Instruction Manual

REF 4062

September 19th 2008

Anti-GBM

- 96 determinations -

IVD *In-vitro diagnostic device*

CE

Enzyme immunoassay for the determination of IgG antibodies to glomerular basement membrane (GBM) in human serum

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

GA GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telefon: +49 (0) 33708-9286-0
Fax: +49 (0) 33708-9286-50

www.genericassays.com

INTENDED USE

Anti-GBM is used for the quantitative determination of IgG antibodies to glomerular basement membrane protein (GBM) in human serum for the diagnosis of Goodpasture syndrome.

Goodpasture syndrome is an autoimmune kidney disorder characterized by the coexistence of proliferative glomerulonephritis with a fatal lung hemorrhage and the formation of anti-glomerular basement membrane antibodies. The American pathologist Ernest Goodpasture was the first to describe this disease in the year 1919.

The incidence of the Goodpasture syndrome ranges between 0.5 to 1 cases per million inhabitants per year and - in case of no treatment - leads to a case fatality rate of 75 to 90 % due to renal and respiratory insufficiency. Chances of survival significantly increase when plasma is exchanged and patients respond to an immunosuppression therapy (1).

The key diagnostic parameter of Goodpasture syndrome is the detection of the pathogenic circulating autoantibodies to glomerular basement membrane. Glomerular basement membrane is an anatomical barrier between kidney epithelia and connective tissue and plays an important role in hemo-ultrafiltration. Antibodies specific for Goodpasture syndrome are directed against the 29 kDa NC1 domain at the C-terminus of the α-3 chain of type IV collagen (NC1 α-3 (IV)) (2). Collagen IV is exclusive to the basement membrane of glomeruli. Identification of this antigen formed the basis of the development of ELISA test systems with specificities and sensitivities of 98 to 99 % to the Goodpasture syndrome.

Rapid progressive glomerulonephritis (RPGN) is a common feature of many autoimmune disorders. Differential diagnosis of autoimmune nephritides requires the determination of antibodies to GBM together with the determination of antibodies to Proteinase 3 (characteristic for Morbus Wegener), to Myeloperoxidase (characteristic for vasculitis-associated RPGN), and ANA (characteristic for Lupus nephritis).

(1) Levy JB, Turner AN, Rees AJ, Pusey CD: Long-term outcome of anti-glomerular basement membrane antibody disease with plasma exchange and immunosuppression. Ann Int. Med. 2001, 134 (11) 1033-44

(2) Hellmark T, Burkhardt H, Wieslander J: Goodpasture Disease: Characterization of a single conformational epitope as the target of pathogenic autoantibodies. J. Biol. Chem. 1999, 274 (36), 25862-8

PRINCIPLE OF THE TEST

Anti-GBM is used for the quantitative determination of autoantibodies to glomerular basement membrane (GBM) in human serum.

The antibodies of standards control and diluted patient samples react with GBM antigen immobilized on the solid phase of microtiter plates. The use of recombinant human GBM protein coated on the microtiter plate guarantees the specific binding of Goodpasture antibodies of the specimen under investigation. Following an incubation period of 30 min at room temperature, unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 30 min incubation at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic or contaminated samples should not be run.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v),***
e.g. 10 µl sample + 1 ml sample diluent (C), prior to assay.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 WELLS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells coated with recombinant human glomerular basement membrane protein (GBM)	1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 50 x	20 ml concentrate capped white
C DIL	**Concentrated sample diluent** sufficient for 100 ml solution 5 x	20 ml concentrate capped white
D CONJ	**Conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped blue
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxid	15 ml ready for use capped black
F HCl	**Stop solution** 1.0 M hydrochloric acid 1.0 M	15 ml ready for use capped white
1-6 CAL	**Standards** (human serum diluted) conc.: 0, 3, 10, 30, 100, 300 U/ml	1,5 ml each ready for use
P CONTROL	**Positive Control** (human serum diluted) Conc. see data sheet +	1,5 ml ready to use capped red
N CONTROL	**Negative Control** (human serum diluted) Conc. see data sheet -	1,5 ml ready for use capped green

Materials required

- micropipettes
- multi-channel pipette or multi-pipette
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- glassware
- microplate reader with wavelength for 450nm and 620 nm or 690 nm
- eppendorf reaction tubes (2ml)

