

# Direct immunofluorescence demystified: Essential insights and recent advances for dermatologists

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## Abstract

Direct immunofluorescence (DIF) is widely used in dermatopathology for the diagnosis of autoimmune blistering diseases (AIBDs), cutaneous vasculitis, and connective tissue disorders. Although it is easy and useful to perform, it needs technical expertise and experience for proper interpretation. The yield of DIF depends on multiple factors including the adequacy, transportation, storage, processing, and interpretation of the biopsy specimen. Effective collaboration between the dermatologist and dermatopathologist along with meticulous clinico-pathological correlation is crucial for accurately interpreting DIF in the appropriate clinical context.

In this narrative review of DIF in dermatology, we discuss the indications of DIF, recent updates on the selection of optimum biopsy sites, basic techniques of DIF including the classical transport medium and its alternatives, processing and staining technique, patterns in various diseases, advancements such as serration pattern analysis, and latest recommendations on the use of DIF in cutaneous disorders.

**Key words:** Direct immunofluorescence, autoimmune blistering diseases, cutaneous vasculitis, serration pattern

## Introduction

The American pathologist Albert Coons first employed immunofluorescence (IF) to visualise pneumococcal antigens in 1941.<sup>1</sup> The use of DIF in various dermatologic diseases including lupus erythematosus was first reported by Burnham et al in 1963.<sup>2</sup> In 1967, Jordan's group presented a groundbreaking study demonstrating the detection of basement membrane zone (BMZ) antibodies using DIF and IIF in bullous pemphigoid (BP).<sup>3</sup> IF has since revolutionised the classification of autoimmune bullous disorders and has proven invaluable for diagnosing and prognosticating disorders such as cutaneous connective tissue disorders, genetic blistering diseases, and vasculitis.

IF is now an essential diagnostic tool in dermatology. Based on the technique used, IF is classified as:

- (1) Direct immunofluorescence (DIF)
- (2) Indirect-immunofluorescence (IIF)
- (3) Indirect immunofluorescence complement-fixation (IIF-CF)
- (4) Immuno-electron microscopy and
- (5) Double immunofluorescence technique.<sup>4,5,6</sup>

In this review of DIF in dermatology, we cover its indications, recent updates on biopsy site selection, basic techniques (including classical transport medium and alternatives),

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processing and staining techniques, disease patterns, advancements like serration pattern analysis, and the latest recommendations for DIF usage in cutaneous disorders.

## Principles of DIF

DIF uses fluorophore or fluorochrome labelled antibodies which interact with a patient's tissue when incubated to form antigen-antibody conjugates. These can then be visualised under an ultraviolet microscope.<sup>7,8</sup> Ideal fluorophores possess high retention and stability, establishing covalent bonds with proteins and emitting fluorescence within the visible light spectrum.<sup>9</sup> The most frequently utilised fluorophores include fluorescein isothiocyanate (FITC), generating an apple green hue, and tetramethyl-rhodamine isothiocyanate (TRITC), which emits a red fluorescence.<sup>10</sup> Antibodies to IgG, IgG4, IgM, IgA subclasses and sometimes to fibrinogen and C3 (complement 3) are used in DIF.

## Indications for DIF

DIF serves as the gold standard for diagnosing and monitoring disease activity in autoimmune bullous disorders (AIBDs). As DIF findings may be similar in many sub-epidermal AIBDs, additional diagnostic techniques such as serration pattern analysis, IIF on salt-split skin, enzyme-linked immunosorbent assay (ELISA), and immunoblotting are often employed to differentiate these conditions.

DIF is also useful as a supportive investigation in connective tissue disorders such as SLE, and cutaneous vasculitis (especially IgA vasculitis).<sup>11,12</sup> Immunofluorescence mapping (a modification of DIF), is used for the diagnosis of epidermolysis bullosa.

## Site of Biopsy and transport for DIF

The selection of an appropriate biopsy site is critical for obtaining a representative specimen. Table 1 outlines recommended biopsy sites for various disorders.

Formalin, commonly used in histopathology for tissue fixation, can degrade antigens in specimens intended for DIF analysis. Therefore, the biopsy sample for DIF should be taken prior to obtaining samples for routine histopathology in order to prevent contamination of forceps and DIF specimens with formalin. In case of accidental contamination, the specimen should be removed immediately from formalin and rinsed with normal saline, or better still, a fresh biopsy specimen should be obtained.<sup>13</sup>

### Oral cavity as a site of biopsy

Oral biopsies for DIF are technically difficult to perform due to poor accessibility, the need for minimal tissue handling, and the innate fragility of the mucosa. A recent study has shown that a routine buccal punch biopsy of the uninvolved mucosa is as sensitive as a perilesional biopsy (within 1 cm of lesion) in the diagnosis of oral pemphigus vulgaris and multi-site mucous membrane pemphigoid.<sup>14</sup>

The intact epithelium is easier to obtain from the buccal mucosa as compared to the thin and friable gingiva. Gingival biopsies often result in a falsely negative DIF due to chronic inflammation around the gingiva. Additionally, they may cause a permanent periodontal defect leading to plaque accumulation.<sup>14</sup>

