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DIAGNOSTIC ACCURACY OF SALIVARY MICROBIOLOGICAL TESTS FOR PERIODONTITIS AND CARIES: A SYSTEMATIC REVIEW

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Abstract

Saliva is an attractive diagnostic medium for oral diseases because it is non-invasive, inexpensive, and easy to collect at scale. This systematic review evaluated the diagnostic accuracy of salivary microbiological tests for periodontitis and dental caries. We searched MEDLINE (PubMed), Scopus, and Web of Science from inception to 26 October 2025 for original studies that measured salivary microorganisms against accepted clinical reference standards for periodontitis or caries. Twelve original studies met inclusion criteria (periodontitis, n=6, caries, n=6). For periodontitis, multiplex qPCR panels and 16S-based models showed good to excellent discrimination: area under the receiver operating characteristic curve (AUC) commonly ≥0.90 for multifeature indices, with sensitivity,specificity typically >80% in internal validation. Single-species cut-offs performed inconsistently. For caries, very high salivary Streptococcus mutans by culture predicted longitudinal caries progression in preschoolers, and several 16S, qPCR models discriminated severe early childhood caries with moderate-to-good accuracy. Study quality was limited by spectrum bias, small samples, and scarce external validation, pre-analytic variation and batch effects were under-reported. Overall, salivary microbiological testing shows promising accuracy for screening and risk stratification of periodontitis and early childhood caries, but broader, multicenter validation and standardized workflows are required before routine clinical adoption.

Keywords: Saliva, Salivary Diagnostics, Oral Microbiome, Periodontitis, Dental Caries, qPCR, 16S rRNA Sequencing, Diagnostic Accuracy, ROC, Screening.

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INTRODUCTION

Saliva contains microbial DNA shed from multiple oral niches and is easy to obtain repeatedly, positioning it as a candidate medium for point-of-care oral diagnostics. Contemporary guidance underscores both the potential and the current limits: as of October 2023, there are no FDA-approved salivary tests for evaluating risk of periodontal disease or dental caries, chiefly due to concerns about marker specificity, pre-analytic variability, and clinical validation requirements [1]. Reviews of salivary diagnostics outline how proteins, nucleic acids, and metabolites, including microbial components, can reflect disease biology while also being susceptible to collection and processing variability [2,3].

A central question for microbiology-based testing is whether saliva sufficiently captures subgingival dysbiosis responsible for periodontitis. Work at population and patient levels indicates that salivary microbiota composition shifts with overall oral health status, and that models can distinguish gradients of dental, periodontal pathology from health [4,5]. Multiplex biomarker approaches, combining microbial and host analytes, can outperform single markers for classifying periodontitis under the 2017 classification, yet still require larger validation cohorts [6]. Direct evidence that saliva reflects subgingival change continues to accumulate, including longitudinal and treatment-response designs where salivary profiles tracked subgingival shifts across health, gingivitis, and staged periodontitis [7].

For dental caries, early childhood caries (ECC) is a priority because of its burden and rapid progression. Studies show salivary microbiome alterations and metabolomic signatures in SECC, supporting diagnostic modeling, at the same time, conventional culture-based risk markers, very high salivary Streptococcus mutans (S. mutans) have shown strong longitudinal predictive utility in preschoolers [8–10]. Updated caries risk assessment reviews encourage integrating objective indicators (microbiological and others) with clinical predictors to improve calibration and practicality [11].

Given rapid methodologic advances and the persisting translational gap, we systematically synthesized the diagnostic accuracy of salivary microbiological tests for periodontitis and caries. We highlight analytic strategies, performance metrics, and sources of bias to inform research and implementation.

METHODS

Protocol and reporting. We followed PRISMA guidance for diagnostic test accuracy syntheses. The question was framed as: in patients undergoing evaluation for periodontitis or dental caries (participants), do salivary microbiological tests (index tests) accurately detect disease status or risk (target condition) compared with clinical reference standards (reference tests).

Eligibility criteria. Inclusion: original human studies (cross-sectional, case-control, cohort, or interventional with baseline classification) that (1) measured microorganisms in saliva or mouth-rinse (culture, species-specific qPCR, or microbiome sequencing with taxon-based features), and (2) reported diagnostic performance (AUC, sensitivity, specificity,

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odds ratios with cut-offs) against accepted clinical reference standards for periodontitis (probing depths, clinical attachment radiographic bone loss per contemporaneous criteria) or caries (DMFT, ICDAS, or clinical diagnosis and progression). Exclusion: non-human studies, non-salivary matrices, purely host biomarkers, editorials and reviews, studies without any performance metric against a clinical standard.

We searched MEDLINE (PubMed), Scopus, and Web of Science from inception to 26 October 2025 using terms for saliva, salivary, microbiome, microbiota, Streptococcus mutans, periodontal pathogens, qPCR,16S, periodontitis, caries, and accuracy metrics (ROC, AUC, sensitivity, specificity). Reference lists of included papers and relevant reviews were screened. No language restrictions were applied at search, English-language full texts were required for data extraction.

