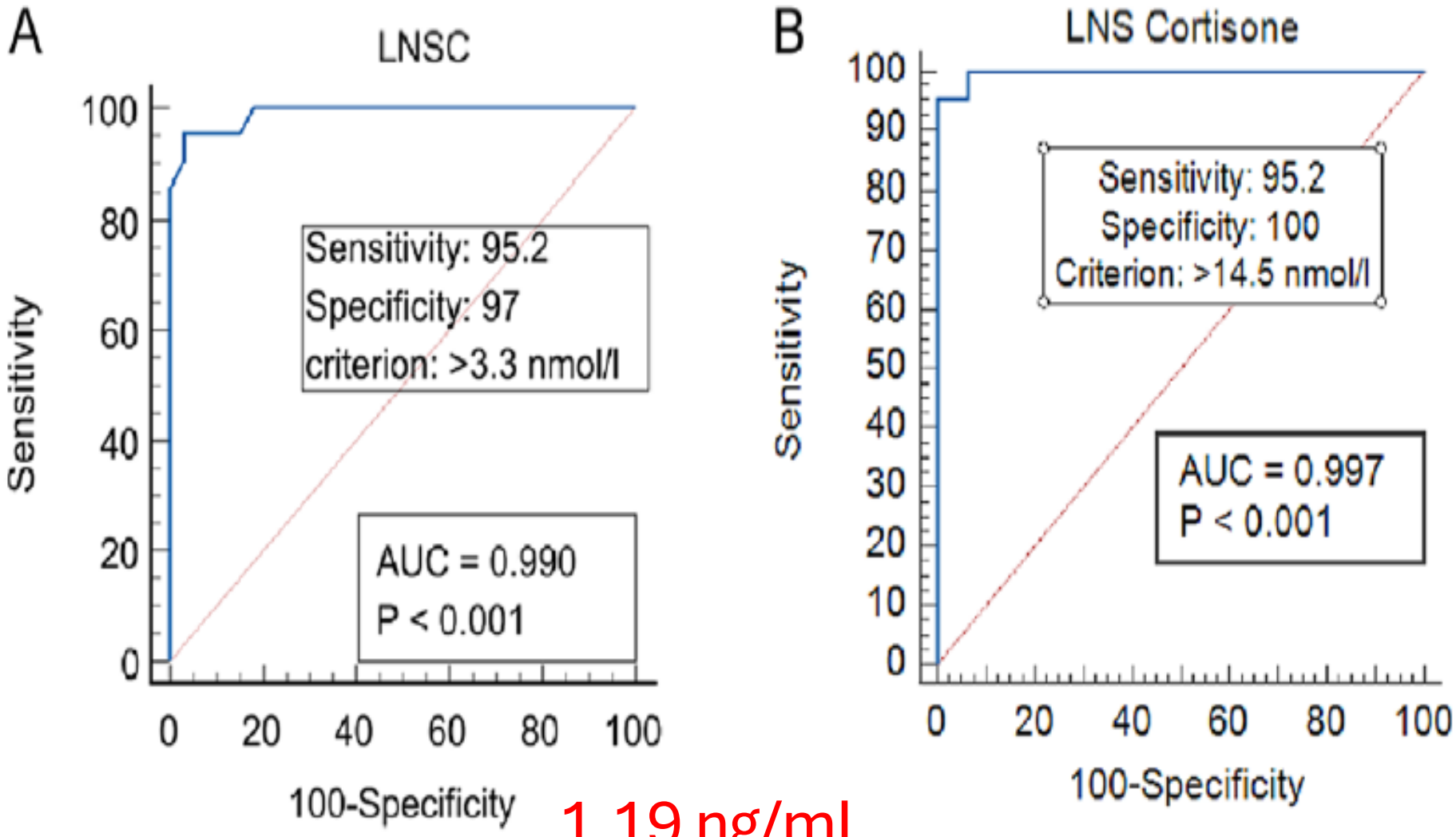
- The amount of salivary cortisone is significantly greater than salivary cortisol and an increased cortisol/cortisone ratio reflects exposure to both endogenous and exogenous cortisol.
- Salivary cortisone can be reliably measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (7).

Results

Table 3 Comparison of diagnostic accuracy of investigations for Cushing's syndrome.