In pure gingival mucous membrane pemphigoid, a biopsy from the reflected alveolar mucosa adjacent to the area of gingival inflammation is preferred. Obtaining gingival specimens with an intact epithelium is critical for histological and DIF evaluation. In addition to the standard perilesional 3-mm punch biopsy, a "stab-and-roll" technique and a "peeling" technique have also been used to obtain a mucosal biopsy.<sup>11</sup>

### Other substrates for DIF

#### 1. Hair

The outer root sheath of the hair follicle is structurally analogous to the epidermis. The diameter of scalp

**Table 1: Site of biopsy for DIF in various dermatological disorders**

Disorder	Site of biopsy	Type of biopsy
Autoimmune bullous disease.	Perilesional skin (within 1 cm of bulla). Avoid: 1. Bullous lesions (immune reactants consumed here due to ongoing inflammation so DIF may be falsely negative 2. Biopsy from uninvolved skin distant from bulla and from lower extremity (in case of BP) may give false negative results. <sup>13</sup>	Punch biopsy of 3–4 mm
Dermatitis herpetiformis	Perilesional skin (within 1 cm of bulla).	Broad, deep shave biopsy (instead of punch biopsy)
Vasculitis	Recent purpuric lesion (< 24 hours old) located most proximally	Punch biopsy of 3–4 mm
DLE, lichen planus and porphyria cutanea tarda	Lesional biopsy	Punch biopsy of 3–4 mm
SLE	Skin from both lesional and non-lesional areas, including from sun-exposed (e.g., shoulder) and non-sun-exposed sites (e.g., volar forearm/buttock) <sup>8,12,13</sup>	Punch biopsy of 3–4 mm

DLE: Discoid lupus erythematosus, SLE: systemic lupus erythematosus

terminal hair is almost twice that of vellus hair at other body sites. Thus, the total volume of desmosomal structures is greater per unit area of the scalp than at any other epidermal site. Pemphigus antigens are found throughout the outer root sheath of the hair follicle and in the dermal bulb matrix.

Wilson *et al.* first described the intercellular deposition of immunoreactants in the outer root sheath of the terminal hair follicle in longitudinal sections of follicular-oriented scalp biopsies.<sup>15</sup> Subsequently, Tanasilovic *et al.* showed the utility of DIF of plucked hair in the diagnosis of pemphigus and observed that the sensitivity and specificity of DIF of plucked hair are comparable with that of anti-desmoglein 1 and 3 antibodies as detected by ELISA.<sup>16</sup>

Five or more anagen hairs, plucked with forceps, are collected. The DIF of both fresh and frozen hair samples (stored for two weeks at  $-20^{\circ}\text{C}$ ) showed positive results in all patients with active PV and PF. Mounting entire hairs, rather than sections is easier. The sensitivity of DIF of plucked hair is 80–100% and positive results have been documented in cases with only mucosal involvement.<sup>17,18</sup>

When monitoring immunological remission in pemphigus, plucked hair provides a simpler, more specific, sensitive, and less invasive alternative to skin biopsy regardless of scalp involvement, thereby avoiding repeated skin biopsies.<sup>17,18</sup> However, inadequate samples or lack of expertise in processing hair specimens may result in negative outcomes.

## 2. Formalin fixed paraffin-embedded (FFPE) tissue

If only formalin-fixed paraffin-embedded (FFPE) tissue is available (e.g., immunobullous or connective tissue disease is not initially suspected, or if frozen tissue is unavailable) DIF can be performed after digestion with proteinase or protease K. However, DIF performed on FFPE samples may show decreased staining and sensitivity and the presence of artefactual background staining of IgG in the epidermis could potentially result in a misdiagnosis of pemphigus.

This technique shows best results in diagnosing bullous pemphigoid and pemphigoid gestationis (with a sensitivity of 79%).<sup>19</sup> The diagnosis of IgA vasculitis and dermatitis herpetiformis can pose challenges due to the difficulty in demonstrating IgA deposits.

## 3. Tzanck smears

DIF may also be performed on Tzanck smears particularly in settings where skin biopsy facilities are not available or lesions are located on mucosal sites that are not easily accessible for biopsy. It provides a straightforward, quick, and non-invasive option compared to skin or mucosal biopsy, with sensitivity

ranging from 40% to 87.8%.<sup>20,21</sup> The antigenicity in the air-dried smears kept at room temperature is retained for up to 10 days. Positive smears show bright green fluorescence at the margins of the acantholytic cells or in the intercellular spaces if clumps of acantholytic cells are present. However, non-specific background staining in smears prepared from inflammatory lesions is an important limitation.

