Salivary vitamin D testing: promising science, elusive clinical utility

Saliva can detect vitamin D metabolites, but the method remains far from replacing blood tests. While LC-MS/MS-based studies demonstrate that 25-hydroxyvitamin D_3 [25(OH)D_3] is measurable in saliva at concentrations roughly **1,000-fold lower than serum**, correlation with blood levels varies dramatically — from near-zero to r = 0.91 — depending on assay technology, collection protocol, and population studied. No commercially available salivary vitamin D test exists as of 2026, no regulatory body has cleared one, and no clinical reference ranges have been established. The field sits at a crossroads: advancing biosensor technologies could eventually enable point-of-care salivary testing, but fundamental analytical challenges — ultra-low concentrations, flow-rate variability, and contamination from gingival crevicular fluid — remain unresolved.

Forty years of searching for vitamin D in spit

The scientific pursuit of salivary vitamin D began in the 1980s with conflicting results that foreshadowed decades of inconsistency. Trafford and Makin (1983) could not reliably detect 25(OH)D in saliva using HPLC, even with 100 mL samples, (VitaminDWiki) and estimated the theoretical concentration at roughly **8 pmol/L (~3.2 pg/mL)** based on the known protein-binding characteristics of 25(OH)D. (VitaminDWiki) They calculated this by analogy with cortisol's behavior: since 25(OH)D is approximately **99.97% protein-bound** in blood (85–88% to vitamin D binding protein, 10–15% to albumin), (PubMed Central) only a vanishingly small free fraction would be available for passive diffusion into saliva.

Fairney and Saphier (1987) reported somewhat higher concentrations (105–1,000 pg/mL) using a competitive protein-binding assay in 55 adults and found a modest correlation with serum ($r = 0.45$, $p < 0.001$). (VitaminDWiki +2) However, a critical and often overlooked finding from that study was that salivary 25(OH)D showed **no correlation with directly measured serum free 25(OH)D** (VitaminDWiki) (Cambridge Core) — a paradox if saliva were truly reflecting only the unbound fraction. (Cambridge Core)

The breakthrough came in 2008, when Higashi and colleagues applied LC-MS/MS with PTAD chemical derivatization to saliva for the first time. Working with just 1 mL of unstimulated saliva from 20 adults, they achieved a limit of quantitation of **2.0 pg/mL** (Springer) and measured salivary concentrations of **3–15 pg/mL** — roughly 1,000 times lower than serum and far below the values reported by earlier immunoassays. (ScienceDirect) Crucially, they found an r^2 of **0.830 ($r = 0.911$, $p < 0.01$)**, the strongest saliva-serum correlation reported to date. (ScienceDirect) (Springer) They also demonstrated that salivary 25(OH)D_3 rose following vitamin D_3 supplementation, confirming the biological plausibility of the measurement.

Correlation evidence is strikingly method-dependent

The strength of the saliva-serum relationship depends almost entirely on how vitamin D is measured. Studies using LC-MS/MS consistently report stronger correlations than those using ELISA or competitive binding assays, which likely suffer from cross-reactivity with other lipophilic compounds in the salivary matrix.

Study	Year	n	Method	Correlation	Significance
Fairney & Saphier	1987	55	Competitive binding	$r = 0.45$	$p < 0.001$
Higashi et al.	2008	20	LC-MS/MS	$r = 0.91$	$p < 0.01$
Clarke et al. (unadjusted)	2019	6	LC-MS/MS	$r = 0.57$	Not significant
Clarke et al. (3-day avg, flow-adjusted)	2019	6	LC-MS/MS	$r \geqslant 0.88$	$p < 0.05$
Sari et al.	2021	56	ELISA	$\rho = 0.42$	Moderate
Squillacioti et al.	2025	62	ELISA	No correlation	Not significant