	LNSC	LNS cortisone	LDDST	ODST	UFC
Sensitivity	95% (76–99%)	86% (64–97%)	100% (75–100%)	100% (77–100%)	60% (26–88%)
Specificity	91% (76–98%)	100% (89–100%)	83% (52–98%)	77% (55–92%)	100% (48–100%)
AUC	0.931 (0.83–0.98)	0.929 (0.83–0.98)	0.917 (0.74–0.99)	0.886 (0.74–0.97)	0.8 (0.52–0.96)
Positive likelihood ratio	10.5 (3.6–31)		6 (1.7–21.3)	4.4 (2–9.5)	
Negative likelihood ratio	0.05 (0.01–0.36)	0.14 (0.05–0.41)	0	0	0.4 (0.19–0.86)
Positive predictive value	87% (70–95%)	100%	87% (65–96%)	74% (56–86%)	100%
Negative predictive value	97% (82–98%)	92% (85–99%)	100%	100%	56% (37–73%)

AUC, area under the curve; LNS cortisone, late-night salivary cortisone; LNSC, late-night salivary cortisol; LDDST, 48-h low-dose dexamethasone suppression test; ODST, overnight dexamethasone suppression test; UFC, 24-h urinary free cortisol.


A
LNSC
Sensitivity
100
80
60
40
20
0
Sensitivity: 95.2
Specificity: 97
criterion: >3.3 nmol/l
AUC = 0.990
P < 0.001
0
20
40
60
80
100
100-Specificity
B
LNS Cortisone
Sensitivity
100
90
80
70
60
50
40
30
20
10
0
Sensitivity: 95.2
Specificity: 100
Criterion: >14.5 nmol/l
AUC = 0.997
P < 0.001
0
20
40
60
80
100
100-Specificity

1.19 ng/mL

Table 4 Diagnostic test accuracy for the diagnosis of Cushing's disease first presentation and recurrent Cushing's disease with late-night salivary cortisol and late-night salivary cortisone.

	CD first presentation + recurrent CD	CD first presentation	Recurrent CD
Late-night salivary cortisol			
Sensitivity	100% (79–100%)	100% (69–100%)	100% (54–100%)
Specificity	91% (76–98%)	91% (76–98%)	91% (76–98%)
AUC	0.96 (0.85–0.99)	0.96 (0.84–0.99)	0.96 (0.84–0.99)
Positive likelihood ratio	11 (3.7–32.4)	11 (3.7–32.4)	11 (3.7–32.4)
Negative likelihood ratio	0	0	0
Positive predictive value	84% (64–94%)	77% (553–91%)	67% (40–86%)
Negative predictive value	100%	100%	100%
Late-night salivary cortisone			
Sensitivity	88% (62–99%)	90% (55–99%)	83% (36–99%)
Specificity	100% (89–100%)	100% (89–100%)	100% (89–100%)
AUC	0.94 (0.83–0.99)	0.95 (0.84–0.99)	0.92 (0.78–0.98)
Positive likelihood ratio			
Negative likelihood ratio	0.13 (0.03–0.46)	0.1 (0.02–0.64)	0.17 (0.03–0.99)
Positive predictive value	100%	100%	100%
Negative predictive value	94% (81.8–98%)	97% (84–99%)	97% (85–99%)

AUC, area under the curve; CD, Cushing's disease.

- Our data confirm that ***LNSC*** has high sensitivity and high NPV when used in a tertiary referral setting.
- By contrast, UFC had very low sensitivity and NPV, thus making it unreliable as a screening tool for CS.
- A recent guideline update on CS diagnosis and management suggests LNSC has 97% sensitivity and 97.5% specificity in diagnosing CS
- and 75–90% sensitivity and 93–95% specificity in diagnosing recurrent CD.

- LNSC is not affected by ***BMI*** or ***cortisol binding globulin*** and therefore accurately reflects serum cortisol concentrations.
- There is conflicting evidence regarding LNSC levels in *polycystic ovarian syndrome*.

Recurrent CD after TSS

- It also appears that LNSC detects recurrent CD earlier than UFC thus making it the ideal test to follow up patients after successful pituitary surgery for CD (6, 11, 13).

Carroll *et al.*

- A meta-analysis by Carroll *et al.* concluded that LNSC is a robust and convenient test to screen and diagnose CS.

- Similar to our own analysis, the authors found that LNSC had a diagnostic sensitivity of 92% and a specificity of 96% (18).

ELISA

- However, many centres do not have access to LC-MS/MS.
- There is also the option of measuring LNSC by immunoassay which is much easier and cheaper with similarly excellent results (22).

MACS

- Multiple sequential LNSC measurements are very useful to investigate diagnostically challenging patients with cyclic Cushing's disease.
- However, the clinical assessment of an experienced endocrinologist remains key to make a correct diagnosis (23).

LNSC + LNS Cortisone

- Garrahy *et al.* showed that a combination of LNSC and LNS cortisone had a sensitivity of 94% compared to LNSC and LNS cortisone alone which had a sensitivity of 92 and 87%, respectively.

LNS CORTISONE

- We fully agree that LNS cortisone should be routinely included in CS workup where available.