## Handling, Transportation, Processing and Storage of Specimens for DIF

### Transportation

1. **Michel's medium (MM)** Michel's medium is a pH-neutral buffered solution containing ammonium sulfate, N-ethylmaleimide, potassium citrate buffer, magnesium sulfate, and distilled water. It is commonly utilised to transport specimens for DIF testing. Unused media should be stored in a refrigerator ( $2-8^{\circ}\text{C}$ ). If there is ammonium sulfate precipitation, crystals should be dissolved at room temperature before use. The expiration date of the media ranges from 12 to 18 months. Specimens preserved in Michel's solution can be stored at room temperature for at least 2 weeks and potentially for 1 to 6 months without compromising signal integrity.<sup>11</sup>

2. **Saline:**

Phosphate buffered saline (PBS) or normal saline is routinely used to transport biopsy samples that can be transported to the laboratory within a few hours after taking the biopsy. Normal saline has shown superior results in DIF than in MM.<sup>22</sup>

Advantages of saline as a transport medium include its inexpensive nature and ready availability in all clinics. It significantly reduces background fluorescence in the dermis, particularly with IgG, and may enhance the cutting properties of skin biopsies. Also, saline could potentially induce an artificial split at the dermo-epidermal junction (DEJ) facilitating the detection of immunoreactants at the basement membrane zone (BMZ) and subepidermal blood vessel walls.

Disadvantages include the need to be transported to the laboratory within 24 hours, as immunoreactants undergo rapid degradation in saline after 48 hours especially in tropical and hot climates. Additionally, it is not a suitable medium for antigen mapping in genetic disorders because it may induce an artificial split at the DEJ.<sup>4,11</sup>

3. **Honey:**

Honey has recently been shown to serve as an effective transport medium for DIF specimens in resource-poor settings. It is believed to preserve cells by preventing autolysis and putrefaction. The optimum preservation time for samples is approximately two weeks; beyond

this period, there is a deterioration of immunoreactants in the biopsy sample.<sup>23</sup>

### Tissue processing and staining

Processing and staining of skin biopsy specimens for DIF has been detailed in Figure 1.

### Other special techniques:

#### 1. Direct-salt split technique:

Immunofluorescence of salt-split skin serves as an effective tool for differentiating various subepidermal autoimmune blistering diseases. Immune binding



**Figure 1:** Processing and staining skin biopsy for DIF.

on either the epidermal (roof) or dermal (floor) side provides crucial diagnostic insights. Both direct and indirect IF can be performed on salt-split skin, with the latter being more commonly used. In the indirect method, patient serum is incubated with control salt-split skin.

However, in the direct salt-split technique the patient's skin biopsy is incubated in 10–15 mL of 1 mol/L sodium chloride at 40°C for 72 hours, after which the epidermis is separated using fine forceps.<sup>24</sup>

## 2. DIF of hair:

Details of this technique have been outlined earlier in the section on “Hair” under “Other substrates for DIF”

### Slide storage

After staining, the slides should be stored between 2–8 °C in a refrigerator and should be kept in the dark. As the fluorescence fades in 10–14 days, the slides should be interpreted soon after staining. However, using permanent mounting media and antifade reagents, the DIF slides can be preserved for up to 30 days at 2–8°C.<sup>25,26</sup>

### Interpretation of DIF

Slides are evaluated qualitatively and semi-quantitatively on a 0–3+ or 0–4+ scale, as per the convenience of the

pathologist. A semi-quantitative assessment of intensity of staining is given as none (0), trace-mild (1+), moderate (2+), and strong (3+/4+). Photographic records are obtained for pertinent positive staining and case-specific positive staining.

DIF is interpreted based on the following points:

1. Type and intensity of immune deposits
2. Pattern of immune deposits (linear/granular/ band like)
3. Location of immune deposits (epidermis, BMZ, dermis, blood vessels, adnexal structures, etc.)
4. Clinical and histological correlation.

The DIF patterns in different conditions have been depicted in Table 2 and Figures 2–13.

Figure 14 illustrates the approach to subepidermal AIBDs based on DIF and salt split IIF findings.

