Diagnostic Utility of Immunofluorescence in Oral Lesions: a Systematic Review

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ABSTRACT

Objectives: This systematic review aims to evaluate the diagnostic utility of direct and indirect immunofluorescence of oral lesions in comparison with conventional diagnostic aids.
Material and Methods: The diagnostic utility of immunofluorescence in various oral lesions was evaluated. Relevant data from 37 studies, including study characteristics, patient population, test details, and outcomes, were systematically extracted. The search was performed analysing studies across multiple electronic databases including MEDLINE (PubMed), Embase, Scopus and Google Scholar, published from January 15, 2024 until May 15, 2024. Risk of bias was assessed using a modified QUADAS-2 tool.
Results: Most studies demonstrated a low risk of bias in most domains, indicating overall methodological rigor. Comparative analysis showed that direct immunofluorescence (DIF) consistently outperformed indirect immunofluorescence. DIF exhibited high sensitivity and specificity for pemphigus vulgaris (87.8% and 100%), mucous membrane pemphigoid (92% and 98%), and desquamative gingivitis oral ulcers overlapping with oral lichen planus (OLP) (81% and 98.9%). For OLP, DIF showed moderate sensitivity (64.3%) and high specificity (88%).
Conclusions: This review highlights the superior diagnostic utility of direct immunofluorescence over indirect immunofluorescence in evaluating oral lesions. Direct immunofluorescence's higher performance makes it the preferred technique for conditions requiring direct visualization of tissue-bound immune deposits. The combined use of direct immunofluorescence and indirect immunofluorescence can enhance the evaluation and management of various oral pathologies.

Keywords: direct immunofluorescence; immunofluorescence techniques; indirect immunofluorescence; oral neoplasms; systematic review.

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INTRODUCTION

Oral lesions encompass a wide range of pathological conditions, including benign, premalignant, and malignant entities. Accurate diagnosis of these lesions is crucial for appropriate management and optimal patient outcomes [1]. Conventional diagnostic modalities, such as clinical examination and histopathological analysis, remain the mainstay in the evaluation of oral lesions [2]. However, these techniques can sometimes be limited in their ability to provide a definitive diagnosis, particularly in cases where the clinical presentation is atypical or the histopathological features are ambiguous.

In recent years, the application of advanced diagnostic techniques, such as immunofluorescence (IF), has gained increasing attention in the field of oral pathology. IF is a sensitive and specific laboratory method that utilizes fluorescently labelled antibodies to detect the presence and distribution of specific antigens within tissue samples [3]. This technique has the potential to provide additional information that can complement the findings from clinical and histopathological examinations, leading to more accurate and timely diagnosis of various oral lesions.

The diagnostic utility of IF has been explored in the evaluation of numerous oral pathologies, including autoimmune disorders, infectious diseases, and neoplastic conditions [4]. By identifying the expression patterns of specific biomarkers or molecular targets, IF can aid in the differentiation of similar-appearing lesions, the assessment of disease progression, and the evaluation of treatment responses [5].

This systematic review aimed to comprehensively evaluate the current evidence on the diagnostic utility of immunofluorescence in the evaluation of oral lesions. By synthesizing the available literature, the review aims to provide a critical appraisal of the performance characteristics, clinical applications, and limitations of this diagnostic modality in the management of various oral pathologies. The findings of this systematic review would contribute to the understanding of the role of immunofluorescence in the enhanced diagnosis and management of oral lesions, ultimately leading to improved patient care and outcomes.

MATERIAL AND METHODS

Protocol and registration

Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement criteria were followed for framing this systematic review [6]. The review adhered to the recent PRISMA guidelines for systematic reviews to ensure transparency and completeness in reporting.

Focus question

The review question for this systematic review on the diagnostic utility of IF in oral lesions was framed in the Primary Immune Regulatory Disorders (PIRD) format (Table 1). In PIRD format, the review question could be stated as: "What is the diagnostic utility of IF (I) compared to conventional diagnostic methods (R) in the evaluation of patients with various types of oral lesions (P), in terms of diagnostic accuracy, sensitivity, specificity, and clinical utility (D)?"

Information sources

To conduct a comprehensive systematic review on the diagnostic utility of IF in oral lesions, a thorough and well-structured search strategy was employed. The search was performed analysing studies across multiple electronic databases, including MEDLINE (PubMed), Embase, Scopus and Google Scholar, published from January 15, 2024 until May 15, 2024, to ensure a broad coverage of the relevant literature.

Search

The search strategy utilized a combination of controlled vocabulary (e.g., MeSH terms in MEDLINE) and free-text keywords related to the key components of the review question: oral lesions,

Table 1. Primary Immune Regulatory Disorders (PIRD) framework

Component	Description
Population (P)	Patients with various types of oral lesions, including but not limited to premalignant, malignant and inflammatory/autoimmune conditions
Index test (I)	Immunofluorescence diagnostic testing
Reference test (R)	Conventional diagnostic methods such as clinical examination and histopathological analysis
Diagnosis/prognosis (D)	Diagnostic accuracy, sensitivity, specificity and clinical utility of immunofluorescence in the evaluation of oral lesions

immunofluorescence, and diagnostic performance. Relevant terms such as "oral cavity," "mouth diseases," "immunofluorescence," "diagnostic techniques," "sensitivity and specificity," "accuracy", "prognosis" and "treatment outcome" were used, along with appropriate Boolean operators (e.g., AND, OR) to optimize the search (Table 2).

The search was limited to studies published in English, without any restrictions on the publication date. This decision was based on the anticipated rapid advancements in the field of IF and the need to capture the most recent evidence. Additionally, the search was restricted to studies involving human participants to ensure the clinical relevance of the included evidence.

To ensure the comprehensiveness of the search, the reference lists of the included studies and relevant review articles were manually screened to identify any additional eligible studies that might have been missed in the initial database searches.

Selection of studies

The selection of articles for inclusion in the systematic review was conducted in a stepwise manner. First, two independent reviewers (S.A. and B.R.) screened the titles and abstracts of all retrieved records to identify potentially relevant studies. Second, the full-text articles of the selected studies were reviewed to determine their eligibility based on the predefined inclusion and exclusion criteria. Title and abstract screenings were performed using an online screening tool Rayyan® (Qatar Computing Research Institute; HBKU, Doha, Qatar [www.rayyan.ai]).

Cohen's kappa coefficient (κ) values for 10% of the publications were calculated to assess the inter-rater reliability of the reviewers.

Types of publication

This systematic review covered human studies that were published in the English language.

Types of studies

The review included all diagnostic accuracy studies, from inception until July 2024 which reported the diagnostic utility of IF compared to conventional diagnostic methods in the evaluation of patients with various types of oral lesions.

Type of population

Patients with various types of oral lesions (premalignant, malignant, and inflammatory/autoimmune conditions).

Inclusion and exclusion criteria for the selection

Inclusion criteria

- Studies that evaluated the diagnostic utility of IF in the evaluation of oral lesions.
- Studies that compared the performance of IF to conventional diagnostic methods (e.g., clinical examination, histopathological analysis).
- Studies that reported at least one of the following outcome measures: diagnostic accuracy, sensitivity, specificity, or clinical utility of IF.

Table 2. Search and screening

Concept	Keywords and search terms
First concept (P): oral lesions	"Oral Lesions"[Mesh] OR "Mouth Diseases"[Mesh] OR "Oral Pathology"[TW] OR "Oral Epithelial Dysplasia"[TW] OR "Oral Lichen Planus"[TW]
Second concept (I): immunofluorescence	"Immunofluorescence"[Mesh] OR "Direct Immunofluorescence"[TW] OR "Fluorescent Antibody Technique"[TW]
Third concept (R): conventional diagnostic methods	"Biopsy"[Mesh] OR "Histopathology"[TW] OR "Cytology"[TW] OR "Clinical Examination"[TW]
Fourth concept (D): diagnostic accuracy, sensitivity, specificity, clinical utility	"Diagnostic Accuracy"[TW] OR "Sensitivity and Specificity"[TW] OR "Clinical Utility"[TW]
Final search combination	("Oral Lesions"[Mesh] OR "Mouth Diseases"[Mesh] OR "Oral Pathology"[TW] OR "Oral Epithelial Dysplasia"[TW] OR "Oral Lichen Planus"[TW]) AND ("Immunofluorescence"[Mesh] OR "Direct Immunofluorescence"[TW] OR "Fluorescent Antibody Technique"[TW]) AND ("Biopsy"[Mesh] OR "Histopathology"[TW] OR "Cytology"[TW] OR "Clinical Examination"[TW]) AND ("Diagnostic Accuracy"[TW] OR "Sensitivity and Specificity"[TW] OR "Clinical Utility"[TW])

Mesh = Medical Subjects Headings; TW = text word.

- Study designs including randomized controlled trials, cohort studies, case-control studies, and cross-sectional studies.

Exclusion criteria

- Case reports, case series, letters, editorials and review articles.
- Studies that did not focus on the diagnostic performance of IF in oral lesions.
- Studies with insufficient data or unclear methodology.
- Any discrepancies between the reviewers during the screening and selection process were resolved through discussion and, if necessary, the involvement of a third reviewer to reach a consensus.

Data extraction

Data extraction for this systematic review was carried out systematically to ensure the accuracy and completeness of the gathered information. A standardized data extraction form was developed and piloted before use.