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The Journal of Clinical Endocrinology & Metabolism 90(10):5730–5736

doi: 10.1210/jc.2004-2264

Reproducibility of Nighttime Salivary Cortisol and Its Use in the Diagnosis of Hypercortisolism Compared with Urinary Free Cortisol and Overnight Dexamethasone Suppression Test

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Clinic for Endocrinology, Diabetes, and Clinical Nutrition (A.V., J.J.P., H.Z., U.K., B.M.) and Department of Central Laboratories (P.H.), University Hospital Basel, 4031 Basel, Switzerland

- In the absence of published data on its day-to-day variability, we assessed the reproducibility of NSC by repeated measurements in healthy volunteers.

- Its diagnostic performance was compared with 24-h urinary free cortisol (UFC) and 1 mg overnight dexamethasone suppression test in : 12 patients with CS , 20 healthy volunteers , 14 referred patients in which CS was excluded or not firmly established, 16 obese patients, and 20 women in late pregnancy.

Results:

- NSC showed a superior reproducibility in healthy volunteers with a low day-to-day variability as reflected by an intraclass correlation coefficient of 0.78.

- The receiver operating characteristic curve-estimated cutoff of ***6.1 nmol/liter (0.22 g/dl)*** demonstrated a sensitivity and specificity of ***100%*** (area under the receiver operating characteristic curve, 1.0 ; 95% confidence interval, 0.94–1.0) in the diagnosis of CS.

- NSC , 24-h UFC [after adjusting the local laboratory cutoff to 504 nmol/d (183 μg/d)] , and the urinary cortisol/creatinine ratio showed a tendency to be superior to 1 mg dexamethasone suppression test in correctly identifying CS.

- In late pregnancy, the preserved diurnal variation at a higher level of salivary cortisol reduced the specificity of NSC to *75%*.

CONCLUSION:

- Based on its remarkable reproducibility, easy noninvasive nature, and at least similar diagnostic performance, NSC appears to be a preferable alternative to 24-h UFC as a first-line screening test for CS.

- The cutoff values of NSC, 24-h UFC, and urinary cortisol/creatinine ratio have to be carefully adjusted using assay and center-specific reference ranges of sufficiently large populations.

(*J Clin Endocrinol Metab* 90: 5730–5736, 2005)

LNSC IN CHILDREN

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doi:10.1210/jendso/bvab033
Clinical Research Article

Clinical Research Article

Bedtime Salivary Cortisol as a Screening Test for Cushing Syndrome in Children

Grethe Å. Ueland,[1,2] Ralf Kellmann,[1] Melissa Jørstad Davidsen,[1,3] Kristin Viste,[1] Eystein S. Husebye,[2,3,4] Bjørg Almås,[1] Helen L. Storr,[5] Jørn V. Sagen,[1,3] Gunnar Mellgren,[1,3,6] Petur B. Júlíusson,[3,7,8] and Paal Methlie[1,2]

Methods:

- Bedtime and morning salivary samples were collected from 320 healthy children aged 4 to 16 years.
- Fifty-four patients from the children's outpatient obesity clinic and 3 children with pituitary CS were used for validation.
- Steroid hormones were assayed by LC-MS/MS.

- Cutoff levels for bedtime salivary cortisol and cortisone were defined by the 97.5% percentile in healthy subjects.

Results:

- Bedtime cutoff levels for cortisol and cortisone were 2.4 and 12.0 nmol/L, respectively.
- Applying these cutoff levels on the verification cohort, 1 child from the obesity clinic had bedtime salivary cortisol exceeding the defined cutoff level, but normal salivary cortisone.
- All 3 children with pituitary CS had salivary cortisol and cortisone far above the defined bedtime cutoff levels.
- Healthy subjects showed a significant decrease in salivary cortisol from early morning to bedtime.

