BULLOUS DISEASES: INDICATED DIRECT IMMUNOFLUORESCENT (DIF) AND SERUM STUDIES*

SKIN LESIONS

If pemphigoid or epidermolysis bullosa acquisita is suspected, take skin biopsy with ~2/3 normal skin and ~1/3 edge of lesion. For best DIF results, take second biopsy ~3mm from lesion.

If pemphigus is suspected, take skin biopsy with \sim 2/3 normal skin and \sim 1/3 lesion edge plus serum for best diagnostic results. Also do IgG4 DIF. For best results take 2nd biopsy as for pemphigoid.

If dermatitis herpetiformis is suspected, take normal skin \sim 3 mm from lesion for best results. Serum tests by IIF-IgA EmA and tTG ELISA increase sensitivity.

If porphyria or pseudoporphyria is suspected, take skin biopsy with ~2/3 normal skin and ~1/3 lesion edge for best DIF results.

If in doubt, take two biopsies – one perilesional, as for pemphigoid, and one normal, as for DH, for best results.

If eruptions with other non-disease specific immune deposits are suspected, including lichenoid, psoriasiform or factitious lesions, or related disorders, take biopsy as for porphyria for DIF and lesional biopsy for light microscopy.*

MUCOSAL LESIONS

If pemphigoid is suspected, take normal mucosa ~3 mm from lesion or Nikolsky sign. For best DIF results, take second biopsy 3-10mm from lesion.

If pemphigus or paraneoplastic pemphigus is suspected, take normal mucosa ~3 mm from lesion or Nikolsky sign **plus serum**. For best diagnostic results, take two biopsies as for pemphigoid.

If erosive lichen planus (LP) is suspected, take mucosal biopsy with $\sim 2/3$ normal mucosa and $\sim 1/3$ edge of lesion or of Nikolsky sign for best DIF results.

HEREDITARY EPIDERMOLYSIS BULLOSA (EB)

If hereditary EB needs to be classified or confirmed, take biopsy of **induced** lesion in normal skin.

CONNECTIVE TISSUE DISEASES: INDICATED DIRECT IMMUNOFLUORESCENT (DIF) AND SERUM STUDIES

If SLE is suspected, take biopsy of sun-exposed normal skin of forearm for DIF for LE band test and blood in red top tube for serum studies for ANA and tests for ARA criteria.

If DLE is suspected, take biopsy of lesion in sun-exposed area of 3 or more months duration for DIF and for light microscopy.* Non-sun-exposed areas are of little value.

If SCLE or Sjogren's syndrome is suspected, take sun-exposed skin lesion biopsy for DIF for in vivo ANA and blood in red top tube for serum tests for ANA, Ro (SS-A) and La (SS-B).

If scleroderma is suspected, take biopsy of sun-exposed skin for DIF and C+DIF studies and blood for serum studies (Profile E).

IMMUNE COMPLEX MEDIATED VASCULITIS: INDICATED DIRECT IMMUNOFLUORESCENT STUDIES*

If leukocytoclastic vasculitis is suspected, (or most other immune complex vasculitides), take biopsy for DIF either of a fresh lesion, less than 48 hours old, or of the edge of a lesion plus adjacent normal skin.

If Henoch Schoenlein purpura is suspected, take biopsy of a fresh lesion (less than 48 hours old).

If stasis dermatitis is suspected, take biopsy for DIF of edge of skin lesion plus adjacent normal skin.

MOLECULAR TESTING FOR T CELL NEOPLASMS

If T cell neoplasm is suspected, send (a) 3-5ml whole blood in EDTA, ACD or heparin tube, or (b) skin biopsy in RPMI media, or (c) 5 unstained skin section slides.

^{*} Light microscopic studies of lesional biopsy sites are indicated, in most cases of vesiculobullous, connective tissue disease, vascular disorders and T cell disorders.



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DATA ON SKIN AND MUCOSAL BIOPSIES