Clarke et al. (2019) used a CDC-certified LC-MS/MS assay and highlighted a critical operational finding: a single-day saliva sample correlated poorly with serum ($r = 0.57$, non-significant), but **averaging results across three consecutive days and adjusting for saliva flow rate** pushed the correlation to $r \geqslant 0.88$. ScienceDirect PubMed This multi-day averaging requirement substantially undermines practical utility. The most recent large study — Squillacioti et al. (2025) in *Biomolecules*, with 62 healthy adults — found **no correlation whatsoever** between ELISA-measured salivary 25(OH)D and serum levels measured by chemiluminescent immunoassay, while urinary 25(OH)D showed a moderate correlation ($r = 0.44$). MDPI A small 2025 pilot study in diabetic patients even reported a **negative** correlation ($R = -0.65$), MDPI suggesting disease states may further distort the relationship. News-Medical

The pattern is clear: **LC-MS/MS with careful collection protocols yields promising correlations, while immunoassays produce unreliable results in the salivary matrix.** ELISA-reported salivary values (100–1,000 pg/mL) exceed LC-MS/MS values (3–15 pg/mL) by one to two orders of magnitude, almost certainly reflecting antibody cross-reactivity rather than true 25(OH)D.

What saliva actually contains — and why it's complicated

The primary vitamin D metabolite detectable in saliva is **25(OH)D_3**, the same form used clinically to assess vitamin D status. 25(OH)D_2 is theoretically detectable but has not been separately quantified in salivary studies due to even lower concentrations. **1,25-dihydroxyvitamin D** [1,25(OH)$_2$D] has been measured in saliva (Sari et al., 2021), though its correlation with serum 25(OH)D was weak ($\rho = 0.34$). PubMed Central +3 Despite having a higher free fraction in blood (~0.4% vs. ~0.03% for 25(OH)D), the extremely low total circulating concentration of 1,25(OH)$_2$D makes salivary measurement impractical.

The mechanism by which vitamin D enters saliva mirrors that of other steroid hormones: the **free (unbound) fraction passively diffuses** through the lipophilic membranes of salivary gland acinar cells from blood into the ductal lumen. nih +2 This is well-established for cortisol, testosterone, and estradiol, where salivary levels reliably reflect circulating free hormone concentrations. PubMed Central +3 However, vitamin D presents three complications that these other steroids do not:

- **Vitamin D binding protein (DBP) is actively secreted by mucous salivary glands** into saliva, PubMed Central meaning salivary vitamin D is not purely "free" but exists in a mixture of bound and unbound forms. Clarke et al. (2019) found that normalizing for salivary DBP helped reconcile differences between collection methods. ScienceDirect PubMed
- **Gingival crevicular fluid (GCF)**, a serum exudate leaking from the gum-tooth margin, introduces protein-bound 25(OH)D into the oral cavity. nih Stimulating saliva by gum-chewing increases GCF contribution and falsely elevates measured levels ScienceDirect +2 (Higashi et al., 2013).
- **The 0.03% free fraction of 25(OH)D is far smaller** than cortisol's ~4% free fraction, Wiley Online Library PubMed Central creating concentrations at the very edge of analytical detectability.

These factors mean that **salivary vitamin D does not cleanly represent the "free" or "bioavailable" fraction** — an assumption central to its theoretical justification. The clinical significance of measuring free vitamin D itself remains debated; most studies in healthy populations find that free and total 25(OH)D correlate well, with free 25(OH)D offering added value primarily in conditions where DBP levels are altered PubMed Central (pregnancy, liver cirrhosis, nephrotic syndrome). MDPI

Biosensors offer a potential path to practical testing

The most exciting recent developments involve novel biosensor technologies that could eventually sidestep the need for laboratory-based LC-MS/MS:

- **Electrochemical aptasensor (Park et al., 2021)**: A sandwich-type sensor using MoS_2/ErGO-modified electrodes achieved a limit of detection of **0.02 ng/mL in buffer and 0.6 ng/mL in real saliva**, with selectivity for 25(OH)D_3 via aptamer recognition rather than antibodies. ScienceDirect
- **Dual vitamin C/D bioelectronic chip (Sempionatto, Wang et al., 2021)**: Developed at UC San Diego in collaboration with DSM Nutritional Products, this compact chip detects both vitamins simultaneously Phys.org in **10 µL of saliva within 25 minutes**. PubMed +2 It successfully tracked temporal vitamin D profiles following 2,000 IU supplementation ResearchGate and remained stable for 30 days at 4°C.
- **Metal oxide transistor microarray (Sharma et al., 2024)**: An In_2O_3/ZnO heterojunction thin-film transistor achieved detection of vitamin D_3 at **100 pM–120 nM in saliva within 60 seconds**, with a limit of detection of approximately 7 pM. PubMed Central +2
- **MXene-based sensor (Ali et al., 2025)**: Antibody-functionalized $Ti_3C_2T_x$ MXene nanosheets achieved a limit of detection of **1 pg/mL**, approaching the sensitivity needed for salivary 25(OH)D_3 measurement. Nature

These technologies are all at the laboratory proof-of-concept stage. None have undergone clinical validation against serum 25(OH)D in meaningful sample sizes, and none are commercially available. The gap between demonstrating analytical sensitivity and proving clinical diagnostic accuracy remains wide.

No commercial salivary test exists, and practical barriers are significant

Despite the research activity, **no FDA-cleared, CE-marked, or commercially available salivary vitamin D test exists as of 2026.** Companies like ZRT Laboratory offer combined panels using saliva for steroid hormones alongside dried blood spots for vitamin D Testkitsusa — the vitamin D component explicitly requires blood. Walk-In Lab ZRT Laboratory All current home vitamin D tests (Everlywell, LetsGetChecked, Thorne, and others) use finger-prick blood samples.

The practical barriers to salivary vitamin D testing are substantial. The requirement for **LC-MS/MS with chemical derivatization** to achieve reliable results eliminates any cost or accessibility advantage over blood testing. Clarke et al.'s finding that three-day averaging and flow-rate correction are needed for acceptable correlation ScienceDirect adds logistical complexity that undermines the convenience argument. PubMed Food intake introduces confounding — Fairney and Saphier showed that a vitamin D-rich meal produced a marked salivary rise 5–8 hours later. VitaminDWiki Cambridge Core Sample processing requires dual centrifugation steps (before and after freezing) to remove mucin. nih ScienceDirect And the

lack of established reference ranges means that even an accurate measurement cannot yet be clinically interpreted. MDPI

For population-level screening, these limitations are compounded by the fact that the **USPSTF (2021) does not recommend universal vitamin D screening** even with validated blood tests, finding insufficient evidence to assess the balance of benefits and harms. US Preventive Services Tas... Dried blood spots — minimally invasive, self-collectable, stable at room temperature, commercially available, and correlating with serum ScienceDirect at r ≈ 0.90 — represent a far more mature and validated alternative for any scenario where venipuncture is impractical. Nature VitaminDWiki

Conclusion

Salivary vitamin D measurement occupies an unusual scientific space: the underlying biology is sound, and sensitive enough analytical tools exist, yet consistent clinical utility has not materialized across four decades of research. The strongest evidence comes from LC-MS/MS studies showing correlations of r = 0.88–0.91 with serum 25(OH)D, ScienceDirect but these relied on **small samples (n = 6–20), multi-day collection, and flow-rate adjustment** — conditions that negate the practical advantages of saliva. ELISA-based studies, which would be more practical, produce unreliable results in the salivary matrix.

Three developments could change this picture. First, emerging biosensor platforms — particularly the UC San Diego dual-analyte chip and metal oxide transistor arrays — could deliver the sensitivity of LC-MS/MS in a point-of-care format. Second, large-scale validation studies using these newer platforms could establish whether salivary vitamin D genuinely tracks clinical vitamin D status across diverse populations. Third, if the clinical significance of free or bioavailable vitamin D becomes clearer, saliva's potential reflection of this fraction could gain unique diagnostic value.

Until these developments mature, **dried blood spots remain the most practical minimally invasive alternative to venipuncture** for vitamin D assessment. Salivary testing should be viewed as an active research frontier rather than a clinical tool — one where the analytical chemistry has advanced faster than the clinical validation needed to make it useful.