Data items

- Study characteristics: authors, publication year, study design, and sample size.
- Patient (population): demographic details (age, gender), type of oral lesions (benign, premalignant, malignant, inflammatory/autoimmune), and diagnostic context.
- Index test details: type of IF (direct or indirect), specific antibodies used, antigen targets, and methodology (including tissue processing and antigen retrieval techniques).
- Reference test details: conventional diagnostic methods used for comparison, such as clinical examination and histopathological analysis.
- Outcome measures: diagnostic accuracy metrics (sensitivity, specificity, positive predictive value, negative predictive value), clinical utility (impact on diagnosis, treatment planning, and patient outcomes), and any reported adverse effects or limitations of IF.
- Key findings: summary of the main results, including any reported improvements in diagnostic accuracy or clinical decision-making.

Two independent reviewers performed data extraction, and discrepancies were resolved through discussion or consultation with a third reviewer. This approach ensured a high level of reliability and minimized the risk of bias in the data extraction process.

Risk of bias assessment

The risk of bias in the included studies was assessed using a modified version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [7]. This tool evaluates the risk of bias across four key domains: patient selection, index test, reference standard, and flow and timing. The assessment process involved:

1. Patient selection: evaluating whether the study population was representative of the general population with oral lesions, and whether selection criteria were clearly defined and appropriate.
2. Index test: assessing the potential for bias in the conduct and interpretation of the IF test, including blinding of the test results and consistency in the application of the test protocol.
3. Reference standard: examining the appropriateness and reliability of the reference standard (clinical examination and histopathological analysis) used for comparison, and whether the reference standard results were interpreted independently of the index test results.
4. Flow and timing: reviewing whether all patients received the same reference standard, the interval between the index test and reference standard, and the handling of any missing data or patient withdrawals.

Each domain was rated as having a low, high, or unclear risk of bias. Two reviewers independently assessed the risk of bias, with any disagreements resolved through discussion or consultation with a third reviewer. The results of the risk of bias assessment were summarized narratively and presented in a tabular format to provide a clear overview of the quality of the included studies.

Synthesis of the results

Data from the aforementioned studies were systematically collected and tabulated into the following fields: study characteristics, patient population, index test details, reference test details, outcome measures and key findings.

Statistical analysis

The level of agreement between the two raters in selecting abstracts and studies were measured using Cohen's kappa coefficient (κ). Quantitative synthesis of the results from the included studies was not possible due to high heterogeneity observed across the included studies.

RESULTS

Study selection

In the current systematic review, an extensive search across multiple databases identified a total of 654 records. Prior to screening, 234 duplicate records were removed, resulting in 420 unique records to be screened. Following the initial screening process, 200 records were excluded based on predefined criteria, leaving 220 reports for further assessment. All 220 reports were successfully retrieved and evaluated for eligibility. Out of these, 183 reports were excluded due to various reasons such as not meeting the inclusion criteria or insufficient data quality and unclear methodology. Ultimately, 37 studies were deemed eligible and included in the final review. This process follows the PRISMA 2020 guidelines, ensuring a rigorous and transparent methodology in the selection of studies for the review [7]. The level of agreement between two authors (S.A. and B.R.) in the selection of abstracts was measured at $\kappa = 0.88$. The detailed workflow for study selection is presented in Figure 1.

Exclusion of studies

One hundred and eighty three articles were not included in this review since they were not relevant to the review question of the current systematic review.

Study characteristics

The characteristics of included studies are presented in Tables 3A, 3B and 4A, 4B, 4C 4D. The included studies [8-44] consist of a mix of prospective and retrospective designs, addressing various aspects of oral mucosal diseases. Among them, fourteen studies were prospective [9,11,13,14,17,18,29-32,34,36,37,42] and twenty three were retrospective [8,10,12,15,16,19-28,33,35,38-41,43,44]. The studies collectively cover a wide range of conditions, with multiple studies focusing on oral lichen planus (OLP) [21,42-44]. Other conditions investigated include cicatricial pemphigoid [13], oral pemphigoid [17], and pemphigus vulgaris [41] (Table 3).

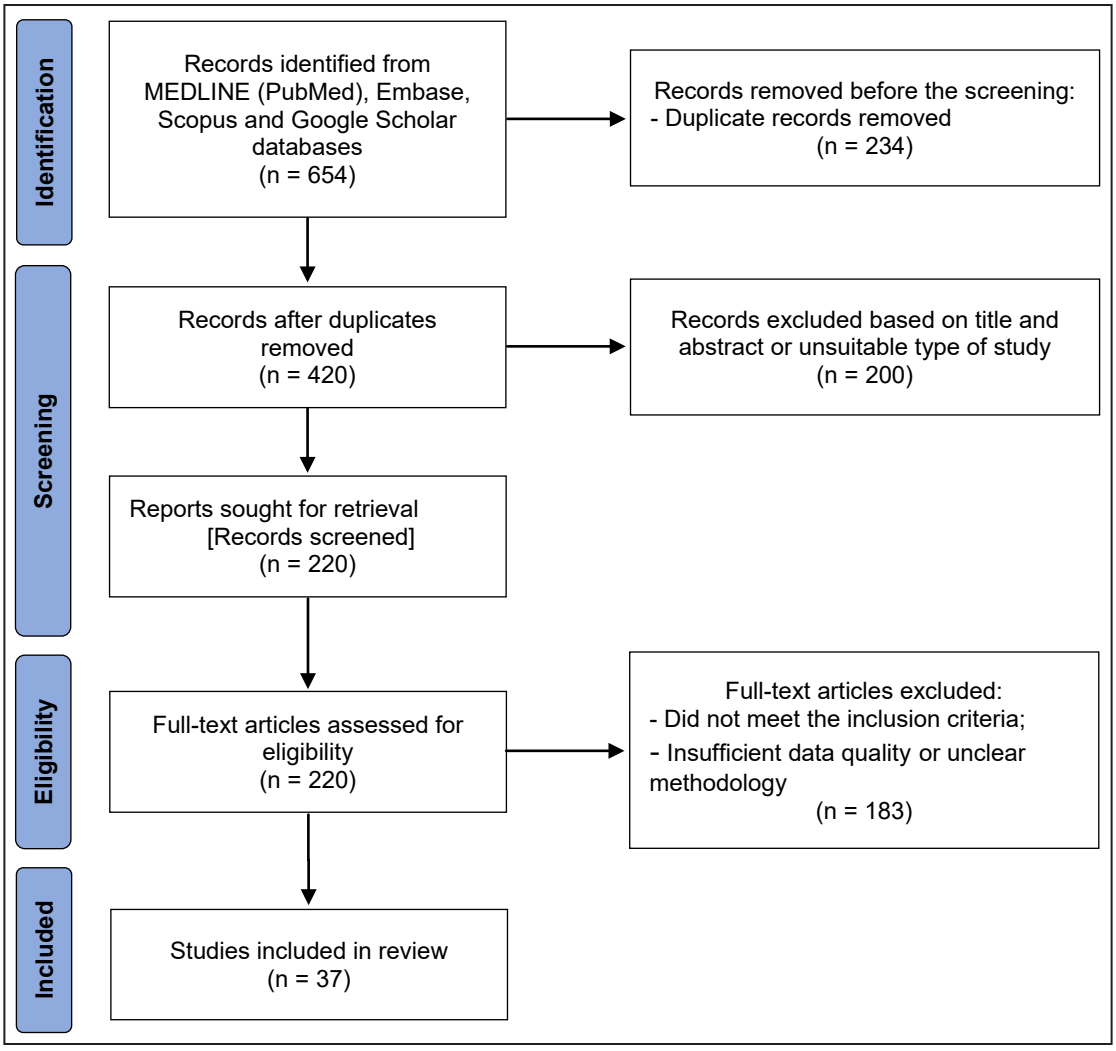

Figure 1. PRISMA flow diagram summarising the search strategy and study selection.