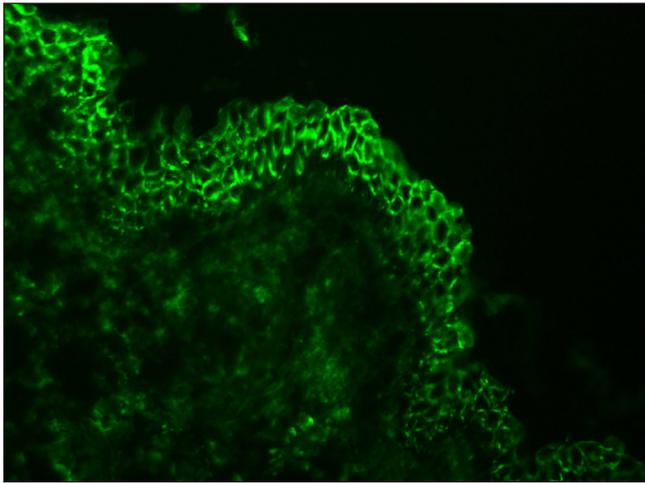
### Reliability of DIF

#### Sensitivity of DIF and causes of false negative results

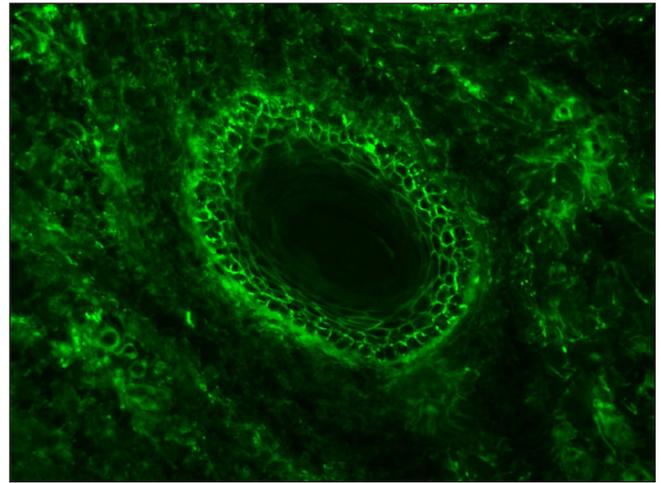
In general, DIF is highly sensitive for autoimmune bullous diseases (AIBDs) and cutaneous vasculitis (such as IgA vasculitis) (>90%). DIF is highly sensitive but has limited specificity in the pemphigus group of diseases, while it is very sensitive and specific (90–98%) in the pemphigoid group of disorders.<sup>11</sup> However, the sensitivity of DIF is low (27–41%) for the diagnosis of paraneoplastic pemphigus.

**Table 2: DIF staining pattern in different conditions**

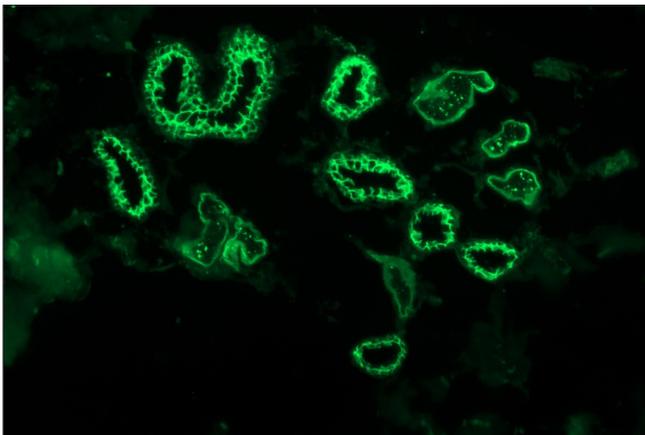
Disease entity	Antibody	Pattern of staining
Pemphigus vulgaris/ foliaceus/ vegetans	IgG and C3	Skin: Linear deposition along intercellular junction in the epidermis and adnexal structures (fishnet pattern) <sup>40</sup> Plucked anagen hair: fishnet pattern of immune deposits along the outer root sheath or hair bulb [Figure 2–5]
IgA pemphigus	IgA and C3	Linear deposition along intercellular junction in the epidermis and adnexal structures (fishnet pattern)
Paraneoplastic pemphigus	IgG and C3	Linear deposition along intercellular junction in the epidermis and adnexal structures (fishnet pattern) and/ or linear deposition along epidermal basement membrane zone [Figure 6] <sup>41</sup>
Pemphigus erythematosus	IgG and C3	Linear deposition along intercellular junction in the epidermis and adnexal structures (fishnet pattern) and granular deposition along epidermal basement membrane zone
Bullous pemphigoid/mucous membrane pemphigoid	IgG and C3	Linear deposition along epidermal/ epithelial basement membrane zone in 'n' serration pattern [Figures 7–8] <sup>35,36,37,42,43</sup>
Epidermolysis bullosa acquisita	IgG and C3 (+ IgA)	Linear deposition along epidermal/ epithelial basement membrane zone in 'u' serration pattern
Dermatitis herpetiformis	IgA + C3	Granular deposition along tip of papillary dermis [Figure 9] <sup>44-46</sup> Also, linear streaks (ie, fibrillar pattern) and IgA deposition within vessel walls, around hair follicles and along the BMZ of adnexal structures <sup>11</sup>
Linear IgA disease/ chronic bullous dermatosis of childhood	IgA + C3	Linear deposition along epidermal basement membrane zone [Figure 10]
Discoid lupus erythematosus/ systemic lupus erythematosus	IgG, IgA, IgM, C3, C1q (in various combination)	Granular deposit along epidermal basement membrane zone, adnexal structures, and dermal blood vessels [Figures 11 and 12]
IgA vasculitis	IgA + C3	Granular deposit along wall of the dermal capillaries [Figure 13] <sup>29,30</sup>
Porphyria and pseudoporphyria	IgG, IgM, C3, membrane attack complex	Homogeneous deposition along epidermal basement membrane zone and dermal blood vessels



