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Cutaneous Immunofluorescence Testing

Immunofluorescence (IF) tests can be performed on sera or tissues obtained in the physician's office. The direct IF test is performed on skin or mucosal biopsy specimens. All biopsy specimens are examined for the presence of bound immunoglobulins (IgG, IgM, IgA), third component of complement (C3), and fibrinogen. The indirect IF test is performed on serum to detect the presence of circulating antibodies. IF testing is particularly useful for confirmation of the following: blistering diseases, connective tissue diseases, and vasculitis. IF tests may be diagnostic when dermatopathologic studies are only suggestive, nonspecific, or negative. The diagnostic value of direct and indirect IF is illustrated in the following chart "Results of IF Testing."

Results of IF Testing*

Disease	Direct IF	Indirect IF
Bullous lupus erythematosus	BMZ: linear/bandlike IgG and C3 (>90%); IgM and IgA also (50-60%); occasionally granular or fibrillar pattern	ANA*: rarely BMZ (IgG) antibodies on split-skin substrate (dermal or combined pattern)
Bullous pemphigoid	BMZ: linear IgG and C3; occasionally IgM and/or IgA	BMZ antibodies (IgG) in 75% of patients; application of BMZ positive serum on split-skin substrate results in staining of epidermal side of separated skin in most cases of BP as compared with the dermal side in EBA; dermal or combined pattern also seen rarely in bullous LE
Chronic bullous disease of childhood	BMZ: linear IgA	BMZ antibodies (IgA) in 70% of cases
Chronic cutaneous (discoid) lupus erythematosus	BMZ: granular IgM, IgG, C3 (involved skin >90%, uninvolved negative)	None (ANA* rarely)
Cicatricial pemphigoid	BMZ: linear IgG and C3; occasionally linear IgA	BMZ (IgG) antibodies in <25% of patients
Dermatitis herpetiformis	Dermal papillae: granular or fibrillar IgA, other Igs or C3 may be present	IgA class anti-endomysial antibodies in 70% of patients with DH or celiac disease; increased incidence of positive results in patients with gluten-sensitive enteropathy who are not following gluten-free diet
Drug reactions	Dermal vessels, cytoids: IgG and/or IgM, C3	None
Epidermolysis bullosa acquisita	BMZ: linear IgG, usually also C3; occasionally IgA and/or IgM	BMZ antibodies (IgG) in 25-50% of cases; may be distinguished from BP by dermal pattern on split-skin substrate
Erythema multiforme	Dermal vessels, cytoid bodies; IgM, C3; also IgG, rarely IgA	None
Herpes gestationis (pemphigoid)	BMZ: linear C3 (100%); IgG (10%); rarely IgA/IgM	HG IgG (HG factor) in most patients BMZ (IgG) in 25% of cases
Lichen planus	Cytoid bodies: IgM characteristically; also IgG, C3, fibrinogen; BMZ: shaggy fibrinogen	None
Lichen planus pemphigoides	Changes of lichen planus with linear IgG and/or C3	BMZ antibodies (IgG) in 50% (epidermal side of split-skin substrate)

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Results of IF Testing* *continued*

