Immunobullous Disease Antibody Panel

ARUP LABORATORIES

Patient: [redacted]
DOB: [redacted] Age: [redacted] Sex: [redacted]
Patient Identifiers: [redacted]

Visit Number (FIN): [redacted]

Client: ARUP Example Report Only
500 Chipeta Way
Salt Lake City, UT 84108

Physician: [redacted]

ARUP Test Code: 3001409

Collection Date: 01/22/2024
Received in lab: 01/01/1900
Completion Date: 01/25/2024

Immunodermatology Serum Test Report Navigation Guide

The Immunodermatology TESTING REPORT from the University of Utah follows "See Note" and is arranged as outlined below on the following pages:

CLINICAL INFORMATION
This content is provided by the ordering clinician and includes the reason for testing.

Specimen Details
This includes specimen identification with collected and received dates.

DIAGNOSTIC INTERPRETATION
This is a synopsis of key findings from the testing and their diagnostic relevance.

RESULTS
This section reports the discrete finding and value of each test component, along with the reference range.

COMMENTS
Specific

These comments provide an explanation of the test results as they relate to clinical considerations, and include reference to any concurrent and/or previous testing.

General

These comments summarize fundamental information about the test(s) and the component(s) assessed to aid in interpretation of their clinical applicability.

TESTING METHODS
The section lists the procedures performed, the test source(s), and the applicable laboratory developed test disclaimer(s).

TEST RESULTS SUMMARY CHART
A chart tabulating results of tests ordered for the patient by the same client is included if previous and/or concurrent testing has been performed.

ELISA RESULTS GRAPH
A graph of ELISA results also is included if previous and/or concurrent testing has been performed; the graph may be found on a subsequent page.

For testing algorithm and additional information, refer to:
arupconsult.com/content/immunobullous-skin-diseases-screening

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IMMUNODERMATOLOGY LABORATORY REPORT

[redacted]

Submitter

ARUP Sendouts

Immunobullous Disease Antibody Panel (Final result)

TESTING REPORT follows "See Note"
See Note

CLINICAL INFORMATION
Widespread blisters and erosions, urticarial lesions, mucosal involvement. Presumptive diagnosis is immunobullous disease.

Specimen Details
[redacted] - ; Collected: 1/22/2024; Received: 1/22/2024

DIAGNOSTIC INTERPRETATION

Consistent with pemphigoid:

- Positive IgG, including IgG4, basement membrane zone antibodies, epidermal (roof) localization with split skin substrate (salt split skin), and negative IgA basement membrane zone antibodies by indirect immunofluorescence;
- Increased IgG BP180 and IgG BP230 antibody levels and normal IgG type VII collagen antibody level by ELISAs;

- Negative/normal IgG, including IgG4, and IgA cell surface/intercellular substance (pemphigus) antibodies

(See Results and Comments)

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Page: 1 of 8

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RESULTS
Indirect Immunofluorescence (IIF)

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

IgG: Positive, titer greater than 1:40,960 (H), monkey esophagus substrate
Positive, epidermal pattern (roof), titer 1:40,960 (H), human split skin substrate

IgG4: Positive, titer greater than 1:40 (H), monkey esophagus substrate
Positive, epidermal pattern (roof), titer greater than 1:40 (H), 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
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, some types of 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

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

IgG: Negative, monkey esophagus substrate

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        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)
  ------------------------------------------------------------
  Bullous Pemphigoid (BP)180 and BP230 IgG Antibodies

  IgG BP180 antibody level:  14 U/mL (H)

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

  IgG BP230 antibody level:  83 U/mL (H)

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

     (H) = high/positive
      U = antibody level in ELISA units

  ------------------------------------------------------------
  Type VII Collagen IgG Antibodies

  IgG type VII collagen antibody level:  2 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

     (H) = high/positive
      U = antibody level in ELISA units

  ------------------------------------------------------------
  Desmoglein (DSG) 1 and 3 IgG Antibodies

  IgG desmoglein 1 antibody level:  0 U/mL
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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: 2 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

(H) = high/positive
U = antibody level in ELISA units

COMMENTS

Specific

The findings in this testing, demonstrating positive IgG, including IgG4, basement membrane zone antibody reactivity with monkey esophagus substrate and epidermal localization (roof) with human split skin substrate (also known as salt split skin) by indirect immunofluorescence and increased IgG BP180 and IgG BP230 antibody levels by ELISAs, support the diagnosis of pemphigoid. The IgG type VII collagen antibody level is normal by ELISA, and, together with the lack of dermal localization (floor) of IgG basement membrane zone antibody reactivity on split skin substrate by indirect immunofluorescence, is against the diagnosis of epidermolysis bullosa acquisita. Although IgA basement membrane zone antibodies can be co-expressed with IgG antibodies, no positive IgA basement membrane zone antibody reactivity is detected by indirect immunofluorescence to indicate this or to support a diagnosis of linear IgA disease.