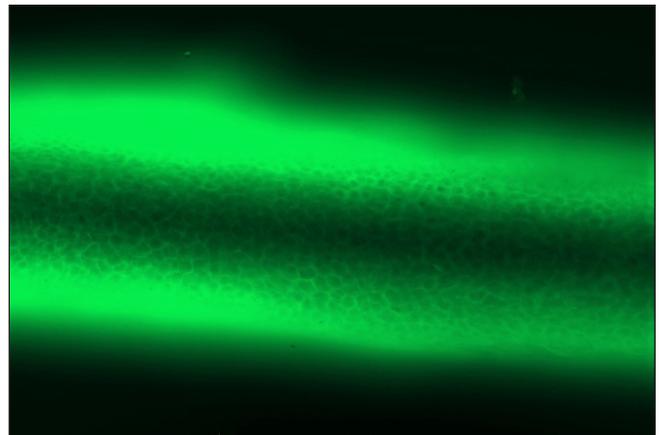
**Figure 2:** Pemphigus vulgaris showing linear IgG deposition in the epidermis along an intercellular junction giving 'fishnet' appearance (fluorescein isothiocyanate, 200x).



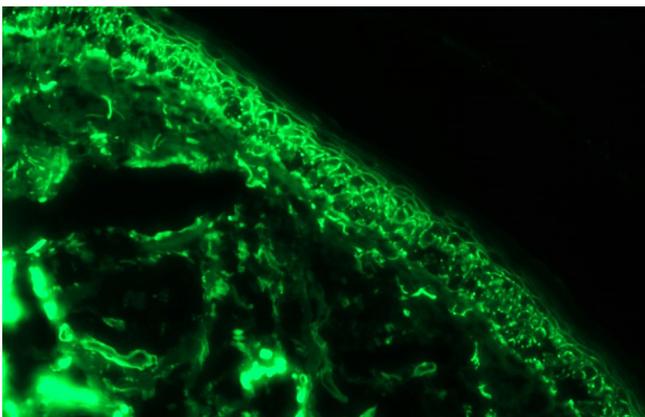
**Figure 3:** Pemphigus vulgaris showing linear IgG deposition in the hair follicle along an intercellular junction giving 'fishnet' appearance (fluorescein isothiocyanate, 200x).



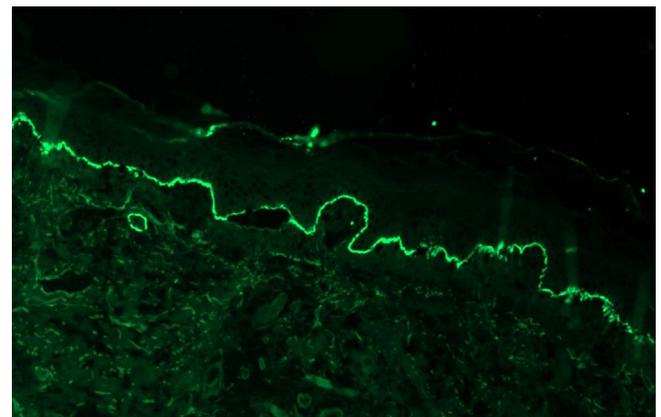
**Figure 4:** Pemphigus vulgaris showing linear IgG deposition in the eccrine ducts along an intercellular junction (fluorescein isothiocyanate, 200x).



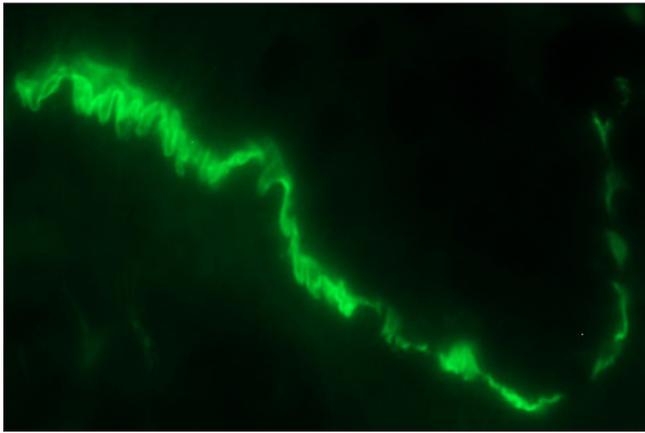
**Figure 5:** DIF on plucked anagen hair of pemphigus vulgaris patient showing linear IgG deposition along intercellular junction in the outer root sheath (fluorescein isothiocyanate, 200x).



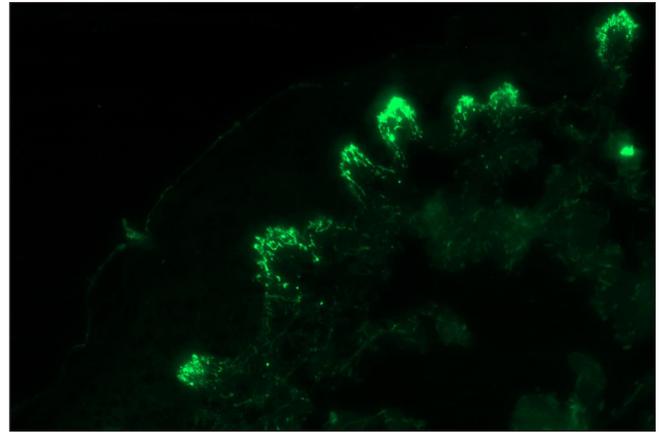
**Figure 6:** Paraneoplastic pemphigus showing linear IgG deposition in the epidermis along intercellular junction and linear deposition along dermo-epidermal junction (fluorescein isothiocyanate, 200x).