Interpretation of direct immunofluorescence (IF) of tissue specimens requires knowledge of the location of the biopsy relative to the clinical lesion. FOR ALL DIRECT IF SPECIMENS, PLEASE FILL OUT THIS PANEL. Biopsy of: ☐ skin ☐ oral mucosa ☐ conjunctiva ☐ other Specific location: ☐ Lesional site: ☐ Normal site: ☐ Perilesional site: For Lupus: □ sun exposed □ sun protected If applicable please draw the biopsy site as it relates to the lesion. 3mm **LESION NOT NEAR** or NIKOLSK A LESION SIGN See "Suggested biopsy sites" for major bullous and collagenvascular diseases on panels to the left. Fixative used: ☐ Beutner Labs ☐ Other: (Biopsies in formalin can NOT be used for DIF.) Clinical diagnoses: ICD-10 Code (Required): ___ Clinical findings: Therapy:_ ANA titer Anti Ro(SS-A) / La(SS-B): _____ ANA pattern____ **ARA CRITERIA FOR SLE** ☐ Malar rash ☐ Discoid rash ☐ Photosensitivity ☐ Oral or nasopharyngeal ulceration ☐ Arthritis ☐ Serositis (pleuritis or pericarditis) ☐ Renal disorder (proteinurea, casts) ☐ Neurologic disorder (psychosis or seizures) ☐ Hematologic disorder (hemolytic anemia, leukopenia, lymphopenia, thrombocytopenia) ☐ Immunologic disorder (anti-DNA, anti Sm, antiphospholipid antibodies, lupus anticoagulant, false positive

serologic test for syphilis with confirmation)

☐ Abnormal titer of antinuclear antibody (ANA)



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(Bx, Sr, H&E)

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TEST REQUEST FORM

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	COMPLETE ALL NT PER HIPAA RE	
Patient Last Name		
First Name		MI
Parent Name (if patient is a	a minor)	
Address		
City	State	Zip
DOB (M/D/Y)	Age:	Sex: ☐ M ☐ F
Date of Specimen		
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REQUEST KITS FREE		
☐ Biopsy Kits	☐ Serum Kits	☐ 4-Tube Kits

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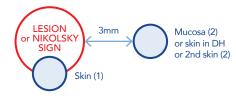
BULLOUS DISEASES

SKIN AND MUCOSAL BIOPSY STUDIES

- ☐ Direct IMMUNOFLUORESCENCE (DIF) for IgG, IgA, IgM, fibrin and C3. If needed, IgG4 and/or IgG1 are also added for greater sensitivity.
- ☐ Differentiation of bullous pemphigoid from epidermolysis bullosa acquisita by DIF of 1 M NaCl split biopsy (with no vesicles)
- ☐ LIGHT MICROSCOPY (H&E)
- ☐ LIGHT MICROSCOPY (H&E) CONSULT ONLY

Please fill in data on back of form.

BLISTERING AND OTHER ERUPTIONS



- (1) Skin biopsy in most pemphigus/pemphigoid cases.
- (2) Mucosal biopsy or skin biopsy for DH or 2nd skin biopsy for pemphigus or pemphigoid cases.

SERUM STUDIES

- ☐ Pemphigus/pemphigoid antibody titer on monkey esophagus by IgG & IgG4 indirect IF (Mk. esoph-IIF)
- ☐ Split skin, pemphigoid/epidermolysis bullosa acquisita (EBA) differentiation by IgG & IgG4 indirect IF (Split-IIF)
- ☐ Desmoglein (Dsg3 and Dsg1) ELISA for pemphigus antibodies
- ☐ BP230 (BPAG1) and BP180 (BPAG2) ELISA for pemphigoid antibodies
- ☐ Paraneoplastic pemphigus antibody titer (on rat bladder) (PPA-IIF)
- ☐ Pemphigus antibody titer prognostic test comparing old and new sera for IgG and IgG4 (Mk. esoph-IIF)
- ☐ Pemphigoid gestationis (HG) factor (HG-IIF)
- ☐ IgA anti endomysial antibodies (AEmA-IIF)
- ☐ IgA tissue transglutaminase antibodies (tTG-ELISA)
- ☐ IgA epidermal transglutaminase antibodies (eTG-ELISA)
- $\hfill \square$ Antibodies to collagen VII (ELISA) to rule out EBA

BASIC PEMPHIGUS - PEMPHIGOID SCREEN

SERUM PROFILES

☐ Basic P-P Screen

Mk. esoph. for IgG and IgG4 antibodies by indirect immunofluorescence (IIF).

Do other profiles if positive \Box Yes \Box No

PEMPHIGUS PROFILE

☐ Profile #1

Mk. esoph. for IgG and IgG4 antibodies by IIF and Dsg 3/1 ELISA.

PARANEOPLASTIC PEMPHIGUS

☐ Profile #2

Mk. esoph. for IgG, IgG1 & IgG4 antibodies by IIF plus IIF on rat bladder and Dsg3/1 ELISA and envoplakin ELISA.

PEMPHIGOID PROFILE

☐ Profile #3

Mk. esoph. and split skin for lgG & lgG4 antibodies by IIF and BP180 plus BP230 ELISA.

