

## Introduction:

Demonstration of Glomerular Basement Membrane antibodies (GBM), by immunofluorescent methods may enable serologic assessment and possible detection of kidney disease. The presence of a histologically defined circulating antibody to the glomerular basement membrane can aid in the patient diagnosis and possible diagnosis in Goodpasture's Syndrome. A linear fluorescence along the Glomerular Basement Membrane can be observed in most Goodpasture's patients.

## Principles:

The primary reaction involves circulating anti-glomerular basement membrane antibodies present in the patient's serum. The binding of the antibody to its homologous antigen site occurs during the first incubation period, while the serum covers the antigen surface. A second reaction follows after the unbound antibody is removed by a PBS rinse. The secondary reaction involves the binding of fluorescein labeled anti-human globulin, commonly referred to as conjugate. The unbound fluorescein conjugate then removed, by a PBS rinse, leaving only specifically bound sites to be observed through the microscope. Bright (3-4+) fluorescence of the glomerular basement membrane indicates a positive reaction. Care should be taken to locate vessel staining or staining outside the glomerulus, where smooth muscle activity could be accidentally reported as a positive reaction.

## Materials Provided:

### Storage & Stability of Components:

1. FITC Conjugate No. 1502L (3.0 ml) with Evans Blue Counterstain is to be stored at 2-8 C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label. This reagent contains antibodies which will react with the human IgG, IgM and IgA Immunoglobulin classes.
2. The antigen slides of monkey kidney sections must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

3. GBM positive control No. 5602L (1.0 ml) should be stored at 2-8 C upon receipt. Check label for specific expiration date.
4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8 C upon receipt. Check label for specific expiration date.
5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stable at room temperature storage for 5 years. The reconstituted Buffer does not contain preservatives and should be stored at 2-8 C. Care should be taken to avoid contamination.
6. Mounting Medium No. 1610 is stable when stored at 2-8 C. Check label for specific expiration date.

## Additional Materials Required but not Provided:

Test tubes and rack or microtiter system  
Disposable pipettes  
Staining Dish and Slide Forceps  
Moisture Chamber  
Volumetric Flask (500 ml)  
Distilled H<sub>2</sub>O  
Fluorescence Microscope  
Paper Towels - lint free

## Reagent Preparation:

1. Buffer Pack No. 1601. Rehydrate buffer with 1 liter of sterile distilled water.

## Specimen Collection:

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8 C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20 C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

## Test Instruction:

**Screening:** dilute test serums 1:4 in PBS. **Titration:** set up doubling dilutions of serum starting at 1:4, 1:8, 1:16, 1:32, etc.

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (20 to 30 µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24 C).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.
5. Wash in PBS for five minutes. Repeat using fresh PBS.
6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. **Do not allow tissue to dry.** Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.
7. Deliver 1 drop (20-30 µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

## Quality Control:

1. Positive and negative serum controls must be included in each day's testing to confirm repro-

ducibility, sensitivity and specificity of the test procedure.

2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

## Results:

A positive result is observed as a bright 3-4+ linear staining of the Glomerular basement membrane. This antibody is seen in many Goodpasture Syndrome patient, and may have significance in the patient profile, as well as aid the diagnosis and prognosis.

## Precautions:

1. All human components have been tested by radioimmunoassay for (HB<sub>s</sub>Ag) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB<sub>s</sub>Ag or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.

5. For In Vitro Diagnostic Use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

**BIBLIOGRAPHY**

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## GLOMERULAR BASEMENT MEMBRANE TEST SYSTEM

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