Components of this Immunobullous Disease Antibody Screening Panel are IgG and IgA epithelial basement membrane zone (BMZ) and cell surface (CS) antibodies by indirect immunofluorescence (IFA) on basement membrane zone split human skin, intact human skin, and monkey esophagus substrates, and IgG BP 180, IgG BP 230, IgG Type VII Collagen, IgG Desmoglein 1, and IgG Desmoglein 3 antibodies by ELISAs. See Compliance Statement A: www.aruplab.com/CS.

BMZ IgG and IgA antibodies - serum is progressively diluted beginning at 1:5 in three two-fold dilutions for IFA screening. Each dilution is layered on sections of human skin split at the basement membrane zone and monkey esophagus substrates, and stained with fluorescein-conjugated anti-IgG and anti-IgA using Analyte Specific Reagents (ASRs). Positive antibody-containing serum is retested and titrated by two-fold dilutions to the end point of antibody detection by IFA.

CS IgG and IgA antibodies - serum is progressively diluted in calcium-containing buffer beginning at 1:10 in three two-fold dilutions for IFA screening. Each dilution is layered on sections of intact normal human skin and monkey esophagus substrates, and stained with fluorescein-conjugated anti-IgG and anti-IgA using Analyte Specific Reagents (ASRs). Positive antibody-containing serum is retested and titrated by two-fold dilution to the end point of antibody detection by IFA.

BP 180, BP 230, and Type VII Collagen IgG serum antibodies – IgG BP 180 and IgG BP 230 antibody levels are determined by FDA-approved enzyme-linked immunosorbent assays (ELISA). (MESACUP BP180 ELISA Kit and MESACUP BP230 ELISA Kit, MBL BION); IgG Type VII Collagen serum antibody level is determined by ELISA (Anti-Type VII Collagen ELISA, MBL International) developed and performance characteristics determined by the Immunodermatology Laboratory at the University of Utah.

Desmoglein 1 and desmoglein 3 IgG serum antibodies – IgG desmoglein 1 and IgG desmoglein 3 antibody levels determined by FDA-approved ELISAs. (DSG 1 & DSG 3 ELISA Test System, MBL BION).

Use
Aid in the diagnosis of pemphigoid, epidermolysis bullosa acquisita, linear IgA disease (including linear IgA bullous dermatosis and chronic bullous disease of childhood), bullous lupus erythematosus, pemphigus foliaceus, pemphigus vulgaris, other IgG-variant pemphigus, IgA pemphigus variants (including subcorneal pustular dermatosis type and the intraepidermal neutrophilic type), and mixed immunobullous disease, especially with overlapping clinical features.

Considerations
Recommend use of this panel along with Endomysial IgA antibody test to initially diagnose and distinguish various immunobullous disorders in patients suspected or known to have any type of immunobullous disease.

Recommend perilesional skin biopsy in Michel's or Zeus' medium for cutaneous direct immunofluorescence.

For antibody monitoring after initial testing:
Use Pemphigoid Antibody Panel or, in some patients, IgG Bullous Pemphigoid Antigens (180 kDa and 230 kDa) antibody testing to monitor pemphigoid disease activity and response to therapy; Use Epithelial Basement Membrane Zone Antibody IgG or, in some patients, IgG Type VII Collagen antibody levels, to monitor epidermolysis bullosa acquisita or bullous lupus erythematosus disease activity; Use Pemphigus Antibody Panel IgG or the IgG desmoglein 1 and desmoglein 3 assay testing to monitor pemphigus vulgaris and pemphigus foliaceus disease activity and therapeutic response. Use separate IgA Pemphigus Antibody test to monitor disease activity in IgA pemphigus.

Repeat Immunobullous Disease Antibody Screening Panel for indeterminate results and/or continuing clinical consideration of persisting and/or worsening unexplained disease activity.

Use Paraneoplastic Pemphigus Antibody Screen for patients who may have paraneoplastic pemphigus.