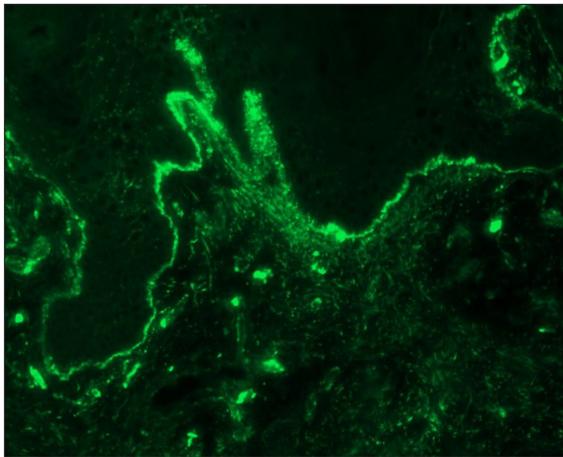
**Figure 7:** Bullous pemphigoid showing linear IgG deposition along dermo-epidermal junction (fluorescein isothiocyanate, 100x).



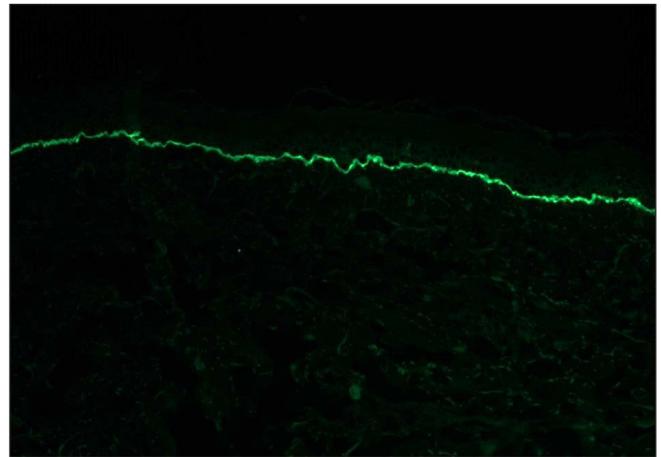
**Figure 8:** Bullous pemphigoid showing 'n' serration pattern of IgG deposition along dermo-epidermal junction (fluorescein isothiocyanate, oil immersion, 1000x).



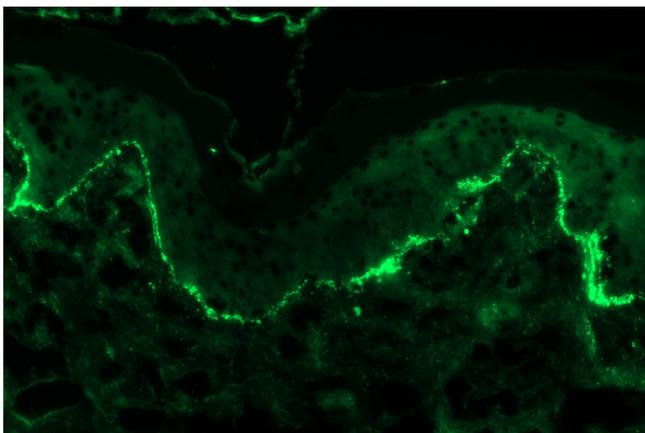
**Figure 9:** Granular IgA deposition along the tip of the papillary dermis in a case of dermatitis herpetiformis (fluorescein isothiocyanate, 400x).



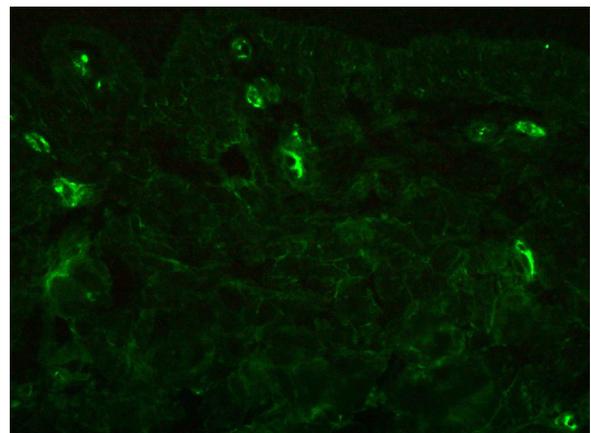
**Figure 10:** Linear IgA disease showing linear IgA deposition along dermo-epidermal junction (fluorescein isothiocyanate, 100x).