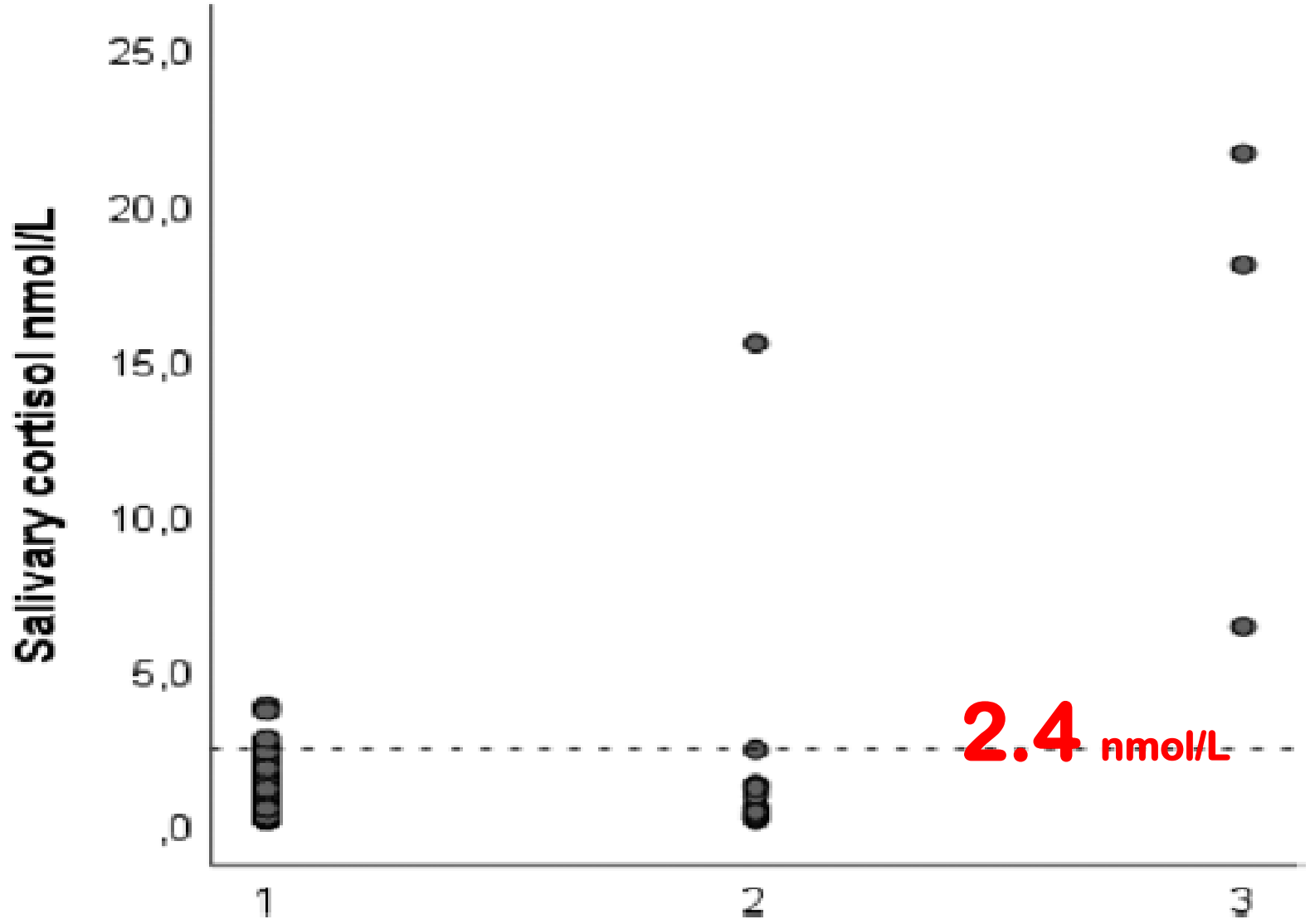

Figure 2. Dot plot showing the distribution of bedtime salivary cortisol (nmol/L) for (1) healthy children, (2) children from the validation cohort, and (3) patients with Cushing syndrome. Dotted line indicating the cutoff level for bedtime salivary cortisol found in this study.

Conclusions:

- We propose that bedtime salivary cortisol measured by LC-MS/MS with a diagnostic threshold above 2.4 nmol/L can be applied as a screening test for CS in children.

- Age- and gender-specific cutoff levels are not needed.

Endocrine Reviews, 2022, Vol. 43, No. 5, 852–877
https://doi.org/10.1210/endrev/bnab046
Review

Review

Molecular Derangements and the Diagnosis of ACTH-Dependent Cushing's Syndrome

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ORCiD number: 0000-0003-0534-8025 (L. K. Nieman).

Late Night (Bedtime) Salivary Cortisol

- Factors that influence the choice of this test include the following:
- *False negative results:*

  i. Patients with **cyclic CS** will have normal results if tested during a nadir period.

False positive results:

- **i. Patients who have inconsistent sleep-wake cycles — these patients do not have normal diurnal rhythms (130).**
- **Thus, this test is not advisable for individuals with inconsistent shift work or those with large variations in sleep onset time, who risk a false-positive result.**

In all cases, the instructions should call for collection at bedtime and not at a specific clock time.

- *Travel over multiple time zones:* Healthy individuals have abnormal diurnal rhythms when crossing time zones: rhythms tend to lag initially closest to the home time zone response, with gradual resolution, like shift workers (131), potentially leading to a false-positive result.

False Positive Results

- iii. *Other reasons for false positive results:* Salivary cortisol can be elevated by excitement (132), contamination with hydrocortisone in skin creams (133), chewing tobacco with licorice, and cigarette smoking (due to inhibition of 11βHSD 2 in salivary glands) (134).

- To avoid the preventable and theoretical increases, patients are told to collect on a "quiet" evening, without engaging in anything exciting, to refrain from flossing or brushing teeth until after the collection, and to spit directly into the collection container or to move the pledget to and from the collection container with their mouth (ie, to not touch it with their fingers).

- The potential contamination with exogenous cortisol (or subnormal 11βHSD2) can be evaluated by measuring both cortisol and cortisone by tandem mass spectrometry; the cortisol level will be much higher than the cortisone level in these situations (133).