Table 3A. Characteristics of included studies

Study	Year of publication	Study design	Sample size	Gender distribution	Age (years)	Type of oral lesions	Diagnostic context	Type of IF	Specific antibodies used
Hasler et al. [8]	1972	Case series	4 patients	3 males, 1 female (case 1: one black woman; case 2 to 4: one Caucasian man in each case)	Case 1: 40; case 2: 59; case 3: 34; case 4: 65	DG, erosive and ulcerative lesions of the oral cavity	PV and bullous pemphigoid	IIF	Intercellular space antibodies in PV, antibodies against BMZ antigen in bullous pemphigoid
Donatsky et al. [9]	1974	IF study	24 patients with simple type of RAS, 24 controls without RAS	-	-	Unkeratinized buccal human oral mucosa from a RAS patient, unkeratinized buccal human oral mucosa from a control, adult human skin, adult human smooth muscle, adult guinea-pig oral mucosa	Humoral immunity to adult human oral mucosa in RAS of the simple type	Double-layer IF staining method	Humoral antibodies
Torabinejade et al. [10]	1979	Cross-sectional	25 dental periapical lesions	-	-	Dental periapical lesions	Patients undergoing periapical surgery	Anti-complement IF	Fluorescein-conjugated goat anti-guinea pig complement (C3 fraction)
Acosta et al. [11]	1981	Observational, comparative study	36 (10 active oral lesions, 3 in remission, 7 recurrent oral ulceration [aphthous ulcers], 2 MMP, 3 erosive lichen planus, 1 erythema multiforme, 10 clinically healthy oral mucosa)	PV: 7 males, 6 females	48 to 82	PV, aphthous ulcers, MMP, erosive lichen planus - erythema multiforme, clinically healthy oral mucosa	Diagnosis of PV	DIF	FITC-conjugated sheep anti-human IgG, IgA, IgM
Daniels et al. [12]	1981	Retrospective analysis	130 cases of chronic or recurrent ulcerative/erosive oral mucosal diseases	-	64.9	Chronic or recurrent ulcerative/erosive diseases of the oral mucosa	Diagnosis based on history, clinical features, histopathology, and clinical follow-up (DIF results not used to establish diagnosis)	DIF	Goat antisera against human IgG, IgA, IgM, C3 (complement), and fibrinogen
Fine et al. [13]	1984	Descriptive study	10 patients	6 males, 4 females	36 to 84	Cicatricial pemphigoid	Examination of IF and immunoelectron microscopy in diagnosing cicatricial pemphigoid	DIF	Specific antibodies against human IgG, IgA, and C3
Firth et al. [14]	1990	Observational study	165 biopsy specimens	-	-	OMLP	To assess the value of DIF microscopy in diagnosing OMLP	DIF microscopy	Anti-fibrinogen, anti-C3, anti-Clq, anti-IgA, anti-IgG, anti-IgM
Lodi et al. [15]	1997	Cross sectional study	67 patients	-	-	Non-erosive OLP	Diagnosis of OLP based on WHO criteria	IIF	Circulating antibodies against epithelial antigens
Yih et al. [16]	1998	Observational study	72 cases of DG	-	26 to 83	Erosive lichen planus or lichenoid mucositis, benign MMP, linear IgA disease, PV, bullous pemphigoid, paraneoplastic pemphigus (7 cases could not be defined)	Diagnosis of chronic DG using DIF in conjunction with histology	DIF	IgG, IgM, IgA, C3c, fibrinogen
Bhol et al. [17]	2001	Immunological study	20 patients	-	-	Oral pemphigoid	Sera from untreated patients with active pemphigoid disease, limited to the oral cavity	IIF assay	Monoclonal antibodies to human α6 integrin were used to detect the presence of autoantibodies in the sera of patients
Nakano et al. [18]	2002	IF study	2 ameloblastoma samples	-	-	Ameloblastoma	Distinguish ameloblastoma from other lesions, assess tumour progression	IIF	Rat monoclonal antibodies against collagen IV α1, α2, α3, α4, α5, α6 chains
Kolde et al. [19]	2003	Retrospective study	30 diagnostic biopsies from 27 patients	7 males, 20 females	Mean 54	Biopsies were taken from the buccal or gingival mucosa	Differentiating OLP from other immunologically mediated oral mucosal diseases like pemphigoid, PV, and LE	DIF testing	FITC-conjugated polyclonal rabbit antibodies against IgG, IgA, IgM, complement C3, and fibrinogen
Musa et al. [20]	2005	Retrospective study	392 consecutive cases	-	30 to 60	OLP	Clinical diagnosis by contributing clinicians, histologic examination by 3 oral pathologists, DIF diagnosis	DIF	Fibrin, immunoglobulins, and C3
Kulthanan et al. [21]	2007	Retrospective study	72 patients	36 males, 36 females	6 to 76	Lichen planus	Differentiation of LP from other conditions, especially LE, in cases with no specific clinical or histologic characteristics or ambiguous features	DIF	IgG, IgA, IgM, C3, fibrin
Suresh et al. [22]	2012	Retrospective analysis	239 consecutive cases of gingival biopsy with clinical diagnosis of DG	49 males, 190 females	16 to 95	OLP, pemphigoid	clinical associations of DG and utility of DIF for definitive diagnosis	DIF	Humoral antibodies
de Freitas Silva et al. [23]	2014	Experimental study	30 oral leukoplakia, 20 OSCC, 10 normal oral mucosa specimens	41 males, 19 females	-	Oral leukoplakia, OSCC	Evaluation of Twist and E-cadherin expression in the development and progression of OSCC	Double IF	Anti-Twist, anti-E-cadherin
Rameshkumar et al. [24]	2015	Retrospective and prospective analysis	Retrospective: 70 (20 OLP, 20 DLE, 20 PV; 10 MMP). Prospective: 12 (chronic/recurrent ulcerative, erosive or vesiculobullous oral lesions)	-	-	OLP, DLE, PV, MMP, chronic/recurrent ulcerative, erosive or vesiculobullous oral lesions	Clinical features, histopathology and DIF	DIF	Not specified
Hashimoto et al. [25]	2015	Retrospective study	12 cases (granular C3 deposition seen in 10 cases)	2 males and 8 females	52.4	Erythemas, reticular lace-like whitish coating, erosions and ulcers on oral mucosae and lips	Suspected autoimmune bullous diseases	DIF	IgG, IgA
Montague et al. [26]	2015	Retrospective study	164 cases of oral premalignant lesions and squamous cell carcinoma	59 males, 105 females	64.9	Premalignant lesions (verrucous hyperplasia, low-grade dysplasia, high-grade dysplasia) and squamous cell carcinoma	DIF testing to differentiate OLP from other immune-mediated diseases, oral dysplasia, and squamous cell carcinoma	DIF	Fibrinogen reactivity at the BMZ

ACIF = anti-complement immunofluorescence; ANA = antinuclear antibody; BMZ = basement membrane zone; C3 = complement component 3; DIF = direct immunofluorescence; DLE = discoid lupus erythematosus; DG = desquamative gingivitis; FITC = fluorescein isothiocyanate; H&E = hematoxylin and eosin; IIF = indirect immunofluorescence; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M; LE = lupus erythematosus; MIF = multiplexed immunofluorescence; MMO = maximum mouth opening; OLDR = oral lichenoid dysplasia; OLL = oral lichenoid lesions; OLP = oral lichen planus; OMLP = oral mucosal lichen planus; OSCC = oral squamous cell carcinoma; OSF = oral submucous fibrosis; PV = pemphigus vulgaris; RAS = recurrent aphthous stomatitis; T1 = tumour size classification (smallest); T4 = tumour size classification (largest); WHO = world health organization; IF = immunofluorescence.

Table 3B. Characteristics of included studies

Study	Year of publication	Study design	Sample size	Gender distribution	Age (years)	Type of oral lesions	Diagnostic context	Type of IF	Specific antibodies used
Lee [27]	2016	Retrospective study	52 patients	18 males, 34 females	11 to 80	OLP and OLL	Evaluate differences in final diagnosis of OLP and OLL based on different diagnostic criteria, and compare DIF findings between OLP and OLL	DIF	Fibrinogen
Zhou et al. [28]	2016	Single-center retrospective study	33 patients with pemphigus. Control: 61	11 males, 22 females. Control: 23 males 38 females	50 (SD 10.4). Control: 58.5 (SD 12.7)	Chronic erosions, blistering, exudation of oral mucosa	Differential diagnosis of pemphigus from pemphigoid, DLE, erosive lichen planus	IIF	IgG autoantibodies against keratinocyte cell surfaces
Masquijo-Bisio et al. [29]	2017	Observational study	85 cases	Male : female ratio: 4.25 : 1 reticular OLP, 1.72 : 1 plaque like OLP, 0.8 : 1 leukoplakia	19 to 83	Plaque-type OLP, homogenous leukoplakia	Differentiation between plaque-type OLP and homogenous leukoplakia	DIF	Anti-fibrinogen antibody
Yamanaka et al. [30]	2018	Prospective, cross-sectional study	110 patients	26 males, 84 females	19 to 87	Reticular lesions mainly on the buccal mucosa	Differentiation of OLP and OLL	DIF	Fibrinogen, IgM, IgA, IgG, C3
Abdalla et al. [31]	2017	Cross-sectional study	61 patients	-	-	Fibroepithelial polyp, low-grade dysplasia, high-grade dysplasia, T1 OSCC, T4 OSCC	Assessing expression of epithelial and pro-invasive markers in oral dysplasia and OSCC	Quantitative IF	Fully characterized antibodies for E-cadherin, EMP1, 5T4, N-cadherin
Kamaguchi et al. [32]	2018	Prospective study	7 cases	-	51 to 85	Gingival lesions in all 7 patients; buccal lesions in 1 of the 7 patients	Diagnosis of MMP	DIF using non-lesional buccal mucosa	IgG and C3
Bresler et al. [33]	2020	Retrospective study	148 cases	45 males, 103 females	Mean 56	DG, oral ulcers, overlap with OLP	Suspected oral autoimmune bullous disorders	DIF	Not specified
Reyes et al. [34]	2020	Observational study	Not detailed	-	-	Oral dysplasia	Diagnosis and progression of oral dysplasia to OSCC	Tissue IF analysis	Antibodies against β-catenin, GSK3β, Axin, APC, EEA1, Rab5
Tikkhanarak et al. [35]	2019	Retrospective study	53 patients" 17 OLP, 19 OLL, 17 OLDR	Male : female ratio: 14 : 3 OLP, 17 : 2 OLL, 15 : 2 OLDR	OLP: 41.9 (SD 14.4), OLL: 48.9 (SD 16.5), OLDR: 61.6 (SD 6.1)	Red and white lesions	Differentiating OLP, OLL, and OLDR	DIF	IgM, IgA, C3, IgG
Noormohammadpour et al. [36]	2020	Prospective	68 patients	30 males, 38 females	17 to 91	Erosive/ulcerated oral lesions	PV vs. other causes of erosive oral lesions	DIF	IgG/C3
Gupta et al. [37]	2021	Cross-sectional study	105 cases (45 OSF patients, 30 healthy controls with areca nut chewing habit, 30 healthy controls without areca nut chewing habit)	-	-	OSF	Detection of ANAs in OSF patients and healthy controls	IIF	ANAs
Hanna et al. [38]	2021	Retrospective study	58 patients	26 males, 32 females	27 to 79	Oral leukoplakia categorized into localized leukoplakia and proliferative leukoplakia.	Included various degrees of dysplasia: mild, moderate, or severe	MIF	nCounter® PanCancer IO 360™ Panel (NanoString Technologies, Inc., Seattle, WA, USA) for gene expression profiling. MIF was used to analyse immune cell populations, including CD8+ T cells and T regulatory (Treg) cells
Petruzzi et al. [39]	2022	Retrospective cohort study	22 patients with oral pemphigus, 64 controls	22 males, 64 females	Mean 59.5	Oral erosions characteristic of PV	Patients with clinical diagnosis of oral pemphigus referred to a tertiary care center	IIF	Anti-desmoglein 1 (Dsg1) and anti-desmoglein 3 (Dsg3)
Rujitharanawong et al. [40]	2022	Retrospective	147 patients	36 males, 111 females	49.9 (SD 14.5)	Not specified, but included oral and cutaneous lichen planus	Confirmed diagnosis of lichen planus based on clinical and histopathological criteria	DIF	IgM, IgG, fibrin, C3
He et al. [41]	2021	Diagnostic accuracy study	81 patients: 41 with PV and 40 with non-pemphigus vulgaris	16 males, 25 females	53.8 (SD 10.47)	PV	Evaluation of diagnostic efficacy of DIF analysis of oral Tzanck smears in clinical practice	DIF	IgG, C3, IgA, IgM
Mao et al. [42]	2022	Prospective study	42 patients with suspected OLP lesions. Control: 47	OLP: 16 males, 26 females. Control: 35 males, 12 females	OLP: 39.6 (SD 13.7), control: 48.1 (SD 12)	OLP	Evaluation of the diagnostic value of DIF and investigation of immune functions in OLP	DIF	It did not provide details on the specific antibodies used for the DIF analysis
Hansen et al. [43]	2023	Retrospective study	132 patients	36 males, 96 females	61.9 (SD 13.8)	OLP	Patients had a clinical or working diagnosis of OLP indicated by the submitting clinician on the biopsy request form	DIF	Not detailed
Korkitpoonpol et al. [44]	2023	Retrospective study	136 patients	21 males, 115 females	19 to 86	OLP, OLL, OED	Differentiating among OLP, OLL, and OED based on clinical presentations	DIF	Not reported