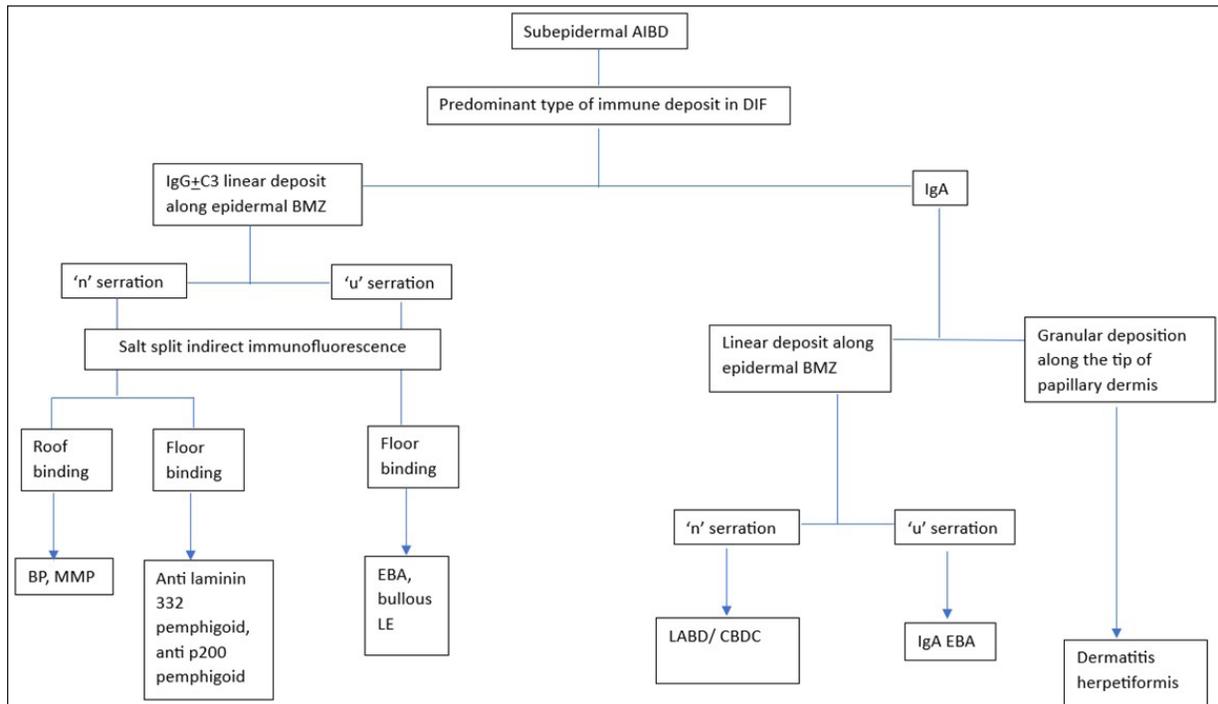
**Figure 11:** A case of discoid lupus erythematosus showing granular IgG deposition along dermo-epidermal junction and dermal capillaries (fluorescein isothiocyanate, 200x).



**Figure 12:** A case of discoid lupus erythematosus showing granular IgA deposition along dermo-epidermal junction (fluorescein isothiocyanate, 200x).



**Figure 13:** A case of IgA vasculitis showing granular IgA deposition along upper dermal capillaries (fluorescein isothiocyanate, 400x).



**Figure 14:** Flowchart showing diagnostic approach to subepidermal autoimmune blistering disorders based on direct immunofluorescence and salt split indirect immunofluorescence. (AIBD: Autoimmune blistering disorder, BP: Bullous pemphigoid, MMP: mucous membrane pemphigoid, EBA: epidermolysis bullosa acquisita, LE: lupus erythematosus, LABD: linear IgA bullous disorder, CBDC: chronic blistering disorder of childhood, BMZ: basement membrane zone, DIF: direct immunofluorescence).

The lupus band test has variable sensitivity and specificity in cutaneous lupus erythematosus. It is more sensitive (80–93%) in SLE as compared to DLE (40–60%), however, it has high specificity (80–90%) for acute cutaneous LE.<sup>27,28</sup> The sensitivity and specificity of DIF is almost similar for non-lesional sun protected as well as sun exposed areas in SLE.<sup>28</sup>

False negative results in DIF can result from:

#### Preanalytical Factors

1. Contamination with formalin
2. Delay in transporting biopsies, especially when sent in saline
3. Use of inadequate transport medium
4. Delay in obtaining biopsies (eg., DIF results are likely to be negative in IgA vasculitis if the sample is obtained more than 7 days after the onset of symptoms<sup>29,30</sup>)
5. Low antibody titres or prior treatment
6. Absence of epidermis or epithelium (common in biopsies taken from the buccal mucosa in autoimmune blistering diseases (AIBDs))
7. Lesional specimens in AIBDs where immunoreactants have already been consumed

#### Analytical Factors:

1. Incubation not done in moist chamber
2. Inadequate washing of sample (especially when it is transported using Michel's medium)

