Client: Example Client ABC123
123 Test Drive

UNITED STATES

Physician: Doctor, Example

Patient: Patient, Example

DOB

Sex:

Patient Identifiers: 01234567890ABCD, 012345

Visit Number (FIN): 01234567890ABCD

Collection Date: 01/01/2017 12:34

Immunobullous Disease Antibody Panel

ARUP test code 3001409

EER Immunobullous Disease Panel

See Note

Authorized individuals can access the ARUP Enhanced Report using the following link:

https:

Immunobullous Disease Panel

See Note

CLINICAL INFORMATION
Blisters, erosions, crusts in patches on skin and mouth lesions.

Specimen Details
S22-IP0000519 - Serum; Collected: ; Received:

DIAGNOSTIC INTERPRETATION

Negative/normal IgG, including IgG4, and IgA basement membrane zone (pemphigoid, epidermolysis bullosa acquisita, linear IgA disease) antibodies, and

Negative/normal IgG, including IgG4, and IgA cell surface (pemphigus) antibodies;

No serum testing evidence for epithelial antibodies to provide support for the diagnoses of pemphigoid, epidermolysis bullosa acquisita, linear IgA disease, or pemphigus

(See Results and Comments)

RESULTS
Indirect Immunofluorescence (IIF)

Basement Membrane Zone (BMZ) IgG, IgG4, and IgA Antibodies

IgG: Negative, monkey esophagus substrate
Negative, human split skin substrate

IgG4: Negative, monkey esophagus substrate
Negative, human split skin substrate

IgA: Negative, monkey esophagus substrate
Negative, human split skin substrate

Reference Range:
Negative - Titer less than 1:10
Borderline - Titer 1:10

H=High, L=Low, *=Abnormal, C=Critical

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        Positive (H) - Titer greater than 1:10

      Localization Pattern on Human BMZ Split Skin:
        Epidermal (roof) or combined epidermal-dermal
        (roof and floor) IgG and/or IgG4 BMZ antibodies
        = pemphigoid (including pemphigoid gestationis,
        bullous pemphigoid, mucous membrane pemphigoid)

        Dermal (floor) IgG and/or IgG4 BMZ antibodies =
        epidermolysis bullosa acquisita or bullous lupus
        erythematosus or anti-laminin-332 pemphigoid or
        anti-p200 (laminin gamma-1) pemphigoid or another
        rare pemphigoid subtype

        Epidermal (roof), combined epidermal-dermal (roof
        and floor), or, dermal (floor) IgA BMZ antibodies =
        linear IgA disease (including linear IgA bullous
        dermatosis and chronic bullous disease of childhood)

     (H) = high/positive

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Cell Surface (CS)/Intercellular Substance (ICS) IgG, IgG4,
and IgA Antibodies

IgG:  Negative, monkey esophagus substrate
      Negative, intact human skin substrate

    IgG4:  Negative, monkey esophagus substrate
           Negative, intact human skin substrate

IgA:  Negative, monkey esophagus substrate
      Negative, intact human skin substrate

      Reference Range:
        Negative - Titer less than 1:10
        Borderline - Titer 1:10
        Positive (H) - Titer greater than 1:10

     (H) = high/positive

Enzyme-Linked Immunosorbent Assay (ELISA)
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Bullous Pemphigoid (BP)180 and BP230 IgG Antibodies

IgG BP180 antibody level:  5 U/mL

    Reference Range:
          Normal (negative) = Less than 9 U/mL
    Increased (H) (positive) = 9 U/mL and greater

IgG BP230 antibody level:  3 U/mL

    Reference Range:
          Normal (negative) = Less than 9 U/mL
    Increased (H) (positive) = 9 U/mL and greater

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Type VII Collagen IgG Antibodies

IgG type VII collagen antibody level:  1 U/mL

    Reference Range:
                Normal (negative) = Less than 7 U/mL
Slightly increased (H) (positive) = 7-8 U/mL
         Increased (H) (positive) = 9 U/mL and greater

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Desmoglein (DSG) 1 and 3 IgG Antibodies
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H=High, L=Low, *=Abnormal, C=Critical

IgG desmoglein 1 antibody level: 2 U/mL

Reference Range:
Normal (negative) = Less than 14 U/mL
Borderline/Indeterminate = 14-20 U/mL
Increased (H) (positive) = Greater than 20 U/mL

IgG desmoglein 3 antibody level: 3 U/mL

Reference Range:
Normal (negative) = Less than 9 U/mL
Borderline/Indeterminate = 9-20 U/mL
Increased (H) (positive) = Greater than 20 U/mL

COMMENTS

Specific

The negative IgG including IgG4 and IgA basement membrane zone antibodies by indirect immunofluorescence testing and the normal IgG BP180, IgG BP230, and IgG type VII collagen antibody levels by ELISAs are against, but do not rule out, the diagnoses of bullous pemphigoid, epidermolysis bullosa acquisita, and linear IgA bullous dermatosis. The results do not rule out the diagnosis of mucous membrane/cicatricial pemphigoid because patients with this pemphigoid subtype may not have detectable circulating basement membrane zone antibodies, although, when present, they can be diagnostically helpful.