DERMATITIS HERPETIFORMIS AND CELIAC DISEASE

- ☐ IgA anti endomysial antibodies (AEmA-IIF)
- ☐ IgA tissue transglutaminase antibodies (tTG-ELISA)
- $\hfill \square$ IgA epidermal transglutaminase antibodies (eTG-ELISA)
- ☐ AEmA, tTG-ELISA and eTG-ELISA

HEREDITARY EPIDERMOLYSIS BULLOSA

- ☐ IF mapping of pencil eraser induced clefts in normal skin away from lesions for HEREDITARY EPIDERMOLYSIS BULLOSA (EB):
 - a) Primary screen for collagen type VII, collagen type IV, & keratin 14 to check for suitable biopsy
 - b) For suitable biopsies, test for collagen XVII, alpha6 integrin, beta4 integrin, laminin 332 and plectin to differentiate dystrophic EB and junctional EB from EB simplex
- ☐ LIGHT MICROSCOPY FOR HEREDITARY EB of an intact, spontaneous vesicle or a freshly induced perilesional cleft for diagnosis of HEREDITARY EB.

MOLECULAR STUDIES (SERUM/BIOPSY)

T CELL NEOPLASMS

- ☐ Gamma receptor rearrangement
- ☐ **Profile** (gamma and beta receptor rearrangement)

LUPUS ERYTHEMATOSUS, CONNECTIVE TISSUE & VASCULAR DISEASE

SKIN AND MUCOSAL BIOPSIES

- ☐ Direct IMMUNOFLUORESCENCE for SLE, DLE & SCLE with tests for IgG, IgA, IgM, fibrin and C3
- ☐ Direct IMMUNOFLUORESCENCE for dermatomyositis with tests for IqG, IqA, IqM, fibrin, C3 and C5b-9
- ☐ Direct IMMUNOFLUORESCENCE for vasculitis with tests for IqG, IqA, IqM, fibrin and C3
- ☐ Complement plus Direct IF (C+DIF) for scleroderma & others with human complement + anti-C3
- ☐ LIGHT MICROSCOPY (H&E) to rule out LE
- ☐ LIGHT MICROSCOPY (H&E) -CONSULT ONLY

COLLAGEN VASCULAR DISEASES





Please fill in data on back of form. See back of form for abbreviations.

- (3) Sun Exposed skin biopsy in most LE cases. Skin biopsy to rule out Henoch Schoenlein purpura and vasculitis (lesion less than 48 hours old)
- (4) Skin biopsy to rule out SLE

SERUM STUDIES*

- ☐ Antinuclear antibody (ANA) titers and patterns on HEp2 cells by standard ANA
- ☐ Antibodies to native (n) DNA
- ☐ Anticentromere antibodies (ACA)
- ☐ Stratified epithelium specific ANA (SES-ANA)/titer for Chronic Ulcerative Stomatitis
 - * All positives are titrated.

ELISA STUDIES: ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS, ETC.

- $\hfill \square$ Sm, RNP, Ro(SS-A) and La(SS-B) -SLE & Others
- ☐ Ro(SS-A) -SLE, SCLE, SjSy & Others
- ☐ La(SS-B) -SLE, SCLE, SjSy & Others
- ☐ Sm -SLE
- ☐ RNP -SLE, MCTD & Others
- ☐ Scl-70 -systemic sclerosis
- $\hfill \square$ Jo-1 -polymyositis & dermatomyositis
- ☐ Antibodies to histone
- ☐ Anti-beta2 glycoprotein I lgG

NOTE: SERUM STUDIES AND PROFILES REQUIRE A MINIMUM OF 2-5ml OF SERUM

SYSTEMIC CONNECTIVE TISSUE DISEASE SERUM STUDY PROFILES

BASIC PROFILE A

☐ Basic Profile A

ANA titers and patterns on HEp2 cells, antibodies to Ro(SS-A) and La(SS-B).

SYSTEMIC LE SCREEN

☐ Profile B

ANA titers and patterns on HEp2 cells, antibodies to nDNA, Ro(SS-A), La(SS-B), Sm and RNP.

SYSTEMIC CONNECTIVE TISSUE DISEASES SCREEN

☐ Profile C

ANA titers and patterns on HEp2 cells, antibodies to nDNA, Ro(SS-A), La(SS-B), Sm, RNP, Jo-1, Scl-70 and beta2 glycoprotein I IgG.

DRUG INDUCED LE/SLE SCREEN

☐ Profile D

ANA titers and patterns on HEp2 cells, antibodies to nDNA, Ro(SS-A), La(SS-B), Sm, RNP and histone.

SYSTEMIC SCLEROSIS SCREEN

☐ Profile E

ANA titers and patterns on HEp2 cells, Scl-70, Sm, RNP and ACA.



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