ACIF = anti-complement immunofluorescence; ANA = antinuclear antibody; BMZ = basement membrane zone; C3 = complement component 3; DIF = direct immunofluorescence; DLE = discoid lupus erythematosus; DG = desquamative gingivitis; FITC = fluorescein isothiocyanate; H&E = hematoxylin and eosin; IIF = indirect immunofluorescence; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M; LE = lupus erythematosus; MIF = multiplexed immunofluorescence; MMO = maximum mouth opening; OLDR = oral lichenoid dysplasia; OLL = oral lichenoid lesions; OLP = oral lichen planus; OMLP = oral mucosal lichen planus; OSCC = oral squamous cell carcinoma; OSF = oral submucous fibrosis; PV = pemphigus vulgaris; RAS = recurrent aphthous stomatitis; T1 = tumour size classification (smallest); T4 = tumour size classification (largest); WHO = world health organization; IF = immunofluorescence.

Table 4A. Outcome variables of included studies

Study	Antigen targets	Methodology	Conventional diagnostic methods	Diagnostic accuracy metrics	Clinical utility	Summary of main results	Improvements in diagnostic accuracy or clinical decision-making
Hasler et al. [8]	Not explicitly stated	Immunofluorescent staining techniques used to detect autoantibodies	Clinical appearance, histopathology, ultrastructural analysis	Not explicitly provided, but IF helped confirm diagnosis in cases where clinical presentation was equivocal	Immunofluorescence provided a valuable diagnostic tool to differentiate between PV, bullous pemphigoid, and other similar bullous disorders	Immunofluorescence helped confirm the diagnosis of PV or bullous pemphigoid in 4 patients with oral lesions, where conventional methods were equivocal	Immunofluorescence allowed differentiation between PV, bullous pemphigoid, and other similar bullous disorders that can present with oral lesions
Donatsky et al. [9]	Unkeratinized buccal human oral mucosa from RAS patient	Immunofluorescence staining, microscopic examination, absorption experiments to ensure specificity	-	Compared antibody endpoint titres between controls and RAS patients, but did not report quantitative accuracy metrics	Supports autoimmune aetiology for RAS	RAS patients had higher antibody titres to adult human oral mucosa compared to controls. No difference in titres using RAS vs control oral mucosa as antigen. Absorption experiments confirmed specificity of positive IF	Provides evidence for autoimmune mechanism in RAS, but does not directly improve diagnostic accuracy or clinical decision-making
Torabinejade et al. [10]	Immune complexes	1. Periapical lesions were frozen, sectioned, and examined using ACIF technique. 2. Complement and anti-complement were used at 1 : 20 dilution. 3. Positive and negative controls were used	Histopathological examination of formalin-fixed sections	-	ACIF technique was able to detect immune complexes in 23 out of 25 periapical lesions, suggesting a role of immune complexes in the pathogenesis of periapical lesions	23 out of 25 periapical lesions were positive for antigen-antibody complexes using ACIF technique. No staining was observed in the 2 lesions diagnosed as periapical scar tissue	ACIF technique was more sensitive than the DIF technique used in previous studies, allowing for better detection of immune complexes in periapical lesions. This could provide insights into the pathogenesis of these lesions
Acosta et al. [11]	Intercellular substance of stratified squamous epithelium	Epithelial cells collected from oral mucosa by scraping. Cells spread directly on slides or washed and cytocentrifuged onto slides. DIF staining performed	Histology. IIF for serum intercellular antibodies	-	DIF on oral cytological smears may be useful for diagnosing PV	IgG deposition was observed on cytological smears from oral lesions in all patients with active PV. No fluorescence was seen in smears from pemphigus patients in remission, patients with other oral diseases, or healthy controls. Washing the cells prior to IF improved specificity by reducing non-specific fluorescence	DIF on oral cytological smears may provide a more specific and less invasive diagnostic method for PV compared to traditional histology and IIF
Daniels et al. [12]	Immunoglobulins, complement components, and other protein substances within affected tissues	Cryostat sectioning, incubation with diluted antisera, washing, mounting, and fluorescence microscopy examination	History, clinical features, histopathology, and clinical follow-up	Not reported (qualitative assessment of DIF patterns in relation to diagnosis)	DIF findings can establish diagnosis of pemphigus, pemphigoid, lichen planus, and LE; absence of characteristic patterns can help rule out these conditions	DIF can provide distinguishing patterns to aid in the diagnosis of chronic ulcerative oral mucosal diseases	DIF findings can strengthen or rule out diagnoses, thereby improving diagnostic accuracy and clinical decision-making for chronic ulcerative oral mucosal diseases
Fine et al. [13]	BMZ antigens	Includes tissue processing and potentially antigen retrieval techniques, but specifics are not provided	Clinical examination and histopathological analysis	Not reported	Enhanced understanding of the disease mechanism, potential impact on diagnosis	Immunofluorescence and immunoelectron microscopy provide detailed insights into the pathology of cicatricial pemphigoid	Better visualization of antigen-antibody complexes and complement deposits in tissue samples, aiding in the differentiation of CP from other similar conditions.
Firth et al. [14]	Fibrinogen, C3, Clq, IgA, IgG, IgM	One portion of the biopsy was processed for routine histopathology (H&E staining), while another portion was frozen, sectioned, and stained with the fluorescent-labelled antibodies for DIF examination.	Clinical features, routine histopathology (H&E staining)	Concordance between clinical, histopathological, and DIF diagnoses	DIF was considered an "integral step" in the diagnosis of OMLP, as it contributed to the diagnosis in 13 cases and helped exclude OMLP in 9 cases where the clinical and/or histopathological findings were equivocal.	In 116 (70%) cases, all three diagnostic modalities (clinical, histopathological, and DIF) agreed on the diagnosis of OMLP. In 13 (7.9%) cases, the diagnosis could only be established by DIF. In 9 (5.6%) cases, DIF helped exclude a diagnosis of OMLP when the clinical and/or histopathological findings were equivocal. DIF was non-contributory in 27 (16.3%) cases.	The study concludes that DIF was an "integral step" in the diagnosis of OMLP, as it provided additional diagnostic information not available from clinical and histopathological evaluation alone.
Lodi et al. [15]	Epithelial antigens on monkey oesophagus substrate	IIF technique using pre-fixed sections of monkey oesophagus	Clinical and histological features of OLP	Not reported	Determine the nature and frequency of circulating antibodies to epithelial antigens in the sera of HCV-positive patients with OLP	There was a significant association between the concomitance of OLP and HCV infection and the presence of circulating antibodies to epithelial antigens	Some patients with HCV-associated OLP may have circulating antibodies to epithelial antigens, although their precise etiological role in the development of this disease in HCV infection remains unknown
Yih et al. [16]	Immunoglobulins and complement component within affected gingival tissues	Biopsy of gingival lesions, with one half processed for H&E histology and the other for DIF using frozen sections	Clinical presentation and routine histological examination	DIF analysis, in combination with histology, was able to provide a definitive diagnosis in 65 out of 72 (90.3%) DG cases	Correct diagnosis is critical for determining appropriate treatment and follow-up, as DG can be caused by a variety of immunological and idiopathic conditions with different prognoses	Improvements in diagnostic accuracy or clinical decision-making	DIF analysis, combined with histology, provided a more accurate diagnosis compared to clinical presentation and routine histology alone, which frequently cannot differentiate between the various DG-causing conditions
Bhol et al. [17]	α6 integrin- BMZ	Sera of patients and determination of their effects on normal human buccal mucosa in organ culture	Clinical symptoms (erosions, vesicles, or bullae in the oral cavity), histological findings (subepithelial cleft with mixed cell infiltrate)	Not specified	Identification of target antigens and understanding their effects on buccal mucosa	Identify the target antigens recognized by the sera of patients with oral pemphigoid and understand their effects on normal human buccal mucosa	Identifying α6 integrin as a target antigen could improve diagnostic accuracy for oral pemphigoid and help differentiate it from other blistering diseases.