False Positive Results

- Some conditions, such as older age (>60 years), diabetes, and hypertension are associated with higher bedtime salivary cortisol values, so that as many as 43% of subjects would be falsely diagnosed with CS if all 3 conditions are present (125,126).
- Consideration should be given to using other tests in this population.

Interpretation

- **A recent large literature review of studies comparing responses of 1102 patients with proven CS and 2039 in whom CS was excluded showed a sensitivity, specificity, positive likelihood ratio, and negative likelihood (with 95% confidence interval estimates) of 94.5 (91.3-96.6), 89.7 (85.9-92.6), 9.2 (6.6-12.8), and 0.06 (0.04-0.10), respectively (128).**

CS-5016.04 | Rev. 4, 28JUL2023

FDA Listed | CE | UKCA

SALIMETRICS®

SalivaBio
Exclusively from Salimetrics

Collection Methods: Passive Drool using the Saliva Collection Aid

- ✓ Use for participants 6 years of age and older*
- ✓ Constructed of polypropylene

Dia.Metra *Saliva Collection Device*

Instructions for Use:

Step 1:

If packaged, open pouch and remove the Saliva Collection Aid (SCA). Otherwise, proceed to Step #2.

Step 2:

Place ribbed-end of the SCA securely into a pre-labeled collection vial *(see Caution 3 above)*.

Step 3:

Allow saliva to pool in mouth. Then, with head tilted forward, **gently** guide saliva through the SCA into the vial. Fill to the required volume.*

Step 4:

Remove and discard SCA. Attach cap to collection vial and tighten.

Saliva Processing Instructions with *Saliva Collection Device Dia.Metra*

1. Let the saliva flow down trought the straw into the centrifuge glass tube.
2. Centrifuge the sample for 15 minutes at 3000 rpm
3. Store at – 20°C for at least 1 hour
4. Centrifuge again for 15 minutes at 3000 rpm
5. The saliva sample is now ready to be tested.
6. Store the sample at 2 - 8°C for one week or at – 20°C for longer time.

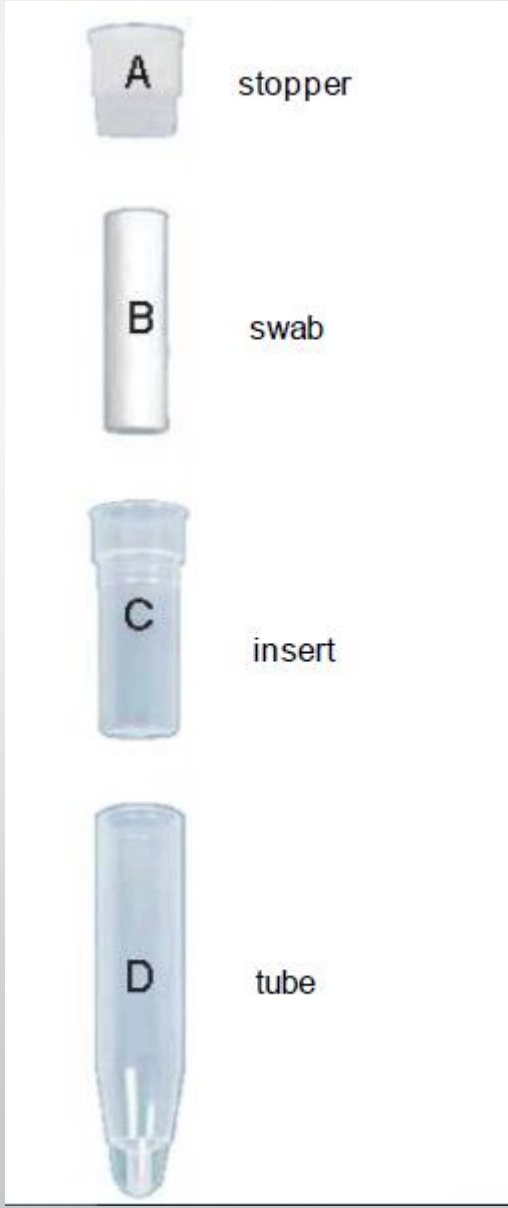
Saliva Cortisol Test Collection Guidelines

- To do a saliva cortisol test, you will need a Salivette® collection device kit.
Do not collect saliva in any other container.

- The collection time is usually between 11 p.m and 1 a.m.

