

Anti-Glomerular Basement Membrane Test System

QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, 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 controls should result in bright 3+ to 4+ fluorescence. If these controls show 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 non-specific 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 Goodpastures Syndrome patients and may be significant in the patient profile, as well as aid in the diagnosis and prognosis.

Positive results can be confirmed on the Bio-Diagnostics Anti-GBM Quantitative Rainbow ELISA (cat. no. 107-007), Anti-GBM Rapid Rainbow ELISA (cat. no. 107-006) or the GBM QuickCard (cat. no. CS012).

TEST LIMITATIONS

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.

LITERATURE REFERENCES

1. Goodpasture, E.W.: The significance of certain pulmonary lesions in relation to the etiology of influenza, In: Am J Med Sci, 1919, pp. 158:863.
2. Stanton, M.C., Tanga, J.D.: Goodpasture's syndrome (pulmonary hemorrhage associated with glomerulonephritis), In: Austr Ann Med, 1958, pp. 7:132.
3. Hudson, B., Wieslander, J., Wisdom, Jr., B., Nelken, M.: Biology of disease Goodpasture Syndrome: Molecular architecture and function of basement membrane antigen, In: Lab Inv, 1989; 61.3: pp. 256-269.
4. Hellmark, T., Johannsson, C., Wieslander, J.: Characterization of anti-GBM antibodies involved in Goodpasture's syndrome, In: Kidney Int, 1994; 46: pp. 823-829.

EC REP

Advena Ltd. Tower Business Centre, 2nd Flr.,
Tower Street, Swatara, BKR 4013 Malta

INSTRUCTIONS FOR USE

Anti-Glomerular Basement Membrane Antibody Test System

K4814 - 48 Tests

K9614 - 96 Tests

Also for: **Monkey kidney slides**

S4202 - 4 well

S8202 - 8 well

S5202 - 5 well

S0202 - 10 well

INTENDED USE

The Bio-Diagnostics Anti-Glomerular Basement Membrane (GBM) Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of antibodies to glomerular basement membrane, in human serum.

SUMMARY AND EXPLANATION

Demonstration of GBM antibodies by immunofluorescent methods may allow serological assessment and possible detection of kidney disease. The presence of a histologically defined circulating antibody to GBM can aid in patient diagnosis and possible diagnosis in Goodpastures Syndrome. A linear fluorescence along the Glomerular Basement Membrane can be observed in most Goodpastures patients.

PRINCIPLE OF THE TEST

The primary test reaction involves circulating anti-GBM antibodies present in the patient's serum, which attach to their homologous antigens. This occurs during the incubation period whilst the serum covers the antigen surface. A secondary reaction then follows a rinsing period that removes the unbound human antibody. The reagent used in the secondary reaction is a fluorescein labelled antihuman globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope.

Anti-Glomerular Basement Membrane Test System

WARNINGS AND PRECAUTIONS

1. This human components of the controls have been screened and found to be negative for HBsAg and antibodies to HIV-1, HIV-2, P24 antigen and HCV. However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The controls included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although these are at low concentrations, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure 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 artefacts.

KIT CONTENTS

SLIDE	Monkey kidney substrate antigen slides (S4202, S8202, S5202, S0202)
CONJ IgG	FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain: J502. This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL +	GBM antibody Positive Control no: C013N-0.5
CONTROL -	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA PBS	Buffer Pack no: R002
MM	Mounting Medium no: R005

Note: All kit components are available separately.
Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED






Test tubes and rack or microtitre system	Disposable pipettes
Staining Dish and Slide Forceps	Moisture Chamber
Volumetric Flask (500 ml)	Distilled Water
Fluorescence Microscope	Paper Towels – lint free

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

 Manufacturer	 Contains sufficient for <n> tests	 Ready for use
 Temperature limitation	 In vitro diagnostic medical device	

Anti-Glomerular Basement Membrane Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents. Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS

Screening: dilute test serums 1/4 (1 part patient sample to 3 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.

Titration: set up doubling dilutions of serum starting at 1/4, (i.e. 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 30µl) 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 18-24°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 onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Carefully dry the back and edges of the slide using a paper towel. **Do not allow tissue to dry.**
7. Deliver 1 drop (20-30µl) 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.