The negative IgG, including IgG4, and IgA cell surface (CS)/intercellular substance (ICS) antibody reactivity by indirect immunofluorescence is against, but does not rule out, the diagnoses of pemphigus vulgaris, pemphigus foliaceus, other IgG pemphigus variants, and IgA pemphigus. The normal IgG desmoglein 1 and IgG desmoglein 3 antibody levels by ELISAs 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, and IgG BP180 antibody levels correlate with disease activity in some patients with pemphigoid. Clinical correlation is needed, including with direct immunofluorescence findings on a biopsy specimen and treatment status. Monitoring antibody profiles by indirect immunofluorescence and antibody levels by ELISAs may be useful in assessing disease activity and expression, including response to therapy.

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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. IgG4 subclass reactivity by indirect immunofluorescence may be more sensitive than IgG in some patients with pemphigoid and epidermolysis bullosa acquisita. 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. Positive serum IgA epithelial basement membrane zone antibodies are highly specific diagnostic markers for linear IgA disease. IgA basement membrane zone antibodies by indirect immunofluorescence may be found in variant presentations of mucous membrane pemphigoid and epidermolysis bullosa acquisita. Moreover, IgA basement membrane zone antibodies may be co-expressed with IgG basement membrane zone antibodies in some patients with pemphigoid including mucous membrane/cicatricial pemphigoid and in linear IgA/IgG bullous dermatosis.

More 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 CS/ICS antibodies may be in low titer or negative in patients whose disease activity is minimal and/or under therapeutic control. CS/ICS antibodies are implicated in the pathophysiology of pemphigus. CS/ICS antibodies are typically not detected in normal individuals or in patients with other immunobullous diseases, although cell surface reactivity may be observed transiently and/or nonspecifically in normal individuals and in patients with drug reactions, infections, and other mucocutaneous diseases. 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. Approximately 40 percent of patients with nonclassical IgG/IgA pemphigus have an underlying systemic disease when diagnosed, malignancy being the most common.

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 located in the hemidesmosomal plaque of basal cells in the epidermis. Serum levels of IgG BP180 and IgG BP230 antibodies are determined by ELISA, which may be more sensitive than indirect immunofluorescence. 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 in the tested 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. ELISA testing for IgG type VII collagen antibodies may be more sensitive than indirect immunofluorescence. 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 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.

Tests that detect antibodies with specificity for laminin-332, p200 (laminin gamma-1), and alpha6beta4 integrin may be more sensitive than indirect immunofluorescence but are not currently available to aid in the diagnosis of pemphigoid subtypes except testing for IgG laminin-332 antibodies in select laboratories. Mucous membrane involvement is predominant in anti-laminin-332 pemphigoid. Recognition of the association of this pemphigoid variant with underlying or developing malignancy (typically solid tumor) in up to one third of cases is critical so appropriate clinical evaluation is conducted. Patients with anti-p200 (laminin gamma-1) pemphigoid tend to be younger than those with bullous pemphigoid and have lesions that clinically resemble both bullous pemphigoid and the inflammatory epidermolysis bullosa acquisita

variant that may include mucosal involvement. For those patients with antibodies to alpha6beta4 integrin, alpha6 epitopes primarily are targeted in oral pemphigoid, and beta4 epitopes primarily are targeted in ocular pemphigoid.

Pathogenic antibodies in serum from individuals with pemphigus bind to desmogleins, which are calcium-dependent adhesion molecules in epithelial desmosomes; such antibodies are detected by ELISA. Specific reactivity to the type of desmoglein may be helpful in determining pemphigus subtypes; IgG desmoglein 1 autoantibodies predominate in patients with pemphigus foliaceus, and IgG desmoglein 3 autoantibodies, with or without accompanying desmoglein 1 autoantibodies, predominate in patients with pemphigus vulgaris. Autoantibody expression to both desmogleins 1 and 3 is associated with both skin and mucosal lesions, often with clinical features of pemphigus foliaceus and pemphigus vulgaris. ELISA testing for IgG desmoglein 1 and IgG desmoglein 3 antibodies is highly sensitive, with greater than 90 percent of patients with IgG-variant pemphigus showing increased levels of one or both antibodies. IgG desmoglein antibody levels also correlate with disease activity in pemphigus foliaceus and pemphigus vulgaris; however, patients with cell surface/intercellular substance antibody-positive pemphigus by indirect immunofluorescence can have normal results on ELISA testing with antibodies to different desmoglein 1 and/or desmoglein 3 epitopes than in the ELISAs or to other desmosomal adhesion molecules.

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

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-

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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) with two limiting dilution, end-point titers (antibody titer X 2)]

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]

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Resulting Laboratory

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