- Before you collect saliva, **do not let** your child:

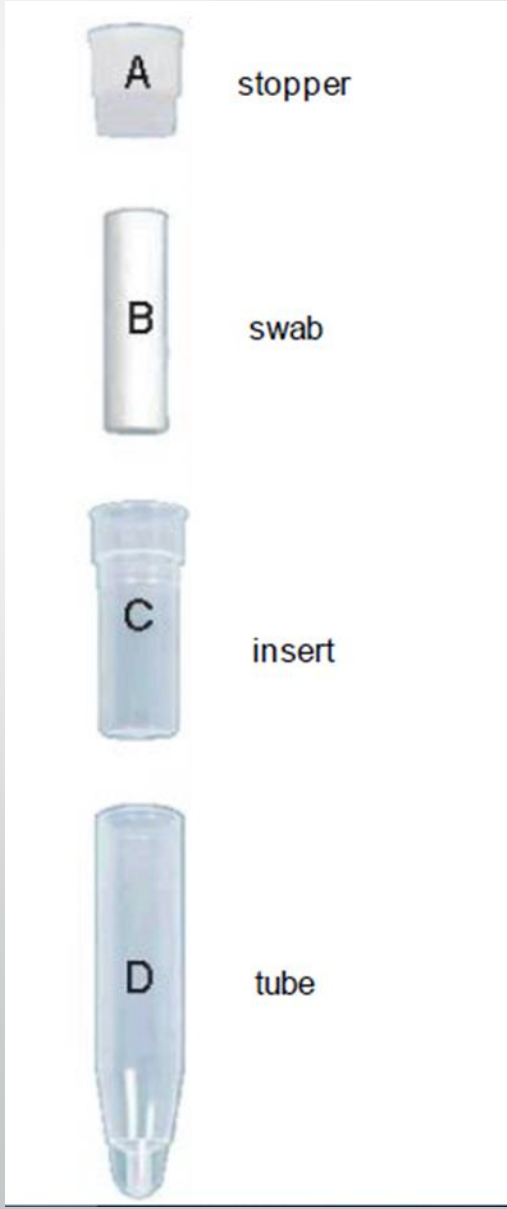
- eat for 60 minutes. (1 hour)
- have any alcohol less than 12 hours before.
- brush or floss teeth or do anything that would cause the gums to bleed.



Salivette®

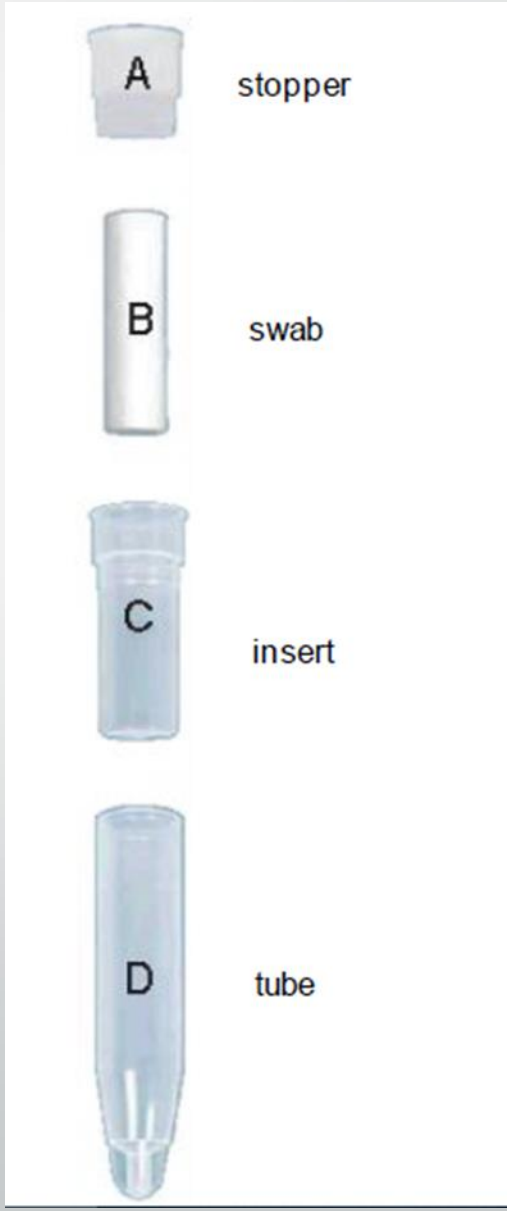
- **Ask your child to rinse their mouth thoroughly with water 1 hour before collecting saliva.**
- **Your child may have to repeat the test if the pH value(s) is more than 9.0 or less than 3.5. Your health care provider will let you know.**
- It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other such extraneous materials.

Wash your hands with soap and water, rinse and dry well before starting the test.



