

# IMMUNOBULLOUS SKIN DISORDERS

## Serological Methods for Detection of Immunobullous Skin Disorders

### INTRODUCTION

The detection of intercellular (IC) and basement membrane zone antibodies (BMZ) in serum aids in the detection, diagnosis, and differentiation between chronic vesiculobullous diseases such as Pemphigus and its various subtypes, Bullous Pemphigoid (BP), Mucous Membrane Pemphigoid (MMP), and Epidermolysis Bullosa Acquisita (EBA).

### SEROLOGICAL DIAGNOSIS OF PEMPHIGUS AND ITS SUBTYPES

Pemphigus refers to a group of potentially life-threatening autoimmune blistering diseases of the skin and mucous membranes. The primary subsets of pemphigus have been identified and include pemphigus vulgaris (PV), pemphigus foliaceus (PF), and paraneoplastic pemphigus (PNP). Each type of pemphigus has distinct clinical and immunopathologic features. The pathophysiology of PV is characterized by circulating autoantibodies directed against desmogleins (DSG 1 & DSG 3), the proteins present in the epithelium binding the cells together. The diagnosis of pemphigus depends on biopsy and serum studies that characterize lesions and detect the autoantibodies that cause them. Serum

studies afford highly sensitive diagnostic aids and can be performed by indirect immunofluorescence (IF) using animal tissues sections and by ELISA.

**immunofluorescence:** Intercellular (IC) antibodies are diagnostic for pemphigus. They occur in over 90% of patients with pemphigus. There is species specificity of IC antibodies between various tissue substrates which helps in differentiation between subtypes of pemphigus. The use of dual substrate of monkey and guinea pig (gp) esophagus distinguish IC antibodies of *pemphigus vulgaris* and *vegetans* which react with monkey esophagus from *pemphigus foliaceus* and *erythematosus* antibodies which react more strongly with gp esophagus. Almost all the patients with pemphigus have circulating IC antibodies. The use of

a dual substrate increases the sensitivity of detecting IC antibodies (Table 1). In addition the use of dual substrate enables immunological differentiation of PV from PF. PV sera usually gave higher titers and brighter staining on monkey esophagus than on gp esophagus, while converse is true for PF sera (Table 2). **IMMCO employs the dual substrate for IC antibody test.**

**ELISA:** Antibodies to DSG1 and DSG3 have been shown to be present in patients with pemphigus. Patients with PF, usually have antibodies to DSG1 and PV mostly DSG3 and sometimes DSG1. Antibody titers correlate with disease activity in many patients. Patients with severe disease usually have higher titer of antibodies to DSG which are expected to decrease with clinical improvement.

TABLE 1. Sensitivity of Indirect IF for IC Antibodies between tissue substrates

Test Substrate	# Tested	# Positive	% Positive
Monkey esophagus alone	123	111	89
Guinea Pig esophagus alone	123	103	81
Monkey and Guinea Pig esophagus fused	123	122	99

TABLE 2. Differentiation of PV from PF by Indirect IF

Higher Titer and/or Brighter Staining on	PV Sera	PF Sera
Monkey esophagus	73	0
Guinea Pig esophagus	0	25
No Difference	2	5

ELISA in comparison to Indirect IF for diagnosis of Pemphigus

Population (number)	DSG-1 Positive	DSG-3 Positive	IIF Positive
Pemphigus vulgaris (n = 28)	21 (75%)	28 (100%)	28 (100%)
Pemphigus foliaceus (n = 23)	23 (100%)	6 (26%)	21 (92%)

## ■ SEROLOGICAL DIAGNOSIS OF PEMPHIGOID

Bullous Pemphigoid (BP) is a heterogenous group of chronic, autoimmune, subepidermal, blistering disease of the skin and mucous membrane. Pemphigoid (MMP) is characterized by the presence of:

1. Immunoglobulin IgG and complement deposits with a linear pattern in the basement membrane zone (BMZ) in the biopsy of the skin and/or the mucus membrane seen by direct IF.
2. Presence of immunoglobulin IgG autoantibodies to BMZ antigens by indirect IF and to BP230 (BPAg1) and BP180 (BPAg2) by ELISA in the serum.

**Indirect IF:** Indirect IF studies for BMZ antibodies on monkey esophagus from salt split normal skin are positive in majority of BP, EBA, Pemphigoid gestationis (PG) and in MMP. Serum studies on split skin in cases where routine indirect IF studies are negative, are useful. IMMCO employs the dual substrate method of detecting antibodies to BMA.

**ELISA:** Antibodies to BP180 and BP230 are present in most patients with pemphigoid. The BP180 ELISA test has a 70.8% and BP230 ELISA test has a 73.4% positive agreement rate when compared to the Indirect IF. This is due to the fact that the indirect IF method detects both BP180 and BP230 antibodies at the same time. When both BP180 and BP230 ELISA tests are performed on the same sample, the positive agreement with the IF method increases to 90%. It is recommended that each patient is tested for both BP180 and BP230 antibodies for the most accurate diagnosis. Antibody titer correlates with disease activity in many patients. Patients with severe disease can usually be expected to have high titer of antibodies to BP and are expected to decrease with clinical improvement.

## ■ IMMCO TESTS

### **Pemphigus Profile:**

*IMMCO Test Code:* 127 (IC and BMZ by IF; Dsg-1; Dsg-3 by ELISA)

*Methodology:* Indirect IF, ELISA

### **Pemphigoid Profile:**

*IMMCO Test Code:* 128 (IC and BMZ; Differentiation of BP from EBA; BP 180; BP 230)

*Methodology:* Indirect IF, ELISA

### **Pemphigus/Pemphigoid Profile I:**

*IMMCO Test Code:* 129 (IC, BMZ; Differentiation of BP from EBA)

*Methodology:* Indirect IF

### **Pemphigus/Pemphigoid Profile II:**

*IMMCO Test Code:* 129 (IC and BMZ; Differentiation of BP from EBA; Dsg-1; Dsg-3; BP 180; BP 230)

*Methodology:* Indirect IF, ELISA

**Schedule/Turnaround Time:** Assay performed daily Mon.-Fri. Report availability is within 48 hours from the time of specimen receipt.

**Specimen Requirements:** Specimen need not be refrigerated or frozen. Collect 5-10 ml of blood in a red top or serum separator tube. If possible, separate serum from clot and place into orange tube provided with IMMCO collection kits. Do not puncture top of orange tube. If separation facilities are not available, the blood can be sent in the tube used for collection.

**Sample Stability:** Sample is stable at ambient temperature during shipment. If sample is stored prior to shipment, it is stable refrigerated (2-8°C) up to five days and frozen (-20°C or lower) up to one year.

## ■ SAMPLE SUBMISSION

Specimen collection kits are available free of charge. **Please call 1-800-537-8378 or e-mail [service@immco.com](mailto:service@immco.com) for supplies.**

Specimen can be shipped by courier services, U.S. Postal service and overnight carriers free of charge. Results are reported

within two business days from receipt of the specimen via mail, fax and through IMMCO online, a HIPAA-compliant web tool at [www.immco.com](http://www.immco.com).

## ■ SUGGESTED READING

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