ACIF = anticomplement immunofluorescence; ANA = antinuclear antibody; BMZ = basement membrane zone; C3 = complement component 3; CB = cytoid bodies; DIF = direct immunofluorescence; DG = desquamative gingivitis; Dsg1 = desmoglein 1; Dsg3 = desmoglein 3; H&E = hematoxylin and eosin; HGD = high-grade dysplasia; IIF = indirect immunofluorescence; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M; LE = lupus erythematosus; LGD = low-grade dysplasia; MMP = mucous membrane pemphigoid; MMO = maximum mouth opening; OLDR = oral lichenoid dysplasia; OLL = oral lichenoid lesions; OLP = oral lichen planus; OMLP = oral mucosal lichen planus; OSCC = oral squamous cell carcinoma; OSF = oral submucous fibrosis; PV = pemphigus vulgaris; RAS = recurrent aphthous stomatitis; T1 = tumour size classification (smallest); T4 = tumour size classification (largest); ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; SCC = squamous cell carcinoma; IF = immunofluorescence.

Table 4.B Outcome variables of included studies

Study	Antigen targets	Methodology	Conventional diagnostic methods	Diagnostic accuracy metrics	Clinical utility	Summary of main results	Improvements in diagnostic accuracy or clinical decision-making
Nakano et al. [18]	Collagen IV α chains	Frozen sections of ameloblastoma, oral mucosa, and tooth germ samples stained with specific antibodies	Histological examination (H&E staining)	Not reported	Collagen IV α chain distribution may serve as markers for ameloblastoma cytodifferentiation and progression	Ameloblastoma expressed α1(IV), α2(IV), α5(IV), α6(IV) chains intensely around neoplastic epithelium, similar to oral mucosa. α4(IV) expression was rare, mainly around nests of primitive tumour cells or potentially invasive sites. Tooth germ showed stage- and position-specific collagen IV α chain distribution	The study suggests collagen IV α chain distribution may be used as diagnostic markers to distinguish ameloblastoma from other lesions and assess tumour progression, but did not provide specific accuracy metrics.
Kolde et al. [19]	Shaggy deposition of fibrinogen along the BMZ and/or IgM-positive cytoid-like bodies	Dewaxed and rehydrated histological sections were treated with pronase E for antigen retrieval prior to IF staining	Histological diagnosis and classification of OLP were made according to WHO criteria	Sensitivity and specificity of IF staining patterns were calculated in comparison to histological diagnosis	IF testing on pronase-treated histological sections improved the often controversial histopathological assessment of OLP	Typical IF staining patterns of OLP (shaggy fibrinogen deposition, IgM-positive cytoid-like bodies) were found in 17 of the 30 biopsies. Immunofluorescence established the diagnosis of OLP in 6 out of 9 biopsies with histological alterations compatible but not evident for OLP	The technique showed high sensitivity and specificity compared to histological diagnosis
Musa et al. [20]	Fibrin, immunoglobulins, and C3	Staining of tissue samples for fibrin, immunoglobulins, and C3 and examination by fluorescence microscopy	Clinical presentation, histopathology (H&E)	Not explicitly reported, but comparison of DIF and histopathology findings	DIF is valuable in establishing OLP diagnosis when histology is not conclusive, and in ruling out OLP when it is not present. However, DIF may misdiagnose some cases of dysplasia, carcinoma in situ, and SCC as OLP	75 cases were positive for OLP by both H&E and DIF, 65 cases were positive for OLP by DIF but not H&E, and 36 cases were suggestive of OLP by H&E but ruled out by DIF. Some cases of dysplasia, carcinoma in situ, and SCC were misdiagnosed as OLP by DIF	DIF can improve diagnostic accuracy by detecting OLP cases missed by histopathology, and by ruling out OLP when not present. However, DIF has limitations and should be used in combination with histopathology to reach the final diagnosis
Kulthanan et al. [21]	Deposits at the DEJ and CBs	Frozen skin biopsy sections stained with fluorescein isothiocyanate-conjugated antibodies and examined under a fluorescence microscope	Clinical and histologic examination	Positive DIF yield in 75% of cases	DIF may be helpful in disease differentiation, especially in cases with no specific clinical or histologic characteristics or ambiguous features	Deposits at the DEJ and CBs were detected in 53% and 60% of cases, respectively. DEJ +CB deposits were found in 38% of cases. IgM +other immunoreactant deposits, including fibrin at the CBs, were found in 56% of cases. Shaggy fibrin deposition at the DEJ was found in 56% of cases, 44% of cases had immunoreactants other than fibrin deposited along the DEJ, which resembled those of LE	DIF may help differentiate LP from other conditions, especially LE, in cases with no specific clinical or histologic characteristics or ambiguous features
Suresh et al. [22]	Humoral antigens	Gingival biopsy subjected to H&E and IF	Conventional H&E microscopy in addition to DIF	Conventional H&E microscopy in addition to DIF	Clinical-pathologic correlation essential for definitive and differential diagnosis of DG cases	Definitive diagnosis in 80% of gingival biopsies.Clinical diagnosis of lichen planus correlated with biopsy findings in 80% of cases, pemphigoid in 60%	Negative DIF cases had significant pathology (dysplasia, carcinoma) missed on H&E alone
de Freitas Silva et al. [23]	Twist, E-cadherin	Immunohistochemistry, Western blotting, double-IF	Histological grading of oral dysplasia based on WHO criteria	Not reported	Possible value of Twist and E-cadherin in predicting the risk of oral epithelium malignant transformation	Significant differences in Twist and E-cadherin immunoexpression were observed between normal oral mucosa and OL, with an inverse relation since the earliest stages of oral dysplasia. Downregulation of E-cadherin was found to occur in a Twist-dependent manner in OSCC	Twist and E-cadherin could be used as potential markers to predict the risk of malignant transformation in oral epithelium, but does not provide specific data on diagnostic accuracy
Rameshkumar et al. [24]	Not specified	Review of clinical features and histopathology (H&E) of 70 subjects; Histopathology (H&E) and DIF performed on biopsy specimens from 12 subjects	Clinical features, histopathology	Consistency between histopathology and DIF in the prospective analysis (66% cases)	DIF can improve diagnostic efficiency of oral mucocutaneous lesions when used in addition to clinical and histopathological evaluation	Retrospective analysis showed similar findings to previous studies except for some differences; prospective analysis, histopathology and DIF were consistent in 66% cases	DIF can enhance diagnostic accuracy when used along with clinical and histopathological evaluation for oral mucocutaneous lesions
Hashimoto et al. [25]	C3 deposition in BMZ	Oral mucosal biopsy, immunoserological tests (IIF, immunoblotting, ELISA)	Clinical, histopathological assessments	Not reported	Granular C3 deposition in oral BMZ may be a characteristic feature of severe OLP	10 out of 12 cases showed granular C3 deposition in oral BMZ on DIF. The 10 cases showed no positive reactivity for IgG or IgA antibodies	DIF for C3 deposition may provide additional diagnostic information for severe cases of OLP
Montague et al. [26]	Fibrinogen	Diagnostic codes, recorded age, gender, diagnosis, DIF findings, and biopsy location; re-examined H&E slides to confirm diagnosis	Clinical and histopathologic examination	Characterizing fibrinogen positivity in premalignant and malignant oral lesions	Fibrinogen positivity on DIF may contribute to diagnostic confusion, especially in cases with mild or reactive premalignant features	68 out of 164 cases showed fibrinogen positivity. Low-grade dysplasia and verrucous lesions made up the majority of fibrinogen positive cases. A lichenoid distribution of the inflammatory infiltrate significantly predicted fibrinogen positivity	Fibrinogen positivity may be seen in premalignant and malignant oral lesions, increasing the risk of misdiagnosis
Lee [27]	Fibrinogen deposition at the mucosal-submucosal interface	Modified WHO diagnostic criteria for OLP and OLL, and criteria proposed by AAOMP. DIF results were evaluated for deposition intensity or pattern of fibrinogen and classified as positive, possibly positive or negative	Clinical and histologic assessment	Not provided	Final diagnosis of OLP could be different depending on the type of diagnostic criteria used. There was no statistical difference in DIF findings between OLP and OLL	Patients diagnosed as OLP were slightly more when using the modified WHO criteria compared to the AAOMP criteria. There was no statistical difference in DIF between OLP and OLL when applying either the modified WHO or AAOMP criteria	Highlights the potential for variability in the final diagnosis of OLP and OLL depending on the diagnostic criteria used, and suggests that DIF may not provide additional diagnostic value in differentiating between these two conditions

ACIF = anticomplement immunofluorescence; ANA = antinuclear antibody; BMZ = basement membrane zone; C3 = complement component 3; CB = cytoid bodies; DIF = direct immunofluorescence; DG = desquamative gingivitis; Dsg1 = desmoglein 1; Dsg3 = desmoglein 3; H&E = hematoxylin and eosin; HGD = high-grade dysplasia; IIF = indirect immunofluorescence; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M; LE = lupus erythematosus; LGD = low-grade dysplasia; MMP = mucous membrane pemphigoid; MMO = maximum mouth opening; OLDR = oral lichenoid dysplasia; OLL = oral lichenoid lesions; OLP = oral lichen planus; OMLP = oral mucosal lichen planus; OSCC = oral squamous cell carcinoma; OSF = oral submucous fibrosis; PV = pemphigus vulgaris; RAS = recurrent aphthous stomatitis; T1 = tumour size classification (smallest); T4 = tumour size classification (largest); ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; SCC = squamous cell carcinoma; IF = immunofluorescence.