Disease	Direct IF	Indirect IF
Linear IgA bullous dermatosis	BMZ: linear IgA essential for diagnosis; linear C3 in some cases	BMZ antibodies (IgA) rarely present (30%)
Mixed connective tissue	Varies with clinical presentation; as in LE or vasculitis; ANA in epidermis	ANA*
Other pemphigoid diseases (desquamative gingivitis, Brunsting-Perry pemphigoid, localized pemphigoid)	BMZ: linear IgG, C3; sometimes IgA and/or IgM	BMZ antibodies (IgG) uncommonly present
Pemphigus (e.g., vulgaris, foliaceus, paraneoplastic)	ICS: IgG, C3 BMZ: granular to linear C3, characteristic of paraneoplastic	ICS antibodies (IgG) ICS antibodies bind to simple, columnar and transitional epithelia; BMZ: antibodies may be present, characteristic of paraneoplastic
Porphyria	Dermal vessels: IgG and IgM; also BMZ staining	None
Subacute cutaneous lupus erythematosus	Particulate intercellular substance IgG, IgM, IgA, C3, one or more conjugate 30%; BMZ: granular IgM or IgG (40% lesional, 10% normal)	ANA*
Systemic lupus erythematosus	BMZ: granular IgM and/or IgG, C3, sometimes IgA (>90% positive involved skin) (50% positive sun exposed, uninvolved) (30% positive unexposed, uninvolved)	ANA*
Urticaria	Patchy staining of connective tissue fibers in dermis with fibrinogen and variable number of eosinophils; dermal vessels (in cases of urticarial vasculitis) as noted below	None
Vasculitis (e.g., leukocytoclastic, Henoch-Schönlein purpura, rheumatoid, urticarial, granuloma faciale)	Dermal vessels: IgG and/or IgM and/or IgA and/or C3 in early lesions; IgA characteristic of Henoch-Schönlein purpura	None

KEY: * ANA, done in immunology laboratory
ANA, antinuclear antibodies
BMZ, basement membrane zone
BP, bullous pemphigoid
DH, dermatitis herpetiformis
EBA, epidermolysis bullosa acquisita
HG, *Herpes gestationis*
ICS, intercellular substance (cell surface)
LE, lupus erythematosus
SLE, systemic LE

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A. Selection of Biopsy Sites

1. Cutaneous immunofluorescence.

a. ***PEMPHIGUS*** and pemphigoid groups (including linear IgA bullous dermatosis and chronic bullous disease of childhood): Biopsy erythematous perilesional skin or mucosa. Avoid erosions, ulcers, and bullae while obtaining tissue adjacent to active lesions. Label as perilesional skin.

b. ***DERMATITIS HERPETIFORMIS:*** Biopsy normal appearing skin, 0.5–1.0 cm away from lesion. Label as perilesional skin.

c. ***LUPUS ERYTHEMATOSUS:*** Involved areas of skin such as erythematous or active borders are preferred biopsy sites to confirm diagnosis of lupus erythematosus, either discoid or systemic. Label as involved skin. Uninvolved, nonexposed skin is the preferred site to exclude systemic lupus erythematosus. Should unexposed skin be desired, buttock or medial thigh is suggested. Label as uninvolved, nonexposed skin. Avoid ulcers, old lesions, and facial lesions, if possible.

d. ***MIXED CONNECTIVE TISSUE DISEASE:*** Biopsy as for lupus erythematosus except when sclerodermoid features are present. For sclerodermoid features, biopsy inflamed area. Label as involved or uninvolved, exposed or nonexposed skin.

e. ***VASCULITIS AND URTICARIA:*** The erythematous or active border of a new lesion is preferred. Avoid old lesions and ulcers. Label as involved skin. If appropriate skin lesion is not present, diagnosis may sometimes be made from uninvolved skin.

f. ***PORPHYRIA CUTANEA TARDA:*** Biopsy involved skin. Avoid old lesions and ulcers. Label as involved skin.

g. ***LICHEN PLANUS:*** Biopsy involved skin. Avoid old lesions and ulcers. Label as involved skin.

2. Cutaneous leukocyte immunophenotype.

a. Biopsy involved skin.

1) This test is used to help differentiate benign from malignant lymphocytic infiltration of the skin as well as to help classify cutaneous lymphomas. For optimal interpretation, a formaldehyde-fixed H & E stained section is required. The test may be used in conjunction with molecular genetics studies for the most accurate assessment of malignancy. It may also be used to help differentiate CD1 positive from CD1 negative histiocytoses, such as X vs. non-X histiocytoses.

2) The test requires that the tissue be snap-frozen in liquid nitrogen and transported on dry ice. (Transportation of the tissue in transport medium is not adequate.) An accompanying permanent H & E section of the tissue is optimal.

