COD 44588 12 x OOOO
STORE AT 2-8ºC
Reagents for the qualitative determination of glomerular basement membrane antibodies Only for *in vitro* use in the clinical laboratory

GLOMERULAR BASEMENT MEMBRANE ANTIBODIES (GBMA)

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BioSystems REAGENTS & INSTRUMENTS

GLOMERULAR BASEMENT MEMBRANE ANTIBODIES (GBMA)

Indirect Immunofluorescence
MONKEY KIDNEY

PRINCIPLE OF THE METHOD

Serum anti-glomerular basement membrane antibodies (GBMA) bind to the corresponding antigen present in a monkey kidney section, after induced exposure of cryptic epitopes. The antigen-antibody complexes are detected by means of a fluorescein labeled anti-human immunoglobulin, and visualized with the aid of a fluorescence microscope[1].

CONTENTS

	COD 44588
A. Slides	12 x 4 wells

COMPOSITION

A. Slides. Monkey kidney sections.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:

– Liquid components: Presence of particulate material, turbidity.
– Slides: rips in the sealing bag, macroscopic defects on the tissue section like scratches or tissue peelings.

AUXILIARY REAGENTS

- Cod 44588 needs the following auxiliary reagents available in a separate form:
  - B. PBS (10x): Sodium phosphate 112.5 mmol/L, potassium phosphate 30 mmol/L, sodium chloride 1.15 mol/L, sodium azide 0.95 g/L. pH 7.2.
  - D. IgG FITC/Evans (M): Anti-human IgG conjugated with fluorescein isothiocyanate (FITC) and adsorbed with monkey serum, Evans blue 0.01g/L, sodium azide 0.95 g/L.
  - E. Mounting Medium: Mowiol 12%, Glicerol 30%, Tris 20 mmol/L, sodium azide 0.95 g/L.
  - H. Glycin 0.1 mol/L pH 2.2.
  - C+. GBMA Positive Control: Human serum containing anti-glomerular basement membrane antibodies, sodium azide 0.95 g/L
  - C–. Negative Control: Human serum, sodium azide 0.95 g/L.

Human sera used in the preparation of the positive and negative controls have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the controls should be handled cautiously as potentially infectious.

REAGENT PREPARATION

PBS: Dilute Reagent B 1/10 with distilled water. Stable for 1 week at 2-8ºC.

All other reagents are provided ready to use.

ADDITIONAL EQUIPMENT

– Moisture chamber
– Wash tray
– Coverslips 24 x 60 mm
– Fluorescence microscope equipped with a 495 nm excitation filter and a 525 nm emission filter for FITC visualization.

SAMPLES

Serum or plasma collected by standard procedures. Stable for 1 week at 2-8ºC.

Dilute samples 1/ 30 in PBS (see Reagent Preparation) before assay.

For titration of positive samples, make two-fold serial dilutions starting from 1/ 30 in PBS.

PROCEDURE

1. Bring the reagents and samples to room temperature.
2. Place 1 drop (50 µL) of Reagent H on each slide well, making sure that it is completely covered (Note 1).
3. Incubate the slide for 30 minutes at room temperature (15-30ºC) into a moisture chamber.
4. Wash thoroughly the slide by immersing in a washing tray filled with PBS for 5 minutes. Change PBS and repeat wash.
5. Carefully dry off the slides by using the blotting paper provided. Keep the tissue section moist along the procedure.
6. Place 1 drop (50 µL) of the diluted sample or Control on each slide well, making sure that it is completely covered (Note 1).
7. Incubate as in step 3.
8. Drain sample drops off by gently tapping the inclinated slide. Avoid cross-contamination of the sera.
9. Rinse gently the slide with PBS (see Reagent Preparation) (Note 2).
10. Wash (step 4) and dry (step 5).
11. Place 1 drop of Reagent D on each well. Incubate the slide for 30 minutes at room temperature (15-30ºC) into a moist chamber.
12. Wash (step 4) and dry (step 5).
13. Place several drops of Reagent E on the slide and cover with a coverslip avoiding the formation of air bubbles.

READING

Examine the slide using the fluorescence microscope (250-400x). For best results, the slides should be read immediately. Select reading fields in the inner part of the tissue section. Fluorescent intensity in the tissue edge is not representative of the slide preparation.

Sera showing a smooth linear staining of the basement membrane in the renal glomerule at the recommended dilution should be considered positive.

Positive sera may be titered.

When none of the specific staining are observed, the result should be considered negative for these autoantibodies.

QUALITY CONTROL

Positive Control (C+) and Negative Control (C–) should be tested together with the patients samples, in order to verify the assay performance.

Positive Control (C+) should give the above described specific staining.

Negative Control (C–) should not give any specific staining.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

ASSAY CHARACTERISTICS

– IgG FITC/Evans (M) conjugate is valued against the WHO International Standard for FITC labeled sheep anti-human immunoglobulin.
– Results obtained with the BioSystems GBMA kit in a comparative study do not show significant systematic differences compared to an ELISA for anti-type IV collagen alpha 3 chain antibodies. Details of this comparative study are available upon request.
– The specificity of the GBMA Positive Control has been verified against an internal reference serum

DIAGNOSTIC CHARACTERISTICS

Indirect immmunofluorescence assay is the conventional method for the determination of anti-glomerular basement membrane antibodies (GBMA).

Antibodies to glomerular basement membrane are present in patients with Goodpasture's syndrome. The disease presents a progressive glomerulonephritis with or without massive pulmonary hemorrhage. The finding of GBMA confirms a diagnosis of a life threatening disease that causes loss of kidney, and indicates a grave prognosis[2,3].

BioSystems Glomerular Basement Membrane Antibodies kit was used to test 106 sera from Goodpasture's syndrome patients as well as healthy donors. The results are described as follows, showing a sensitivity and specificity of 100%.

		BioSystems GBMA	
Patients	***n***	***Positive***	***Negative***
Goodpasture syndrome	*28*	*28*	*0*
Healthy controls	*78*	*0*	*78*

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Avoid touching the tissue section fixed into the wells along the procedure.
2. Use a squeeze bottle or a pipette to wash the slides, avoiding cross-contamination among the adjacent samples.

BIBLIOGRAPHY

1. Melnicoff MJ. Immunofluorescence Methods for Microscopic Analysis. In: Howard GC ed. Methods in Nonradioactive Detection. Appleton & Lange, 1993.
2. Hellmark T, Segelmark M and Wieslander J. Anti-GBM antibodies in Goodpasture syndrome; anatomy of an epitope. Nephrol Dial Transplant (1997) 12: 646–648
3. Hellmark T, Segelmark M, Bygren P and Wieslander J. Glomerular basement membrane autoantibodies. In: James B. Peter and Yehuda Shoenfeld eds. Autoantibodies. Elsevier, 1996.