Table 4C. Outcome variables of included studies

Study	Antigen targets	Methodology	Conventional diagnostic methods	Diagnostic accuracy metrics	Clinical utility	Summary of main results	Improvements in diagnostic accuracy or clinical decision-making
Zhou et al. [28]	Desmoglein 1 and Desmoglein 3 (by ELISA)	IIF, ELISA, Tzanck smear test	Histopathology, DIF	Sensitivity and specificity for IIF, ELISA, Tzanck smear, and serial testing	Tzanck smear and ELISA serial testing recommended as a simple, rapid and reliable way to diagnose pemphigus in dental clinics	Sensitivities: Tzanck smear 96.7%, IIF 84.8%, ELISA 84.8%; specificities: Tzanck smear 60%, IIF 91.8%, ELISA 96.7%; serial testing of Tzanck smear and ELISA showed 82% sensitivity, 98.7% specificity	Serial testing of Tzanck smear and ELISA recommended as a simple, rapid and reliable diagnostic approach for pemphigus in dental clinics
Masquijo-Bisio et al. [29]	Fibrinogen deposition at BMZ	Immunofluorescence staining of biopsy samples	Clinical examination, histopathological analysis	Not reported	Differentiating plaque-type OLP from homogenous leukoplakia when clinical and histopathological features are ambiguous	Fibrinogen deposition at the BMZ was detected in 100% of plaque-type OLP cases, but in 0% of homogenous leukoplakia cases	DIF using anti-fibrinogen antibody can improve diagnostic accuracy when differentiating plaque-type OLP from homogenous leukoplakia
Yamanaka et al. [30]	BMZ, Civatte bodies (apoptotic cells)	Patients with OLP and OLL were compared to patients with MMP, PV, and fibrous hyperplasia	Clinical and histopathological features	Frequency of positive DIF in OLP (73.3%) and OLL (38.4%)	DIF is a key tool in differentiating some lichenoid lesions and could improve the diagnosis of OLP and OLL, especially in lesions showing typical clinical and histological features of OLP	22 patients with OLP (73.3%), 10 with OLL (38.4%), 25 with MMP (96.1%), and all with PV (100%) had positive DIF. The most frequent DIF pattern was linear fibrinogen at the BMZ. A logistic regression model found a statistically significant difference in positive DIF between OLP and OLL	DIF can improve the diagnosis of OLP and OLL, especially in lesions with typical clinical and histological features of OLP
Abdalla et al. [31]	E-cadherin, EMP1, 5T4, N-cadherin	Quantitative IF microscopy to assess marker expression in tissue samples	Not specified	Z-scores calculated to predict abnormal epithelium and classify disease grades (LGD, HGD, T1 OSCC, T4 OSCC)	Markers (E-cadherin, EMP1, N-cadherin) can predict abnormal epithelium and classify disease grades	Loss of E-cadherin is an early event in oral dysplasia. Loss of E-cadherin and EMP1 is an indicator of LGD. Loss of E-cadherin, EMP1 and 5T4 is an indicator of HGD. Expression patterns of E-cadherin, EMP1 and N-cadherin can predict abnormal epithelium and classify disease grades. Significant differences in marker expression observed in safety margins compared to normal tissue	Provides a quantitative, objective approach to assess epithelial markers that can improve diagnosis and classification of oral dysplasia and OSCC
Kamaguchi et al. [32]	BMZ proteins	Biopsy of non-lesional buccal mucosa, DIF analysis	Histological analysis, IIF, ELISA, immunoblotting	DIF of non-lesional buccal mucosa was positive in all 7 cases, while conventional methods failed to detect autoantibodies in any of the cases	DIF using non-lesional buccal mucosa was superior to histological and serological tests for diagnosing MMP	DIF using non-lesional buccal mucosa was able to detect linear deposits of IgG and C3 at the BMZ in all 7 MMP cases, while conventional diagnostic methods failed to detect autoantibodies. This procedure was found to be technically easy and have high diagnostic value	DIF using non-lesional buccal mucosa improved diagnostic accuracy compared to conventional diagnostic methods for MMP. This could lead to faster diagnosis and initiation of appropriate treatment
Bresler et al. [33]	Not specified	Patients underwent biopsies for concurrent routine histologic evaluation and DIF testing	Routine histology	Sensitivity: 0.810, specificity: 0.989, negative predictive value: 0.889, positive predictive value: 0.979	In patients with low clinical suspicion for oral autoimmune bullous disorder, routine histology alone may be sufficient and more cost-effective	57 out of 121 (47.1%) high suspicion cases were consistent with oral autoimmune bullous disorder on DIF. 11 cases in the high suspicion group were histologic false negatives. No histologic false negatives or inconclusive DIF results in the low suspicion group	Routine histology alone may be sufficient in low clinical suspicion cases, avoiding the need for more expensive DIF testing
Reyes et al. [34]	Components of the β-catenin destruction complex, early endosome markers	Tissue IF, cell culture experiments with DOK and non-dysplastic oral keratinocytes (OKF6), Rab5 activity assays, subcellular fractionation, transcription and protease protection assays	Histological assessment of oral dysplasia	Not reported	Understanding the mechanisms underlying nuclear accumulation of β-catenin in oral dysplasia, which is associated with progression to OSCC	Increased Rab5 activity and endosomal sequestration of the β-catenin destruction complex leads to stabilization and nuclear accumulation of β-catenin in oral dysplasia	This study provides insights into the molecular mechanisms involved in the progression of oral dysplasia, but does not directly report improvements in diagnostic accuracy or clinical decision-making
Tikkhanarak et al. [35]	BMZ, cytoid/CBs	DIF analysis to evaluate immunoreactant patterns	Medical history, oral examination, histopathology	Not explicitly reported	DIF interpretation can aid in differentiating OLP, OLP/LE, chronic ulcerative-like lesion, immune-mediated disease, or dysplasia	Atrophic pattern was most common in OLP, OLL, and OLDR groups. DIF confirmed OLP in only 41.2% of OLP cases, with 23.5% each as LP/LE or negative. In OLL, most common DIF was LP/LE or non-specific (31.6% each). In OLDR, DIF was OLP, LP/LE, immune complex-mediated disease, or mixed connective tissue disease. 1 OLDR case showed mild to moderate dysplasia. No significant differences in ANA positivity or patterns between groups	DIF analysis can aid in differentiating OLP, OLL, and OLDR, identifying cases that may not be typical OLP or OLL

ACIF = anticomplement immunofluorescence; ANA = antinuclear antibody; BMZ = basement membrane zone; C3 = complement component 3; CB = cytoid bodies; DIF = direct immunofluorescence; DG = desquamative gingivitis; Dsg1 = desmoglein 1; Dsg3 = desmoglein 3; H&E = hematoxylin and eosin; HGD = high-grade dysplasia; IIF = indirect immunofluorescence; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M; LE = lupus erythematosus; LGD = low-grade dysplasia; MMP = mucous membrane pemphigoid; MMO = maximum mouth opening; OLDR = oral lichenoid dysplasia; OLL = oral lichenoid lesions; OLP = oral lichen planus; OMLP = oral mucosal lichen planus; OSCC = oral squamous cell carcinoma; OSF = oral submucous fibrosis; PV = pemphigus vulgaris; RAS = recurrent aphthous stomatitis; T1 = tumour size classification (smallest); T4 = tumour size classification (largest); ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; SCC = squamous cell carcinoma; IF = immunofluorescence.