The negative IgG, including IgG4, and IgA cell surface antibodies by indirect immunofluorescence testing are against, but do not rule out, the diagnoses of pemphigus vulgaris, pemphigus foliaceus, other types of IgG pemphigus, and IgA pemphigus. The normal ELISA results for IgG desmoglein 1 and IgG desmoglein 3 antibody levels also are against, but do not rule out, the diagnosis of active pemphigus foliaceus or pemphigus vulgaris.

Detection, levels, and patterns of diagnostic antibodies may fluctuate with disease manifestations. IgG4 subclass staining was performed because it may be more sensitive than IgG in some patients with immunobullous diseases but did not provide additional findings. Clinical correlation is needed, including with direct immunofluorescence findings on a biopsy specimen and treatment status. Monitoring serum antibody profiles by indirect immunofluorescence and antibody levels by ELISAs may aid in assessing disease expression and activity, particularly with persistent, progressive, or changing disease and in response to therapy.

General

Approximately 80 percent of patients with bullous pemphigoid, epidermolysis bullosa acquisita, and linear IgA bullous dermatosis have positive antibodies to basement membrane zone components in their sera detected by indirect immunofluorescence. Approximately 50 percent of patients with mucous membrane/cicatricial pemphigoid demonstrate antibodies to basement membrane zone components detected by indirect immunofluorescence. The immunoglobulin class of basement membrane zone antibodies and pattern of antibody localization on split skin substrate distinguish the diseases. IgG4 subclass reactivity by indirect immunofluorescence may be more sensitive than IgG in some patients with pemphigoid and epidermolysis bullosa acquisita.

Greater than 80 percent of patients with pemphigus have positive epithelial cell surface (CS) antibodies, also known as

intercellular substance (ICS) antibodies, in their sera identified by indirect immunofluorescence. IgG4 subclass reactivity by indirect immunofluorescence may be more sensitive than IgG in some patients with pemphigus. Serum antibody titers correlate with disease activity, and cell surface antibodies may be in low titer or negative in patients whose disease activity is minimal and/or under therapeutic control. Cell surface antibodies are implicated in the pathophysiology of pemphigus. IgG CS/ICS antibodies characteristically are positive by indirect immunofluorescence in IgG pemphigus variants, including pemphigus foliaceus and pemphigus vulgaris. IgA CS/ICS antibodies are positive by indirect immunofluorescence in patients with IgA pemphigus and in some pemphigus variants along with positive IgG CS/ICS antibodies.

Major molecular structures in the basement membrane zone to which IgG pemphigoid antibodies bind have been identified and termed "BP180" for a 180 kDa bullous pemphigoid antigen (also known as bullous pemphigoid antigen 2, BPAG2, or type XVII collagen, COL17) and "BP230" for a 230 kDa bullous pemphigoid antigen (also known as bullous pemphigoid antigen 1, BPAG1). BP180 is a transmembrane component of the basement membrane zone with collagen-like domains; the non-collagenous 16A (NC16A) antigenic domain of BP180 has been identified as a main antigenic target. BP230 is in the hemidesmosomal plaque of basal cells in the epidermis. Serum levels of IgG BP180 and IgG BP230 antibodies are determined by enzyme-linked immunosorbent assays (ELISA), and serum levels of IgG BP180 antibodies may correlate with disease activity in pemphigoid, diminishing with treatment response. Up to 7 percent of individuals who do not have pemphigoid, including patients with other immunobullous diseases, have increased levels of IgG BP180 and/or BP230 antibodies by ELISAs. Patients with pemphigoid may show reactivity to multiple basement membrane zone components in addition to or other than the BP180 and BP230 epitopes expressed in these ELISAs.

Type VII collagen is a component of anchoring fibrils within epithelial basement membrane zone (skin and mucous membranes), and patients with epidermolysis bullosa acquisita characteristically develop IgG antibodies to type VII collagen. An increased serum IgG type VII collagen antibody level by ELISA provides support for the diagnosis of epidermolysis bullosa acquisita and also a subset of bullous lupus erythematosus together with dermal localization (floor) of IgG basement membrane zone antibodies on split skin substrate by indirect immunofluorescence. Patients with inflammatory bowel disease, including Crohn disease and ulcerative colitis, with and without mucocutaneous manifestations of epidermolysis bullosa acquisita, may demonstrate increased levels of antibodies to type VII collagen. The major epitopes for antibody reactivity reside in the non-collagenous amino-terminal domain, NC1, with minor epitopes in the non-collagenous carboxy-terminal domain, NC2, of the three identical alpha chains that comprise type VII collagen. The tested ELISA contains combined purified recombinant antigens from both NC1 and NC2 for detection of IgG antibodies. Serum antibody levels above the reference range threshold of 6 U/mL may correlate with disease activity. Patients with epidermolysis bullosa acquisita or bullous lupus erythematosus may develop antibodies to basement membrane zone antigens in addition to or other than the type VII collagen epitopes displayed in this ELISA, and patients with other epithelial antibody-associated disorders may develop overlapping basement membrane zone antibody expression with an increased level of IgG type VII collagen antibodies.