Study selection and data extraction. Two reviewers (you and collaborator) screened titles, abstracts and assessed full texts for eligibility. Data extracted included population, sampling protocol, index test (culture, qPCR targets, or taxonomic features), reference standard, modeling approach, and accuracy metrics (AUC, sensitivity, specificity, predictive values, or adjusted odds ratios with cut-offs).

Risk of bias and applicability. We used QUADAS-2 tailored for diagnostic accuracy of microbiological assays (domains: patient selection, index test, reference standard, and flow,timing). For models lacking explicit thresholds (multifeature machine-learning classifiers), we appraised overfitting risk (internal vs. external validation) and batch,pre-analytic handling as additional concerns.

Synthesis. We summarized periodontitis and caries separately. Given heterogeneity in designs, populations, analytes, and modeling, meta-analysis (pooled AUC or sensitivity, specificity) was not attempted. Instead, we narratively synthesized accuracy ranges and methodological features, emphasizing multifeature vs. single-species performance and validation status.

RESULTS

Study selection and overview. Twelve original studies were included: periodontitis (n=6) and caries (n=6). Periodontitis studies comprised qPCR panels from mouth-rinse, whole saliva and 16S rRNA gene sequencing with ASV-level modeling, caries studies spanned culture-based S. mutans counts, qPCR panels, and 16S-based classifiers. Below, we outline key design features and summarize accuracy.

Periodontitis salivary microbiological accuracy

Panels and indices via qPCR. In a 170-participant study, Kim et al. quantified nine periopathogens by multiplex qPCR from mouth-rinse and proposed a Periodontal Pathogen Index (PPI). Optimized ROC analysis distinguished healthy controls from chronic periodontitis with AUC 0.91 (95% CI 0.87–0.96), distribution across PPI categories tracked clinical severity and attachment loss, supporting use for screening and monitoring [12]. Salminen et al. evaluated four pathogens (Porphyromonas gingivalis,

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Tannerella forsythia, Prevotella intermedia, and Aggregatibacter actinomycetemcomitans) by qPCR in 462 dentate adults. High salivary concentrations of P. gingivalis and T. forsythia were independently associated with moderate–severe periodontitis and with deeper pocket counts and bone loss, combining organisms improved discrimination relative to single targets [13].

16S, ASV-based machine-learning classifiers. A 2024 multi-batch 16S study (n=124) built models discriminating periodontal health vs periodontitis with excellent performance before and after batch-effect removal. Using only 16 ASVs, the best model achieved AUC 0.944, sensitivity 90.7%, specificity 87.2%, after batch correction, AUC 0.935 with higher specificity (91.5%). Notably, robust performance could be retained with as few as 4–20 ASVs exceeding AUC > 0.90, highlighting feasibility for targeted assay development [14]. Ji et al. sequenced V3–V4 regions and then validated nine species via qPCR, showing salivary P. gingivalis, T. forsythia, and Filifactor alocis correlated with full-mouth probing depth sum and were “moderately accurate” at distinguishing staged severity (health, gingivitis, moderate, severe) on ROC analysis [16]. Jung et al. paired subgingival and saliva sampling across health, gingivitis, and staged periodontitis (with post-therapy follow-up) and demonstrated that salivary microbiota mirrored subgingival changes across severity and after non-surgical periodontal therapy, saliva reflected treatment-responsive dysbiosis [7]. These studies support non-invasive surveillance of periodontal status by salivary taxa signatures, provided technical confounders (batch effects) are addressed.

Total burden or “subgingival-specific” signatures in saliva. Studies also assessed whether saliva captures subgingival pathogenic load. Kageyama et al. identified 12 subgingival plaque–specific OTUs and showed their summed relative abundance in saliva strongly correlated with percent of sites with pockets ≥4 mm (r=0.78) and decreased after therapy in parallel with subgingival shifts, supporting clinical utility for whole-mouth status monitoring [11].

Multifeature indices (whether qPCR panels or compact ASV sets) consistently outperformed single-species thresholds. AUCs around or above 0.90 were reported for optimized multifeature models [12,14], single marker performance varied (A. actinomycetemcomitans inconsistent across populations) [13]. Where assessed, internal validation existed (cross-validation, test sets), but external validation across centers was limited, one study explicitly quantified and corrected batch effects, showing small ASV panels can still retain high AUC after batch correction [14].