Table 4D. Outcome variables of included studies

Study	Antigen targets	Methodology	Conventional diagnostic methods	Diagnostic accuracy metrics	Clinical utility	Summary of main results	Improvements in diagnostic accuracy or clinical decision-making
Noormohammadpour et al. [36]	Nuclear constituents of cells	Serum samples from OSF patients and healthy controls were tested for ANA positivity using IIF. ANA positivity was correlated with clinical parameters like MMO and site of involvement	Clinical examination and assessment of MMO and sites of involvement	Prevalence of ANA positivity in OSF patients vs. healthy controls	Presence of autoantibodies like ANAs, female predilection, and alterations in humoral and cellular immunity justify OSF as an autoimmune disease, providing a broader perspective to adopt therapies targeting autoimmune pathways.	Significantly higher incidence of ANA (35.6%) in OSF patients compared to healthy controls. Higher prevalence of ANA positivity in females (68%) than males. Significantly lower MMO in ANA positive OSF patients. Significantly more sites of involvement in ANA positive OSF cases. Speckled, homogeneous and nucleolar fluorescence patterns observed in ANA positive cases	Evidence supporting an autoimmune component in the etiopathogenesis of OSF, which can guide the development of targeted autoimmune therapies for this condition
Gupta et al. [37]	PD-L1, CD8+, FOXP3+ T cells	Tissue processing: formalin-fixed paraffin-embedded whole tissue sections. Antigen retrieval: baked slides for 3 hours at 60 °C, stained using BOND RX™ Automated Stainer (Leica Biosystems; Wetzlar, Germany). Analysis: nCounter® Analysis System (NanoString Technologies, Inc.) for immune gene expression	Pathologic diagnosis verified by an expert oral pathologist, clinical phenotype verification, and gene expression profiling	Cancer-free survival at 5 years, which was significantly lower in the proliferative leukoplakia group compared to the localized leukoplakia group (46.8% vs 83.6%)	Evaluated immune cell infiltration and gene expression profiles to distinguish between proliferative and localized leukoplakia	Proliferative leukoplakia exhibited a higher mean abundance of CD8+ T cells, PD-1+ T cells, and FOXP3+ Tregs compared to localized leukoplakia. PD-L1 expression was significantly increased in proliferative leukoplakia samples. Differential gene expression observed, including cytotoxic T-cell signatures and lower IFNγ expression in proliferative leukoplakia. Specific gene overexpression noted for ICOS and other immune-related genes	Distinct immunologic signature in proliferative leukoplakia, with higher levels of CD8+ T cells, Tregs, and PD-L1 expression compared to localized leukoplakia. This provides a strong rationale for investigating PD-1/PD-L1 axis blockade as a preventative immunotherapy approach for high-risk proliferative leukoplakia
Hanna et al. [38]	Not specified	Tzanck smear evaluated using Giemsa and H&E staining. Histopathology and DIF used for definitive diagnosis	Histopathology, DIF	Sensitivity and specificity of Tzanck smear	Tzanck smear as a rapid, inexpensive screening test for PV	Sensitivity of Tzanck smear (both Giemsa and H&E) was 80.5% for PV. Specificity was 84.6% for Giemsa and 96.3% for H&E staining	Tzanck smear can be used as a rapid screening test for PV prior to definitive histopathology and DIF testing
Petruzzi et al. [39]	Dsg1 and Dsg3	ELISA to detect anti-Dsg1 and anti-Dsg3 antibodies, IIF	Histopathological examination, DIF	Sensitivity, specificity, accuracy, positive predictive value, negative predictive value	Serological tests can be used as adjunctive tools for early detection and diagnosis of oral pemphigus	Anti-Dsg3 ELISA had the best diagnostic performance (75% sensitivity, 100% specificity). Anti-Dsg3 antibody titers correlated with the Oral Disease Severity Score	Anti-Dsg3 ELISA should be considered as a first-line diagnostic test for oral pemphigus detection
Rujitharanawong et al. [40]	DEJ, CBs	DIF technique and slide interpretation performed according to standard criteria	Clinical and histopathological examination	Positive DIF yields: 79.3% in OLP, 93.3% in cutaneous LP	DIF can help differentiate lichen planus from other conditions like LE and lichenoid drug reactions	Deposition of immunoreactants at the DEJ was significantly greater in OLP than cutaneous LP. Fibrin deposition with shaggy pattern at the DEJ was significantly greater in OLP than cutaneous LP. Deposition of immunoreactants at CBs (with or without DEJ) was significantly greater in cutaneous LP than OLP. IgM deposition at CBs was commonly detected in both groups	Fibrin deposition with shaggy pattern at the DEJ may be the best diagnostic indicator of OLP
He et al. [41]	Autoantibodies specific for PV	DIF analysis of oral Tzanck smears; comparison with ELISA and IIF	ELISA and IIF	Sensitivity: 87.8%, specificity: 100%, area under the curve: 0.939	High diagnostic accuracy, less invasiveness, and cost-effectiveness compared to traditional methods	DIF analysis of oral Tzanck smears showed high diagnostic accuracy and clinical utility for diagnosing PV, outperforming ELISA and IIF in terms of sensitivity and specificity	DIF outperforms ELISA and IIF in terms of sensitivity and specificity
Mao et al. [42]	Levels of CD3+ cells CD4+ cells CD8+ cells and the CD4+/CD8+ cell ratio, as well as immunoglobulins) and complement components (C3, C4) in the participants	DIF examination, histopathologic tests	Clinical examination, serologic testing, histopathologic tests	DIF demonstrated 64.3% positive reactivity with 2 distinct distribution patterns and 8 staining patterns	Enhanced understanding of OLP, potential impact on diagnosis and immune function assessment	DIF and serologic testing showed significant differences in immune function markers between OLP patients and controls	Combination of H&E test and DIF was found to be useful for the diagnosis of OLP. 71.2% (42/59) were diagnosed as OLP and 28.8% (17/59) were diagnosed as non-OLP using this approach
Hansen et al. [43]	Not detailed	Tissue processing and antigen retrieval techniques not specified in detail. DIF samples were harvested simultaneously with light microscopic samples	Clinical examination and histopathological analysis using light microscopy	Sensitivity: 0.32, specificity: 0.88, positive predictive value: 0.68, negative predictive value: 0.61	Light microscopy alone is not sufficient for the diagnosis of OLP; combining it with DIF provides more accurate diagnosis and appropriate therapeutic regimen	Light microscopy has low sensitivity but high specificity compared to DIF for diagnosing OLP. The combination of DIF with light microscopy offers improved diagnostic accuracy and better guides treatment planning	DIF combined with light microscopy provides more accurate diagnosis and appropriate therapeutic regimen for OLP
Korkitpoonpol et al. [44]	Shaggy fibrinogen at the BMZ was the most common DIF pattern in all groups	DIF analysis on tissue specimens from patients with clinical presentations of OLP	Histopathological examination	DIF-positivity rates among OLP, OLL, and OED groups	DIF assay alone cannot reliably differentiate among OLP, OLL, and OED; histopathological examination is required	117 out of 136 patients were DIF-positive. The highest DIF-positivity rate was in the OLP group (88.9%), followed by OLL (83.7%) and OED (81%).	DIF assay alone is not sufficient to differentiate among OLP, OLL, and OED; careful clinicopathological correlation and long-term follow-up are necessary to diagnose and manage these lesions

ACIF = anticomplement immunofluorescence; ANA = antinuclear antibody; BMZ = basement membrane zone; C3 = complement component 3; CB = cytoid bodies; DIF = direct immunofluorescence; DG = desquamative gingivitis; Dsg1 = desmoglein 1; Dsg3 = desmoglein 3; H&E = hematoxylin and eosin; HGD = high-grade dysplasia; IIF = indirect immunofluorescence; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M; LE = lupus erythematosus; LGD = low-grade dysplasia; MMP = mucous membrane pemphigoid; MMO = maximum mouth opening; OLDR = oral lichenoid dysplasia; OLL = oral lichenoid lesions; OLP = oral lichen planus; OMLP = oral mucosal lichen planus; OSCC = oral squamous cell carcinoma; OSF = oral submucous fibrosis; PV = pemphigus vulgaris; RAS = recurrent aphthous stomatitis; T1 = tumour size classification (smallest); T4 = tumour size classification (largest); ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; SCC = squamous cell carcinoma; IF = immunofluorescence.

The demographic details of the study populations varied, reflecting a broad spectrum of age groups and gender distributions. For example, Kulthanan et al. [21] included patients aged 6 to 76 years with an equal male-to-female ratio, while Hansen et al. [43] involved an older population with a mean age of 61.9 years, predominantly female. The sample sizes ranged from small cohorts, such as 10 patients in Fine et al. [13], to larger groups, such as 136 patients in Korkitpoonpol et al. [44]. These studies also covered diverse geographic regions, adding to the generalizability of the findings (Table 3).

In terms of diagnostic methodologies, various IF techniques were employed. direct immunofluorescence (DIF) was the most commonly used, featured in studies by Fine et al. [13], Kulthanan et al. [21], Mao et al. [42], and Hansen et al. [43]. Indirect immunofluorescence (IIF) was used in studies such as Lodi et al. [15] and Bhol et al. [17], while de Freitas Silva et al. [23] utilized double-IF (Table 3).

Outcome characteristics

Outcome antibody targets included human IgG, IgA, and complement components (C3), circulating antibodies against epithelial antigens [15], monoclonal antibodies to human α6 integrin [17], and antibodies against Twist and E-cadherin [23] (Table 4).

Antigen targets were also a significant focus. The study by Bhol et al. [17] specifically identified α6 integrin as a key antigen in oral pemphigoid. In total, three studies assessed epithelial antigens [15,17,21], while Fine et al. [13] and He et al. [41] examined various antigens at the basement membrane zone, such as IgG, IgA, IgM, and C3. The study by de Freitas Silva et al. [23] evaluated the expression of Twist and E-cadherin in oral leukoplakia and oral squamous cell carcinoma, adding another layer to the understanding of antigen targets (Table 4).

Conventional diagnostic methods were prominently assessed in the studies. Kulthanan et al. [21] and Hansen et al. [43] emphasized the importance of combining clinical examination with histopathological and IF techniques for a more accurate diagnosis of OLP. Fine et al. [13] used immunoelectron microscopy along with IF to examine cicatricial pemphigoid, demonstrating the value of advanced imaging techniques in enhancing diagnostic precision (Table 4).