## Advantages and disadvantages of DIF

### The advantages of DIF include:

1. Enhanced intensity of illumination
2. Clearer and brighter image resolution
3. Easy to perform
4. Flexibility to utilise different antibodies for fluorescence
5. Signal amplification capabilities
6. Specific targeting ability
7. Diagnostic and analytical capacity (when clinical features or histopathology are inconclusive, a diagnosis can be solely based on DIF findings. The demonstration of immune complexes in skin biopsies at various locations such as intraepidermal, dermoepidermal junction (DEJ), and dermal blood vessels aids in diagnosis)<sup>31</sup>
8. Discrimination between immune-mediated bullous lesions and others
9. Definitive diagnosis in certain diseases, such as the fish-net pattern of pemphigus and IgA positivity in linear IgA dermatosis<sup>11</sup>
10. Facilitates monitoring of response to therapy and can predict relapse in patients in remission (in pemphigus, early positivity in DIF and increased complement deposition indicate relapse in patients)
11. Initial use of DIF on hair for patients in clinical remission to assess immunological status. If positive, DIF on skin may be avoided initially<sup>16,17,32</sup>

**Disadvantages of DIF:**

1. Expensive
2. Photobleaching: Fading occurs under intense illumination
3. Formation of protein cross-links in fixed tissue samples
4. Loss of tissue morphology and relevant antigens
5. Requires strict processing and optimal antibody usage determination
6. Immunoreactivity restoration may be necessary if not stored in darkness<sup>33</sup>

**Advances in DIF**

1. Newly recognized DIF patterns in pemphigus In recent studies examining DIF patterns in pemphigus, researchers have observed deposits of IgG4 using a single-step approach. These deposits appeared as small dots under 400x to 600x magnification, resembling ‘dew drops on a spider web,’ contrasting with the textbook ‘fishnet or chicken wire’ pattern of IgG deposition. They suggest that the spotted pattern seen in both extrafollicular and follicular epithelium could be a distinctive sign of pemphigus, given its regular appearance, and is unlikely to be caused by procedural errors or unrelated phenomena.<sup>34</sup>
2. Serration pattern analysis Serration pattern analysis is an easy, inexpensive, and accurate method for classifying subepidermal AIBDs that provides important diagnostic clues for subepidermal AIBDs. The linear IgG deposition along the dermo-epidermal junction can be categorised into ‘u’ and ‘n’ serration patterns when observed under high magnification (600x to 1000x). Conditions such as EBA and bullous lupus erythematosus typically exhibit a ‘u’ serration pattern, while most pemphigoid disorders (bullous pemphigoid, mucous membrane pemphigoid, anti-p-200 pemphigoid, and anti-laminin-332 pemphigoid) display an ‘n’ serration pattern.<sup>35-37</sup>

**Recommendations for DIF**

Reimann *et al.*<sup>38</sup> in 2021 published recommendations for the evaluation of DIF testing and evaluation as:

1. Limit the panel to IgG, IgA, and C3 in suspected immunobullous disease, and to IgA, C3, and fibrin in suspected vasculitis [Table 3]. The standard practice of reflexive antibody testing using a 6-antibody panel for all DIF biopsies is unnecessary. A DIF protocol tailored to the submitting diagnosis may enhance cost-effectiveness without compromising test sensitivity and specificity.
2. Where the clinical impression is broad or nonspecific (i.e., immunobullous disease), the addition of IgM and fibrin to the basic IgG, IgA, and C3 panel may be necessary.
3. Technicians should be trained to use DIF protocols based on the submitting diagnosis, including lists of

**Table 3: Recommendations for utility and evaluation of DIF in Cutaneous immune mediated disorders.**

Clinical possibility	Biopsy for DIF	Panel of immunoreactants
Immunobullous disorder	Recommended	IgG, IgA, C3
Vasculitis	Recommended, primarily for suspected Henoch-Schönlein purpura	IgA, C3, fibrin
Connective tissue disease	Not routinely recommended	IgA, C3, IgM, IgG
Lichen planus	Not routinely recommended	-
Lichen planus pemphigoides	Recommended	IgG, IgA, C3
Porphyria	Not routinely recommended	-

- specific clinical differential diagnoses for which a 3-antibody panel rather than a full panel is warranted.
4. DIF using non-lesional buccal mucosa was found to be superior to histological and serological tests for diagnosing mucous membrane pemphigoid. The procedure is technically easy and has high diagnostic value.<sup>39</sup>
5. Consider adopting a more cost-conscious and high-value approach to utilising DIF for clinical purposes, which both local and national educational societies should be incorporated into their curriculum planning.
6. Due to the observed lack of utility in diagnosing suspected porphyria, lichen planus (LP), or lupus erythematosus, DIF is not recommended for these conditions.
7. Accurate communication between dermatologists and dermatopathologists are essential.

**Conclusion**

Although DIF is an extremely useful diagnostic tool, it should always be used in conjunction with histopathology and clinical features for best results. DIF microscopy not only serves to confirm the diagnosis in AIBDs but also directs further immunological tests such as enzyme-linked immunosorbent assay (ELISA) and Western blotting. Although DIF can be challenging to perform, clinicians can maximise the yield by carefully selecting the site, employing proper technique, ensuring appropriate tissue handling, and coordinating effectively with dermatopathologists. Importantly, accurate interpretation of the results requires incorporating clinical and histological findings along with good clinico-pathological correlation.

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