Caries salivary microbiological accuracy

Culture-based high-risk marker. In a 5-year retrospective cohort of 200 preschoolers, very high salivary S. mutans (“too numerous to count”) at baseline conferred 6-fold higher odds of caries increment despite preventive care, indicating strong risk stratification potential from a simple culture test [17].

qPCR,16S-based models for SECC. A Sci Rep study proposed a compact two-species qPCR model (S. mutans + Prevotella pallens) trained on 16S guided feature selection to

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flag severe ECC, showing how sequencing inform practical panels [18]. In Brazilian children, Colombo et al. used qPCR for selected taxa and found stepwise increases in bacterial burdens with caries severity, supporting threshold-based classification [19]. A multicenter 16S study reported that geographic variation did not materially degrade the predictive ability of salivary microbiome features for ECC diagnosis, highlighting portability of taxa-based models when bioinformatics is standardized [20]. Han et al. in Shenzhen showed salivary microbiome differences between ECC and caries-free children and built machine-learning classifiers with good accuracy, again supporting diagnostic potential [9]. Lin et al. combined microbiome features with simple clinical variables to screen for high caries activity, models reached AUC 0.842 in validation, suggesting microbiome-augmented screening value [21].

The most robust longitudinal evidence still comes from high S. mutans counts predicting future caries [17]. Sequencing-guided models are promising for ECC detection, sometimes achieving (good) AUCs, and remain attractive for non-invasive community screening. As with periodontitis, combining organisms (or organisms + simple clinical variables) generally outperforms single-species cut-offs. External validation and harmonized pre-analytics are limited. Several studies were cross-sectional, limiting conclusions about true prospective predictive value.

Spectrum bias (case–control designs with clear extremes), small samples, and unreported blinding to reference standards were common. Many 16S-based studies lacked external validation, only one explicitly modeled batch effects and showed their impact on feature selection and accuracy [14]. Pre-analytic variables (stimulated vs unstimulated saliva, timing, mouth-rinse vs whole saliva) and DNA extraction kits were often under-specified, potentially affecting generalizability. Reference standards for periodontitis were appropriate but varied (thresholds for probing depth, attachment loss), complicating cross-study comparisons.

DISCUSSION

This review shows that salivary microbiological assays—especially multifeature approaches—can achieve clinically attractive accuracy for classifying periodontal status and identifying ECC. For periodontitis, two complementary lines of evidence emerge. First, qPCR panels quantifying classic periopathogens (red, orange complexes) can reach AUCs 0.9 under optimized cut-offs [12] and track clinical severity [13]. Second, 16S, ASV-based machine-learning models trained on dozens to hundreds of taxa repeatedly distinguish health from disease, with compact feature sets retaining high AUC even after batch correction [14]. Saliva also mirrors subgingival dysbiosis gradients and treatment responses, supporting its use as a whole-mouth surveillance matrix [7,11]. These findings are consistent with systematic comparisons of saliva and subgingival plaque reporting that, while communities differ, disease-linked taxa shed into saliva in proportion to periodontal burden [5–7].

For caries, the strongest prospective signal remains very high salivary S. mutans culture counts predicting caries increment in preschoolers, an inexpensive, operationally simple

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test [17]. Sequencing-guided qPCR panels (S. mutans with anaerobic partners such as Prevotella pallens) and broader microbiome classifiers offer additional accuracy and scalability, including across geographic cohorts when pipelines are harmonized [18,20]. Multi-omic approaches (microbiome + metabolome) further suggest biological plausibility and potential incremental value [10], while risk-assessment frameworks increasingly encourage integrating objective markers with clinical predictors to improve calibration and actionability [11].

Translational constraints remain. Regulatory summaries emphasize that, as of October 2023, no salivary tests are FDA-approved for caries or periodontal risk, reflecting unresolved issues of analytical validity, clinical validity across diverse settings, and clinical utility (i.e., improved outcomes vs standard care) [1]. Our synthesis also highlights batch effects in 16S studies, modest sample sizes, and limited external validation—factors that can inflate accuracy estimates or undermine portability [6,14]. Standardizing pre-analytics (collection timing, stimulated vs unstimulated, mouth-rinse vs whole saliva), DNA extraction, and bioinformatics, along with reporting calibration and decision-curve analyses, would facilitate implementation. Finally, multifeature microbial panels should be benchmarked against (and integrated with) simple clinical predictors to demonstrate meaningful net benefit in screening pathways [6,11].

Implications. For periodontitis, compact ASV-informed qPCR panels targeting taxa such as P. gingivalis, T. forsythia, F. alocis, and F. nucleatum appear ready for multicenter validation as adjunctive screening tools. For ECC, S. mutans culture or qPCR remains a high-yield starting point, with microbiome-augmented models offering additive value for early detection in preschool populations.

CONCLUSION

Salivary microbiological testing demonstrates promising diagnostic accuracy for both periodontitis and dental caries, particularly when multiple taxa are combined into indices or machine-learning models. Mouth-rinse, whole-saliva assays can track disease severity and treatment response and, for ECC, identify children at high risk. However, current evidence is constrained by variable pre-analytics, small samples, and limited external validation, no assay is yet FDA-approved for these indications. Priorities include multicenter studies with standardized workflows, explicit batch-effect control, and head-to-head comparisons against pragmatic clinical predictors to establish clinical utility and inform guideline adoption [1,6,11,14,17,21].

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