Quality assessment of the included studies

The risk of bias assessment of included studies in this systematic review was conducted using the QUADAS-2 tool, which evaluates bias across four domains: patient selection, index test, reference standard, and flow and timing (Figure 2). The majority of studies demonstrated a low risk of bias in most domains, indicating overall methodological rigor. However, some studies exhibited concerns, particularly in patient selection and flow and timing. Specifically, while domains such as the index test and reference standard consistently showed low risk, variability was noted in patient selection, with a few studies

Study	D1	D2	D3	D4	Overall
Hasler et al. [8]	-	+	-	+	-
Donatsky et al. [9]	+	+	+	+	+
Torabinejade et al. [10]	+	+	+	+	+
Acosta et al. [11]	+	+	+	+	+
Daniels et al. [12]	-	+	-	+	-
Fine et al. [13]	+	+	+	+	+
Firth et al. [14]	+	+	+	+	+
Lodi et al. [15]	+	+	+	+	+
Yih et al. [16]	+	+	+	+	+
Bhol et al. [17]	+	+	+	+	+
Nakano et al. [18]	+	+	+	+	+
Kolde et al. [19]	-	+	-	+	-
Musa et al. [20]	-	+	-	+	-
Kulthanan et al. [21]	-	+	-	+	-
Suresh et al. [22]	-	+	-	+	-
de Freitas Silva et al. [23]	+	+	+	+	+
Rameshkumar et al. [24]	+	+	+	+	+
Hashimoto et al. [25]	-	+	-	+	-
Montague et al. [26]	-	+	-	+	-
Lee [27]	-	+	-	+	-
Zhou et al. [28]	-	+	-	+	-
Masquijo-Bisio et al. [29]	+	+	+	+	+
Yamanaka et al. [30]	+	+	+	+	+
Abdalla et al. [31]	+	+	+	+	+
Kamaguchi et al. [32]	+	+	+	+	+
Bresler et al. [33]	-	+	-	+	-
Reyes et al. [34]	+	+	+	+	+
Tikkhanarak et al. [35]	-	+	-	+	-
Noormohammadpour et al. [36]	+	+	+	+	+
Gupta et al. [37]	+	+	+	+	+
Hanna et al. [38]	-	+	-	+	-
Petruzzi et al. [39]	-	+	-	+	-
Rujitharanawong et al. [40]	-	+	-	+	-
He et al. [41]	+	+	+	+	+
Mao et al. [42]	+	+	+	+	+
Hansen et al. [43]	-	+	-	+	-
Korkitpoonpol et al. [44]	-	+	-	+	-

Figure 2. Risk of bias plot using QUADAS-2 tool.
+ = low; - = some concerns; D1 = patients selection; D2 = index test; D3 = reference standard; D4 = flow and timing.

showing potential biases. Despite these concerns, the comprehensive assessment indicates that the included studies largely adhere to robust methodological standards, ensuring the reliability of the synthesized findings. This careful evaluation underscores the credibility of the evidence base, supporting its use in clinical and research settings.

DISCUSSION

The findings of this systematic review underscore the growing utility of IF in the diagnostic evaluation of various oral lesions. The included studies collectively demonstrate the ability of this technique to complement and enhance the diagnostic capabilities of conventional clinical and histopathological assessments.

One key advantage of IF is its potential to improve the differentiation of similar-appearing oral lesions. Several studies have reported the successful application of IF in distinguishing between clinical mimics, such as pemphigus vulgaris and mucous membrane pemphigoid, or between different subtypes of OLP [45]. For instance, Fine et al. [13] used DIF to identify specific antibody deposits in cicatricial pemphigoid, facilitating accurate differentiation from other similar conditions. Kulthanan et al. [21] demonstrated that DIF was critical in differentiating lichen planus from lupus erythematosus based on unique deposition patterns of immunoreactants. This ability to identify specific expression patterns of various molecular markers and antigens aids clinicians in reaching more accurate and definitive diagnoses, which is crucial for appropriate management and treatment.

Another important aspect of the diagnostic utility of IF is its ability to assess disease progression and treatment response. The reviewed studies have demonstrated the usefulness of IF in monitoring the expression of biomarkers associated with malignant transformation, such as p53 and Ki-67, in premalignant oral lesions. For example, de Freitas Silva et al. [23] utilized double-IF to evaluate the co-expression of Twist and E-cadherin in oral squamous cell carcinoma, providing insights into tumour progression and potential therapeutic targets. Additionally, IF has been employed to evaluate the effects of therapeutic interventions, offering a method to assess treatment efficacy by monitoring changes in the expression of biomarkers.

The comparative analysis of diagnostic parameters reveals notable differences in performance between DIF and IIF across various oral lesions. DIF generally exhibits higher diagnostic performance metrics across conditions such as pemphigus vulgaris, OLP, and desquamative gingivitis oral ulcers overlap with OLP [46].

For pemphigus vulgaris, DIF's high sensitivity (87.8%) and specificity (100%) are attributed to its ability to detect tissue-bound autoantibodies directly in lesional skin or mucosa, providing precise localization of immune deposits. According to He et al. [41] this high level of specificity indicates that DIF is highly reliable in correctly identifying patients without the disease, while its sensitivity ensures most cases are detected. IIF, however, shows slightly lower diagnostic performance due to its reliance on circulating autoantibodies, which may fluctuate based on disease activity and treatment status.

In mucous membrane pemphigoid, DIF is particularly effective in identifying linear deposits of IgG, IgA, or C3 along the basement membrane zone, which are critical for accurate diagnosis. Studies such as those by Genovese et al. [47] emphasize the importance of these specific immune deposits for distinguishing mucous membrane pemphigoid from other blistering disorders. IIF's lower sensitivity in this condition is due to the occasional absence of circulating autoantibodies, as noted in the study by Challacombe et al. [48].

For OLP, DIF effectively detects fibrinogen deposits and colloid bodies. The study by Mao et al. [42] reports a sensitivity of 64.3%, indicating a moderate ability to identify true positive cases. In contrast, Hansen et al. [43] reports a sensitivity of 32% and specificity of 88%, with positive predictive value and negative predictive value of 68% and 61% respectively, suggesting a lower sensitivity but a higher specificity, making DIF more reliable for confirming the disease rather than detecting it. IIF's limited ability to detect specific immunoreactant patterns results in lower sensitivity, as it often fails to identify the fibrinogen deposits characteristic of OLP, leading to lower diagnostic performance.

For desquamative gingivitis oral ulcers overlapping with OLP, the study by Bresler et al. [33] demonstrates a high sensitivity of 81% and specificity of 98.9%, with a positive predictive value of 97.9% and negative predictive value of 88.9%. This high sensitivity and specificity can be explained by the precise localization of immune complexes in tissue samples, which is strength of DIF.

In chronic erosions blistering exudation of the oral mucosa, the IIF study by Zhou et al. [28] shows a sensitivity of 84.8% and specificity of 60%, suggesting a good ability to detect true positive cases but a moderate rate of false positives. This variability in sensitivity and specificity reflects the challenges of

relying on circulating autoantibodies, which can vary in concentration and presence.

Donatsky et al. [9] found that patients with recurrent aphthous stomatitis had higher antibody titres to adult human oral mucosa compared to controls, supporting an autoimmune aetiology for Recurrent Aphthous Stomatitis. However, this did not directly improve diagnostic accuracy or clinical decision-making.

Torabinejade et al. [10] used an anticomplement immunofluorescence (ACIF) technique to detect immune complexes in 23 out of 25 periapical lesions, suggesting the role of immune complexes in the pathogenesis of these lesions. This technique proved to be more sensitive than DIF used in previous studies, enhancing the understanding of periapical lesion pathogenesis.

Acosta et al. [11] highlighted that DIF on oral cytological smears could be a useful diagnostic tool for pemphigus vulgaris, providing a specific and less invasive method compared to traditional histology and IIF. The study by Hansen et al. [43] demonstrated that IF could confirm diagnoses in cases where clinical presentations were ambiguous. This technique was able to differentiate between pemphigus vulgaris and bullous pemphigoid, which are conditions that often present similarly in clinical settings.

The observed diagnostic performance values can be explained by the inherent methodological differences between DIF and IIF. DIF involves direct visualization of immune deposits in tissue biopsies, providing higher sensitivity and specificity due to precise localization of immune complexes [49]. Conversely, IIF relies on detecting circulating autoantibodies in the patient's serum, which can exhibit variability in sensitivity and specificity. The presence and levels of circulating antibodies can fluctuate based on disease activity and treatment status, leading to lower diagnostic performance in some conditions [50].

Limitations

Despite these advantages, the review also underscores the potential limitations and challenges associated with the use of IF in the diagnosis of oral lesions. Optimal tissue processing, antigen retrieval, and antibody selection are crucial for ensuring the reliability and reproducibility of IF results. The interpretation of IF findings can be subjective, requiring experienced pathologists to minimize the risk of false-positive or false-negative interpretations. Additionally, the availability and cost-effectiveness of IF testing may pose practical challenges in certain healthcare settings, particularly in resource-limited regions.

A meta-analysis was not possible due to the heterogeneity of the included studies. Differences in study design, patient populations, diagnostic criteria, and IF methodologies resulted in significant variability in reported diagnostic metrics, precluding a quantitative synthesis of the data. Additionally, variations in antibody selection, tissue processing techniques, and reporting standards across studies further contributed to the difficulty in aggregating results.

Future research should focus on further validating these findings in larger, prospective cohorts, developing standardized protocols, and exploring cost-effective methods to integrate IF into routine clinical practice. The integration of IF with emerging technologies such as digital pathology and artificial intelligence holds promise for further enhancing diagnostic capabilities and improving patient outcomes. The judicious use of DIF and IIF, guided by the specific clinical context and the nature of the suspected oral lesion, can significantly enhance diagnostic accuracy, guide targeted treatment strategies, and ultimately improve patient care in the field of oral pathology.

CONCLUSIONS

1. Direct immunofluorescence shows higher sensitivity, specificity, area under the curve, positive predictive value, and negative predictive value across multiple conditions.
2. Direct immunofluorescence is effective for diagnosing autoimmune and inflammatory oral conditions by visualizing immune deposits in tissues.
3. Indirect immunofluorescence varies in performance due to reliance on circulating autoantibodies, which can fluctuate with disease activity and treatment.
4. Direct immunofluorescence is preferred when precise localization of immune complexes in tissue is essential.
5. Indirect immunofluorescence is valuable in conditions where circulating autoantibodies play a significant role.
6. Both techniques complement each other in diagnosing oral lesions.

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