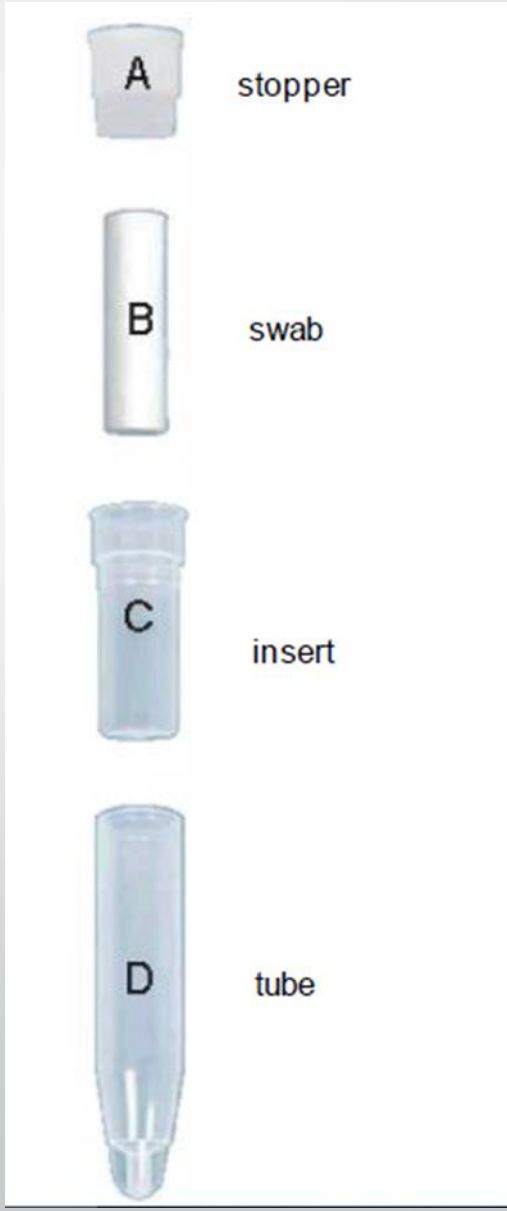
- Take the cap or stopper (A) off the container to get to the swab (B). ***Never touch the swab with your hands***. Always leave the insert (C) inside of the tube (D). The insert is needed to process the specimen at the lab (laboratory).

Tip the tube (D) directly inside the child's mouth. The swab (B) should fall on top of or under the tongue so that it can move freely in the mouth. ***Do not let the swab go in between the cheek and gum.***



- Ask your child to roll the swab inside their mouth for 2 to 3 minutes. It is OK to gently chew it. The swab must be completely wet to do the test right. (It needs have about ¼ teaspoon of saliva.)

Ask your child to carefully spit the wet swab back into the insert (C) ***without touching it.***



- Put the stopper (A) back on tightly. The cap will click when closed correctly.
- Look at the swab to make sure that there is no blood, mucus or bits of food or debris. The specimen should be clear.
- Put the specimen in the refrigerator.

Specimen storage and transport

- **All specimens need to be kept cold in the refrigerator until you take them to the nearest laboratory.**

CE

DiaMetra

care for quality

DCM020-11
Ed. 09/2018

CORTISOL SALIVA ELISA

for routine analysis

Direct immunoenzymatic determination of Cortisol in saliva.

IVD

LOT

See external label

2°C 8°C

Σ = 96 tests

REF DKO020

Saliva Processing Instructions with *Salivette Sardstedt*

- Centrifuge the Salivette for 2 minutes at 1000g (rcf) for saliva generation.

- Remove the insert complete with the swab from the centrifuge vessel and discard. The clear saliva is now ready for analysis (at least 1 mL of saliva should be recovered with this method).

REFERENCE VALUES

A.M.	3 – 10 ng/mL
P.M.	0.6 – 2.5 ng/mL

REFERENCE VALUES

- Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors , such as :

  - specificity and sensitivity of the method used &
  - *type of population* under investigation.

REFERENCE VALUES

- Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the ***indigenous population*** where the laboratory works.

SPECIFICITY

- The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Cortisol	100 %
Prednisolone	46.2 %
11-Deoxycortisol	4 %
Cortisone	3.69 %
Prednisone	3.10 %
11αOH Progesterone	1 %
Progesterone	< 0.1 %
Aldosterone	< 0.1 %
Pregnenolone	< 0.1 %

Endocrine CONNECTIONS

S Tan *et al.* | 12:4 | e230004

RESEARCH

Expanding the use of salivary cortisol as a non-invasive outpatient test in the dynamic evaluation of suspected adrenal insufficiency