B. Choice of Methods for Fixation and Transport of Biopsy Specimens

1. Cutaneous immunofluorescence.

Skin or mucosal specimens can be sent by using either the transport medium or the snap-frozen procedure. The practical value of using Zeus transport medium (Mayo Supply T321) is recognized for direct immunofluorescence testing. However, we have found a loss in sensitivity of approximately 10% with the transport medium as compared with snap-frozen tissue. This may necessitate a repeat biopsy if the result seems to be false-negative. The assay cannot be performed on specimens fixed in formalin.

2. Cutaneous leukocyte immunophenotype.

Skin or mucosal specimens must be sent using the snap-frozen procedure only. The assay cannot be performed on specimens fixed in transport medium or in formalin.

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C. Transport Medium Method for Cutaneous Immunofluorescence Specimens

Supplies and equipment needed — specimen vial containing Zeus medium (Mayo Supply T321), forceps, and biopsy instruments.

1. Use a sharp 4-mm punch. If biopsy specimen is to be divided, use at least a 5-mm punch. An excisional biopsy may be needed. In dividing the specimen, cut with a very sharp razor blade. Do not squeeze or twist the specimen. Make a clean cut. Specimens larger than 5 mm in diameter should be divided for adequate fixation in transport medium.
2. Immediately drop specimen into provided vial of transport medium (Supply T093). Label vial, including patient's name, identification number, biopsy site, and date. Seal tightly.
3. Complete Mayo Medical Laboratories' Dermatopathology/Immunodermatology Request Form (Supply T060). Interpretation of the results is facilitated by having available the following clinical data on the patient: age, sex, clinical diagnosis, biopsy site (anatomic), exposure of site to sun (exposed, unexposed), and relationship to lesional skin (perilesional, involved, uninvolved).
4. Mail in containers (Supply T326) supplied by Mayo Medical Laboratories. Do not mail vials filled with transport medium on dry ice.

D. Snap-Frozen Method for Cutaneous Leukocyte Immunophenotype or Cutaneous immunofluorescence specimens

Supplies and equipment needed — liquid nitrogen, dry ice, specimen vials labeled with control numbers, forceps, biopsy instruments, and aluminum foil (2 x 2 square inch).

1. Pre-label the plastic tube provided, including patient's name, identification number, biopsy site, and date. Be sure tape is securely attached to the plastic tube. Cool the tube.
2. Chill a 2 x 2 inch piece of aluminum foil on the dry ice or in liquid nitrogen.
3. Use a sharp 4-mm punch. If biopsy specimen is to be divided, use at least a 5-mm punch. An excisional biopsy may be needed. In dividing the specimen, cut with a very sharp razor blade. Do not squeeze or twist the specimen. Make a clean cut.
4. Immediately drop the tissue into liquid nitrogen and allow to freeze thoroughly (do not allow specimen to desiccate). If liquid nitrogen is not available, the specimen may be frozen by placing it on a small square of aluminum foil on a block of dry ice. The former method is preferred.
5. Immediately wrap specimen carefully in aluminum foil. At no time should the specimen be allowed to thaw. Wrap as you would a party favor or a piece of taffy candy.
6. Put the wrapped specimen into the prelabeled plastic vial and seal tightly.
7. Fill one of the large cubicle Styrofoam®containers (Supply T328) with dry ice. Put the specimen within the mass of dry ice and seal tightly.
8. Complete Mayo Medical Laboratories' Dermatopathology/Immunodermatology Request Form (Supply T060). Interpretation of the results is facilitated by having the following clinical data on the patient: age, sex, clinical diagnosis, biopsy site (anatomic), exposure of the site to sun (exposed, unexposed), and relationship to lesional skin (perilesional, involved, uninvolved).
9. Mail in containers (Supply T328) supplied by Mayo Medical Laboratories.