For testing algorithm and additional information about immunobullous skin diseases, refer to: arupconsult.com/Topics/ImmunobullousSkinDz.
IMMUNODERMATOLOGY REPORT

Patient: [Redacted]
Medical Record Number: [Redacted]
Gender: F
DOB: [Redacted]
Age: [Redacted]
Physician(s): [Redacted]
Clinic Location: [Redacted]

Accession number: [Redacted]
Procurement Date: 5/7/2019
Received Date: 5/9/2019
Phone: [Redacted]
Fax: [Redacted]

Specimen(s):
1. Serum specimen

Clinical/Diagnostic Information:
No clinical information provided.

DIAGNOSTIC INTERPRETATION

Negative IgG 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

(See Results and Comments)

RESULTS
Indirect Immunofluorescence

Base Membrane Zone (BMZ) IgG and IgA Antibodies

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

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

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

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TRIAL MODE - Click here for more information
IMMUNODERMATOLOGY REPORT

Patient: [Redacted]  
Accession number: [Redacted]

MRN:

Pattern on Human BMZ Split Skin:
- IgG epidermal or epidermal-dermal combined BMZ antibody pattern = pemphigoid
- IgG dermal BMZ antibody pattern = epidermolysis bullosa acquisita
- IgA epidermal, epidermal-dermal combined, or dermal BMZ antibody pattern = linear IgA bullous dermatosis

Cell Surface IgG and IgA Antibodies

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

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

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

(\text{H} = \text{high/positive})

Enzyme Linked Immunosorbent Assay (ELISA)

Bullous Pemphigoid (BP) 180 and 230 IgG Antibodies

IgG BP 180 antibodies: 1 unit

Reference Range:
- Positive (\text{H}) = Greater than or equal to 9 units
- Negative = Less than 9 units

IgG BP 230 antibodies: 2 units

Reference Range:
- Positive (\text{H}) = Greater than or equal to 9 units
- Negative = Less than 9 units

Collagen VII IgG Antibodies

IgG Collagen VII antibodies: 2 units

Reference Range:
- Positive (\text{H}) = Greater than or equal to 9 units
- Slightly increased, positive (\text{H}) = 7-8 units
- Normal/negative = 0-6 units

Desmoglein (DSG) 1 and 3 IgG Antibodies

IgG desmoglein 1 antibodies: 3 units

Reference Range:

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Patient: [Redacted]  Accession number: [Redacted]

MRN:

Positive (H) = Greater than 20 units
Borderline/indeterminate = 14-20 units
Negative = Less than 14 units

IgG desmoglein 3 antibodies: 5 units

Reference Range:
Positive (H) = Greater than 20 units
Borderline/indeterminate = 9-20 units
Negative = Less than 9 units

(H = high/increased; units = units/mL serum)

COMMENTS

Specific

The negative IgG and IgA basement membrane zone antibodies by indirect immunofluorescence testing and normal IgG BP 180, IgG BP 230, 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 a large proportion of patients with this pemphigoid subtype do not have detectable circulating basement membrane zone antibodies.

The negative IgG 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 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. Clinical correlation is needed, including correlation with direct immunofluorescence findings on a biopsy specimen and/or treatment status, with consideration for monitoring serum antibody profiles and levels to aid in assessing disease expression and activity, particularly with persistent, progressive, or changing disease.

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. Approximately 20 percent of patients with mucous membrane pemphigoid, including cicatricial pemphigoid, demonstrate antibodies to basement membrane zone components. The pattern of staining

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Patient: [redacted] | Accession number: [redacted]

on split skin substrate specifies disease.