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AI

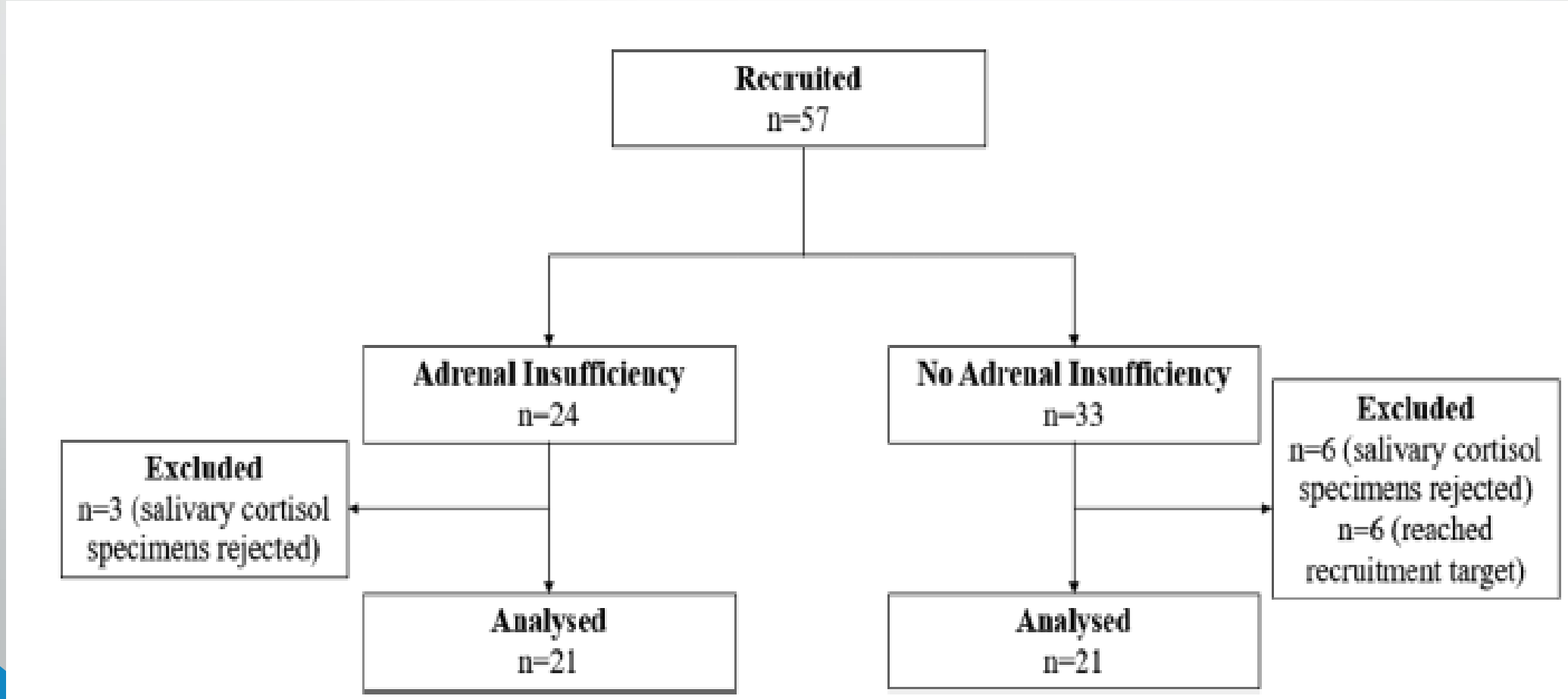
- The first-line diagnostic test, the adrenocorticotrophic hormone (ACTH) stimulation test, measures serum total cortisol.
- However , this is affected in states of altered albumin or cortisol-binding globulin levels, limiting reliability.
- Salivary cortisol reflects free bioactive cortisol levels and is a promising alternative.
- However, few studies are available, and heterogenous methodologies limit applicability.

AI

- Salivary cortisol is an alternative method to measure free cortisol.

- It has been shown that the concentrations of free cortisol in the saliva are at an equilibrium with free cortisol concentrations in the serum, and this relationship is independent of the rate of saliva production (14)

We recruited subjects from the endocrine testing clinic at the Department of Endocrinology at our tertiary centre (August 2020 to January 2022) who were planned for AST for the evaluation of suspected AI.



ACTH stimulation test

- An intravenous cannula was inserted followed by ***the simultaneous*** collection of baseline plasma and saliva samples (0-min sample).

- About 250 µg of ACTH (Synacthen®, Novartis) was then injected intravenously, followed by ***the simultaneous*** collection of serum and salivary cortisol samples at 30 and 60 min.

Results:

- The study recruited 21 (50%) participants with AI and 21 without AI.
- There were no significant differences in baseline characteristics, blood pressure, or sodium levels between groups.
- Following synacthen stimulation, serum and salivary cortisol levels showed good correlation at all timepoints ($R_2 = 0.74$, $P < 0.001$) , at peak levels ($R_2 = 0.72$, $P < 0.001$) , and at 60 min ($R_2 = 0.72$, $P < 0.001$).
- A salivary cortisol cut-off of 16.0 nmol/L had a sensitivity of **90.5%** and a specificity of **76.2%** for the diagnosis of AI.

Conclusion:

- This study demonstrates a good correlation between serum and salivary cortisol levels during the 250 μg synacthen test.
- A peak salivary cortisol cut-off of 16.0 nmol/L can be used for the diagnosis of AI.
- It is a less invasive alternative to evaluate patients with suspected AI.
- Its potential utility in the diagnosis of AI in patients with altered cortisol-binding states should be further studied.

Salivary cortisol & Synacthen test

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Salivary cortisol & Synacthen test

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