Size and storage

Anti-GBM has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the anti-GBM assay have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 50 times with de-ionized or distilled water. For example, dilute 1 ml of the concentrate with 49 ml of distilled water per strip.
The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Prepare a sufficient amount of sample diluent by diluting the concentrated diluent 5 times with de-ionized or distilled water. For example, dilute 10 ml of the concentrate with 40 ml of distilled water. The sample diluent prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **pre-dilute patient sera with sample diluent (made of C) 1 + 100 (v/v),**
  e.g. 10 µl sample + 1 ml sample diluent (made of C)
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl standards (1-6),
   100 µl controls (P) and (N) and
   100 µl diluted patient samples (**1 + 100**) into the respective wells .
3. Seal plate, incubate **30 min** at room temperature (18-25°)
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at room temperature (18-25°)
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **30 min** protected from light at room temperature (18-25°).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the standards 1 - 6 on the ordinate, y-axis, (lin. scale) versus their respective IgG concentrations on the abscissa, x-axis, (log. scale).

Anti-GBM antibody concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

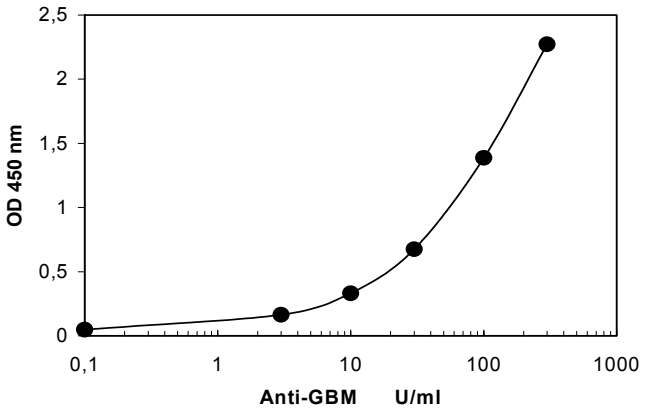
Anti-GBM may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Example of Typical Assay Results

well	OD (a)	OD (b)	OD (mean)	U/ml
Standard **1**	0.045	0.053	0.049	**0**
Standard **2**	0.166	0.162	0.164	**3**
Standard **3**	0.325	0.339	0.332	**10**
Standard **4**	0.689	0.661	0.675	**30**
Standard **5**	1.380	1.394	1.387	**100**
Standard **6**	2.281	2.262	2.272	**300**
Patient **1**	1.053	1.069	1.061	**61**

TYPICAL STANDARD CURVE



Specimens with an OD > standard 6 should be diluted with anti-GBM negative serum and tested again. Results are multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the standard 1 is ≤ 0.15
- the mean OD of the standard 6 is ≥ 1.3

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

GBM antibodies	U/ml
positive	**> 15**
negative	**≤ 15**

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-GBM levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by anti-GBM. However, GBM autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

PERFORMANCE CHARACTERISTICS

Calibration

No international reference material for GBM antibodies is available so the assay is calibrated in arbitrary units.

Linearity

Positive selected serum samples have been tested by this assay and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be sera that do not follow this rule.

Analytical Sensitivity

The analytical sensitivity of this assay has been determined at 1.0 U/ml.

Specificity and Sensitivity

No cross reactivity to other autoantigens have been found.
Antibodies to GBM occur in 65 % of patients with idiopatic crescentic glomerulonephritis, 70 % of patients with microscopic polyangiitis and up to 50 % of patients with Churg-Strauss syndrome.

Precision

Intra-Assay			Inter-Assay		
Sample	**Mean (U/ml)**	**CV (%)**	**Sample**	**Mean (U/ml)**	**CV (%)**
1	24.8	1.5	1	23.6	2.9
2	78.4	3.1	2	75.3	4.5
3	225.0	2.4	3	215.0	3.4

INCUBATION SCHEME

Anti-GBM (4062)

Dilute patients sample	**10 µl sample + 1 ml sample diluent (made of C)**

1	Bring all ready for use reagents to room temperature (18-25°C) before use				
			Standards	Controls	Sera
2	Pipette	Standards (1 – 6) Controls (P + N) 1 + 100 prediluted sera	100 µl	100 µl	100 µl
3	Incubate		30 min, room temperature(18-25°C)		
4	Wash		Decant, dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 min, room temperature (18-25°C)		
7	Wash		Decant, dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		30 min, room temperature (18-25°C)		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 versus 620 (690) nm in between 30 min				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.