GBM Ab ELISA Kit

Catalog Number KA1266
96 assays
Version: 02

Intended for research use only
Introduction

Intended Use

Anti-GBM is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies to glomerular basement membrane (GBM) in human serum or plasma. The assay is intended for research use only as an aid in the diagnosis of autoimmune renal disorders such as Goodpasture syndrome.

Background

Primarily, Goodpasture syndrome is an autoimmune disorder of the kidneys. Ernest Goodpasture, an American pathologist was the first in 1919 who described the coexistence of a fatal lung hemorrhage coming along with proliferative glomerulo-nephritis in a young man. The syndrome is now considered as an autoimmune disorder consisting of the triad of glomerulonephritis, lung hemorrhage and antiglomerular basement antibodies formation.

The incidence of the Goodpasture syndrome is about 0.5 to 1 cases per million inhabitants per year. Patients in the third and seventh decade are mainly affected. Goodpasture syndrome is a medical emergency with a case fatality rate of 75 to 90 % due to kidney and respiratory insufficiency, if not treated. Histologically, the disorder is characterized by continuous linear deposition of immunoglobulins along the glomerular basement membrane (GBM), demonstrable by direct immunofluorescence on kidney biopsies. Nowadays, the determination of circulating autoantibodies against the C-terminal end of the a-3 chain of type IV collagen is considered the diagnostic criterion.

Basement membranes form an anatomical barrier wherever epithelia meets connective tissue. Type IV collagen, that is only found in GBM forms a matrix in which additional molecules (e.g. Laminin, Entactin) are integrated. Three out of six a-chains (polypeptides with more than 1650 amino acids) form a triple helix and characterize the structural subunits of type IV collagen. All C-terminal ends of the a-chains form a globular domain, that can be dissolved from the triple helix by treatment with bacterial collagenase.

The reactivity of Goodpasture specific anti-GBM autoantibodies is directed against the 29 kDa NC1 domain of the a-3 chain of type IV collagen of GBM. After the target antigen has been entirely characterized nowadays the triad of glomerulonephritis, lung hemorrhage and the antibodies against the a-3 chain of type IV collagen of GBM are the essential elements of diagnosis of Goodpasture syndrome. ELISA test systems provided with the corresponding pure antigen exhibit sensitivities and specificities of about 98 to 99%.
**Principle of the Assay**

Highly purified GBM is bound to microwells. Antibodies to this antigen, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically bind to the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.
## General Information

### Materials Supplied

#### List of component

<table>
<thead>
<tr>
<th>Components</th>
<th>Description</th>
<th>Volume / Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro titer strips</td>
<td>Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified GBM. Ready to use.</td>
<td>12</td>
</tr>
<tr>
<td>Anti-GBM Calibrators (A-E)</td>
<td>Anti-GBM Calibrators (A-E) in a serum/buffer matrix (PBS, BSA, NaN₃ &lt;0.1% (w/w)) containing: 0; 20; 40; 80; 200 U/ml. Ready to use.</td>
<td>5 vials, 1.5 mL each</td>
</tr>
<tr>
<td>Anti-GBM Controls</td>
<td>Anti-GBM Controls in a serum/buffer matrix (PBS, BSA, NaN₃ &lt;0.1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.</td>
<td>2 vials, 1.5 ml each</td>
</tr>
<tr>
<td>Sample buffer</td>
<td>(Tris, NaN₃ &lt;0.1% (w/w)), yellow, concentrate (5x).</td>
<td>1 vial, 20 ml</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>(PBS, Proclin 300 &lt;0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use.</td>
<td>1 vial, 15 ml</td>
</tr>
<tr>
<td>TMB substrate solution</td>
<td>TMB substrate solution. Ready to use.</td>
<td>1 vial, 15 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>Wash solution (PBS, NaN₃ &lt;0.1% (w/w)), concentrate (50x).</td>
<td>1 vial, 15 ml</td>
</tr>
</tbody>
</table>

### Storage Instruction

- Store the kit at 2-8°C.
- Keep microplate wells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun or strong light during storage and usage.
- Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8°C.

### Materials Required but Not Supplied

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
Precautions for Use

- Precautions
  - Do not use kit components beyond their expiration dates.
  - Do not interchange kit components from different lots.
  - All materials must be at room temperature (20-28°C).
  - Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
  - Perform the assay steps only in the order indicated.
  - Always use fresh sample dilutions.
  - Pipette all reagents and samples into the bottom of the wells.
  - To avoid carryover contamination change the tip between samples and different kit controls.
  - It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
  - All incubation steps must be accurately timed.
  - Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
  - Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semiquantitatively.

- Limitations of Procedure
  The GBM Ab ELISA is a diagnostic aid and by itself is not diagnostic. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.
Assay Protocol

Reagent Preparation

- Preparation of sample buffer
  Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use.
  Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

- Preparation of wash solution
  Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.
  Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

- Sample preparation
  Dilute all patient samples 1:50 with sample buffer before assay. Therefore combine 10 µl of sample with 490 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

Sample Preparation

✓ Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
✓ Allow blood to clot and separate the serum by centrifugation.
✓ Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
✓ Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
✓ Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
✓ Testing of heat-inactivated sera is not recommended.

Assay Procedure

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet 100 µl of controls and prediluted patient samples (1:50 dilution!) in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28°C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µl of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

✓ Automation
The GBM Ab ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.
Data Analysis

Calculation of Results

• Quality Control
This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not met, the results are invalid and the test should be repeated.

• Calculation of results
For Anti-GBM IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

• Interpretation of results
In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-GBM test:

<table>
<thead>
<tr>
<th>Anti-GBM Ab [U/ml]</th>
<th>normal:</th>
<th>elevated:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 20</td>
<td>≧ 20</td>
</tr>
</tbody>
</table>

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-GBM.
Performance Characteristics

- Parallelism
In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-GBM kit. The assay shows linearity over the full measuring range.

- Precision (Reproducibility)
Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Mean [U/ml]</th>
<th>CV [%]</th>
<th>Sample No</th>
<th>Mean [U/ml]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.6</td>
<td>5.3</td>
<td>1</td>
<td>14.2</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>85.8</td>
<td>5.4</td>
<td>2</td>
<td>90.1</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>143.4</td>
<td>5.7</td>
<td>3</td>
<td>154.4</td>
<td>5.8</td>
</tr>
</tbody>
</table>

- Sensitivity
The lower detection limit for Anti-GBM has been determined at 1.0 U/ml.

- Specificity
The microplate is coated with highly purified GBM as antigen. No crossreactivities have been observed.

- Calibration
Since no international reference preparation for Anti-GBM autoantibodies is available, the assay system is calibrated in relative arbitrary units.

- Interferences:
No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples be avoided.
Resources

References