Major molecular structures in the basement membrane zone to which IgG pemphigoid antibodies bind have been identified and termed "BP 180" for a 180 kDa bullous pemphigoid antigen and "BP 230" for a 230 kDa bullous pemphigoid antigen. BP 180 is a transmembrane component of the basement membrane zone with collagen-like domains and is a principal antigenic target in mucous membrane pemphigoid. BP 230 is located in the hemidesmosomal plaque of basal cells in the epidermis. Serum levels of IgG BP 180 and IgG BP 230 antibodies are in the negative range in normal individuals, and serum levels of IgG BP 180 antibodies may correlate with disease activity in pemphigoid, diminishing with treatment response. Patients with pemphigoid may show reactivity to multiple basement membrane zone components in addition to or other than the BP 180 and BP 230 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 collagen VII. Patients with inflammatory bowel disease, including Crohn's disease and ulcerative colitis, with and without mucocutaneous manifestations of epidermolysis bullosa acquisita and patients with bullous lupus erythematosus also may develop antibodies to collagen VII. The major epitopes for epidermolysis bullosa acquisita 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 collagen VII. This ELISA contains combined purified recombinant antigens from both NC1 and NC2 for detection of IgG antibodies in serum. The reference range for this assay indicates a threshold level at 6 units/mL, and levels above this threshold may correlate with disease activity. The IgG type VII collagen antibody level by ELISA is a sensitive diagnostic marker together with dermal pattern IgG basement membrane zone antibody reactivity on split skin substrate by indirect immunofluorescence in patients with epidermolysis bullosa acquisita and in a subset of patients with bullous lupus erythematosus, although patients with these disorders may demonstrate antibodies to basement membrane zone antigens in addition to or other than the type VII collagen epitopes expressed in this ELISA.

Greater than 80 percent of patients with pemphigus have positive epithelial cell surface antibodies in their sera identified by indirect immunofluorescence. Serum antibody titers correlate with disease activity. Cell surface
antibodies are implicated in the pathophysiology of pemphigus and are not typically detected in normal individuals, in patients with other diseases or in patients with pemphigus whose disease activity is minimal and/or under therapeutic control. IgG cell surface antibodies characteristically are positive by indirect immunofluorescence in IgG pemphigus variants, including pemphigus foliaceus and pemphigus vulgaris, and IgA cell surface antibodies characteristically are positive in IgA pemphigus. They also may be observed in some pemphigus variants along with positive IgG cell surface antibodies.

Antibodies in serum from individuals with pemphigus bind to desmogleins, which are calcium-dependent adhesion molecules in cell surface desmosomes; such antibodies are detected by enzyme linked immunosorbent assay (ELISA) testing. Specific reactivity to the type of desmoglein may be helpful in differentiating 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. Overlapping expression with autoantibodies to both desmogleins 1 and 3 typically is associated clinically 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 pemphigus patients showing increased levels of one or both antibodies. Desmoglein antibodies are not increased in normal individuals. IgG desmoglein levels by ELISA testing also correlate with disease activity.

TESTING METHODS
Indirect Immunofluorescence
----------------------------------------
IgG and IgA Epithelial Basement Membrane Zone and Cell Surface Antibodies

The patient’s 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 stained with fluorescein-conjugated anti-IgG and anti-IgA using Analyte Specific Reagents (ASRs). 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. These tests were developed and their performance characteristics determined by the Immunodermatology Laboratory at the University of Utah. They have not been cleared or approved by the U.S. Food and Drug Administration. ASRs are used in many laboratory tests necessary for standard medical care and generally do not require FDA approval. These tests should not be regarded as investigational or for research only. [Immunofluorescence studies, two antibodies on three substrates]

Enzyme Linked Immunosorbent Assay (ELISA)
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IMMUNODERMATOLOGY REPORT

Patient: [Redacted]  Accession number: [Redacted]

MRN: [Redacted]

IgG BP 180 and IgG BP 230 serum antibody levels determined by U.S. Food and Drug Administration-approved ELISAs (Mesacup, MBL BION).

[Two ELISAs]

Collagen VII IgG serum antibody level determined by ELISA (Mesacup, MBL International). This test 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 U.S. Food and Drug Administration. [One ELISA]

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

[Two ELISAs]

[Redacted] MD
Immunodermatologist
Electronically signed 5/11/2019 11:08:41PM

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