Pathogenic antibodies in serum from patients with pemphigus bind to desmogleins, calcium-dependent adhesion molecules in cell surface desmosomes; such antibodies are detected by ELISAs. Specific reactivity to the type of desmoglein may be helpful in

determining pemphigus subtypes; the IgG desmoglein 1 antibody level is increased in patients with pemphigus foliaceus, and the IgG desmoglein 3 antibody level, with or without an increased IgG desmoglein 1 antibody level, is predominantly increased in patients with pemphigus vulgaris. Overlapping expression with antibodies to both desmogleins 1 and 3 clinically is associated with both mucosal and skin lesions. ELISA testing for IgG desmoglein 1 and IgG desmoglein 3 antibodies is highly sensitive, with greater than 90 percent of patients with pemphigus showing increased levels of one or both antibodies, and IgG desmoglein antibody levels correlate with disease activity. However, patients with cell surface antibody-positive pemphigus by indirect immunofluorescence can have normal results on ELISA testing with cell surface antibodies to different desmoglein 1 and/or desmoglein 3 epitopes than displayed in tested ELISAs or to other adhesion molecules.

Basement membrane zone and cell surface antibodies characteristically develop together in paraneoplastic pemphigus. Mixed antibody profiles, generally, may be found in: concurrent disease presentations with co-dominant autoantibody expression; incidental cross-over antibodies with dominant features of one immunobullous disease; autoimmune diseases in patients who are multiple autoantibody producers; drug reactions; as a spurious result from nonspecific reactivity/interference in an assay; nonspecific expression of one or more of the antibodies; as well as associated with paraneoplastic conditions/malignancy in addition to or other than paraneoplastic pemphigus.

TESTING METHODS
Indirect Immunofluorescence (IIF)

IgG, IgG4, and IgA Epithelial Basement Membrane Zone (BMZ) and Cell Surface (CS)/Intercellular Substance (ICS) Antibodies

Patient serum is progressively diluted beginning at 1:5 in four two-fold screening dilutions, layered on sections of human skin split at the basement membrane zone, intact human skin and monkey esophagus substrates, and reacted with fluorescein isothiocyanate (FITC)-conjugated antibodies to IgG and IgA. When positive, the serum is further diluted in two-fold reductions to the limiting dilution of antibody detection or to a maximum dilution of 1:40,960. The limiting-dilution, end-point titer is reported for each substrate, and the pattern of staining on split skin substrate also is reported. FITC-conjugated anti-IgG4 is tested to increase test sensitivity (maximum serum dilution of 1:40). This indirect immunofluorescence testing was developed and its performance characteristics determined by the Immunodermatology Laboratory at the University of Utah. It has not been cleared or approved by the FDA (US Food and Drug Administration). FDA clearance or approval currently is not required for this testing performed in a CLIA-certified laboratory (Clinical Laboratory Improvement Amendments) and intended for clinical use. [Indirect immunofluorescence, three antibodies on three substrates (IIF X 9)]

Enzyme-Linked Immunosorbent Assay (ELISA)

IgG BP180 and IgG BP230 serum antibody levels determined by U.S. Food and Drug Administration (FDA)-approved ELISAs (Mesacup, MBL BION). [Two ELISAs]

IgG type VII collagen serum antibody level determined by ELISA (Mesacup, MBL International). The performance characteristics of this ELISA testing were determined by the Immunodermatology Laboratory at the University of Utah. The testing has not been cleared or approved by the FDA (US Food and Drug Administration). FDA clearance or approval currently is not required for this testing performed in a CLIA-certified

laboratory (Clinical Laboratory Improvement Amendments) and intended for clinical use. [One ELISA]

IgG desmoglein 1 and IgG desmoglein 3 serum antibody levels determined by U.S. Food and Drug Administration (FDA)-approved ELISAs (Mesacup, MBL BION). [Two ELISAs]

Electronically signed by , MD, on at .
Performed At:

Medical Director: , MD
CLIA Number:

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
EER Immunobullous Disease Panel	22-173-115097			
Immunobullous Disease Panel	22-173-115097			

END OF CHART

H=High, L=Low, *=Abnormal, C=Critical