Durability of direct immunofluorescence (DIF) slides stored at room temperature

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Background: Prior studies suggested that direct immunofluorescence (DIF) slides can be stored at room temperature.

Objectives: We sought to determine the durability of DIF slides stored at room temperature for 5 years.

Methods: This was a retrospective study of 83 DIF slides archived at room temperature during 2010. The pattern of immunoreactants was compared with those noted in the original report.

Results: Loss of reactivity was limited to cases with weak fluorescence at original diagnosis. Loss of IgG was noted in 12.5% of cases, IgA in 12%, C3 in 10%, and IgM in 9.75%. Fibrin showed no loss of reactivity. Preservation of immunofluorescence was not related to site of deposition. Overall, a reliable diagnosis could be made in 75 of 79 archived cases (94.9%).

Limitations: Cases had been archived for periods varying from 4.5 to 5 years. Variations in processing and fluorochromes could affect durability. We have no way of knowing how long slides had been exposed to ultraviolet light at the time of initial examination.

Conclusion: DIF showed excellent durability in slides kept at room temperature for 5 years. (J Am Acad Dermatol 2015;73:1021-4.)

Key words: direct immunofluorescence; room temperature; storing.

Direct immunofluorescence (DIF) staining of cutaneous specimens is a valuable tool in the diagnosis of immunobullous diseases, connective tissue disease, and vasculitis. In the past, many laboratories kept DIF slides refrigerated at 4°C for short-term preservation.[1] Grimwood and Proffer[2] reported the stability of DIF slides at room temperature for up to 30 months. Storing DIF slides at room temperature is more convenient and allows retrospective review of archived material for patient care, research, and teaching. We sought to determine the percentage of DIF slides that still demonstrated diagnostic findings after archiving at room temperature for approximately 5 years.

METHODS

A total of 83 positive DIF cases prepared in 2010 were retrieved from the files of Ackerman Academy of Dermatopathology. Each case was examined by 2 dermatopathologists for fluorescence of IgG, IgM, C3, IgA, and fibrin. The results were compared to original reports in regard to the number of positive immunoreactants, pattern of deposition, and the final diagnosis. Intensity of staining was defined on a scale from 1 to 4+, where a weak or focal staining was scored 1+ and strong continuous staining was scored 4+. This is standard practice in our laboratory.

The DIF slides were processed as follows: fresh skin specimens were placed in Michel transport

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medium (BBC Biochemical, Mt Vernon, WA). Within 48 hours, the specimens were processed for DIF staining for C3-fluorescein isothiocyanate (FITC), IgG-FITC, IgM-FITC, IgA-FITC, and fibrinogen-FITC (Ventana). Skin specimens were rinsed of transport medium using ChemWash (BBC Biochemical) for 5 minutes in a Petri dish. Specimens were then mounted for frozen section using OCT Compound (Sakura). Sections were cut at 5 μM and placed on charged slides. Staining was done on the Benchmark XT (Ventana). Slides were then rinsed in distilled water and coverslipped using an aqueous mounting media (Dako). After the initial reading, slides were stored at ambient temperature.

CAPSULE SUMMARY

- Data on long-term durability of direct immunofluorescence slides at room temperature are limited.
- We demonstrate that slides retain their immunofluorescent pattern up to 5 years.
- Storage at room temperature allows reviewing the archived material for patient care, research, and teaching.

RESULTS

In all, 83 DIF-positive cases were examined. The diagnoses included 29 cases of bullous pemphigoid, 1 case of herpes gestationis, 15 cases of lupus erythematosus, 12 cases of dermatitis herpetiformis, 8 cases of epidermolysis bullosa acquisita, 4 cases of linear IgA dermatosis, 4 cases of Henoch-Schonlein purpura, 3 cases of porphyria cutanea tarda, 2 cases of pemphigus vulgaris, 1 case of pemphigus foliaceous, and 4 cases with nonspecific positive immunofluorescence (Figs 1 to 4).

Preservation of immunoreactants by type

Positive IgG was initially reported in 64 cases. Of these, 8 cases lost reactivity (12.5%). IgM was reported in 41 cases with loss of reactivity in 4 cases (9.75%). C3 was reported in 76 cases, with loss in 5 cases (10%). IgA was reported in 50 cases, with loss in 6 cases (12%). Fibrin was reported in 72 cases with no loss of reactivity in any case. In all cases that showed loss of immunoreactants, the original reactants had demonstrated only 1+ staining intensity. All other immunoreactants retained positivity after storage.

Preservation of immunoreactants by site of deposition

All 3 cases with epidermal deposition showed preservation of the pattern enabling reliable diagnosis after being stored at room temperature for roughly 5 years. Dermoepidermal junction deposition was found in 61 cases, with preservation of the original pattern of immunoreactants in 58 (95%) cases. Of the 19 cases with dermal vasculature deposition, 18 (94.7%) showed preservation of the pattern of deposition. All cases with dermal deposition of fibrin showed good preservation of the original pattern. In short, the site of deposition did not appear to influence preservation of immunofluorescence.

Reliability of diagnosis

Details of the immunoreactants initially present in each case and the reliability of diagnosis in archived slides is summarized in Table I. Overall, a reliable diagnosis could be made in 75 of 79 archived cases (94.9%).

DISCUSSION

We found excellent preservation of immunofluorescent patterns after roughly 5 years of slide storage at room temperature. Loss of immunofluorescence

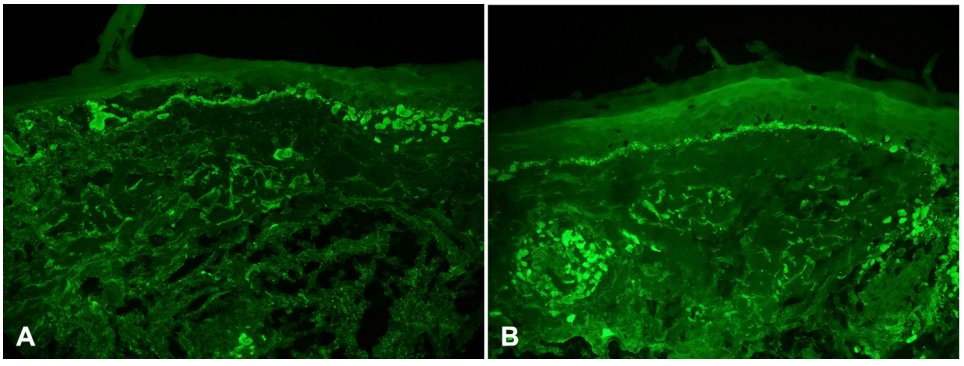

Fig 1. Lupus erythematosus demonstrating preserved pattern for diagnosis; granular deposition of IgM (**A**) and IgA (**B**). Slide prepared October 15, 2010. (Immunofluorescence; original magnification: ×100.)