EUROPLUS Mosaic with kidney (monkey) / GBM BIOCHIPs / lung (monkey)
Instructions for the indirect immunfluorescence test

ORDER NO.	ANTIBODIES AGAINST	SUBSTRATE	SPECIES	FORMAT SLIDES x FIELDS
FA 1250 FA 1250-1 FA 1271-1 (see p. 12)	renal glomeruli (GBM) + renal tubuli glomerular basement membrane (GBM) alveolar basement membrane	kidney GBM EUROPLUS lung	monkey -- monkey	10 x 03 (030) 10 x 05 (050) 10 x 10 (100)

Indication: This test kit provides qualitative or semiquantitative in vitro determination of human antibodies of immunoglobulin class IgG against glomerular and alveolar basement membrane in patient samples to support the diagnosis of Goodpasture's syndrome and glomerulonephritis.

Application: The determination of circulating autoantibodies against the glomerular basement membrane (GBM) of the kidneys, which can also be directed against alveolar basement membrane, is an important aspect in the diagnosis of anti-GBM disease, a progressive glomerulonephritis, frequently with lung involvement (in those cases also known as Goodpasture's syndrome). The combination of the IIFT substrates kidney tissue, lung tissue, and GBM EUROPLUS enables the determination of antibodies and the monospecific confirmation of anti-GBM antibodies in one step.

Test principle: Frozen sections of primate kidney must be pretreated with glycine urea buffer and subsequently incubated with diluted patient sample. If a positive reaction is obtained, specific antibodies of classes IgA, IgG and IgM attach to the antigens. In a second step, the attached antibodies are stained with FITC-labelled anti-human antibodies and made visible with a fluorescence microscope.

Contents of a test kit for 50 determinations (e.g. FA 1250-1005-1):

Description	Format	Symbol
1. Slides, each containing 5 x 2 BIOCHIPs coated with frozen sections of primate kidney/GBM EUROPLUS	10 slides	SLIDE
2. FITC-labelled anti-human IgG (goat), ready for use	1 x 1.5 ml	CONJUGATE
3. Positive control: autoantibodies against renal glomeruli (GBM), human, ready for use	1 x 0.1 ml	POS CONTROL
4. Negative control: autoantibody negative, human, ready for use	1 x 0.1 ml	NEG CONTROL
5. Glycine urea buffer, ready for use	1 x 1.5 ml	GLYCIN UREA
6. Salt for PBS pH 7.2	2 packs	PBS
7. Tween 20	2 x 2.0 ml	TWEEN 20
8. Mounting medium, ready for use	1 x 3.0 ml	GLYCEROL
9. Cover glasses (62 mm x 23 mm)	12 pieces	COVERGLASS
10. Instruction booklet	1 booklet	---

LOT	Lot description		Storage temperature
IVD	In vitro diagnostic medical device	CE	Unopened usable until

Single slides (e.g., EUROIMMUN order no. FB 1250-1005-1) are provided together with cover glasses. Additional positive control (e.g., order no. CA 1251-0101) and negative control (e.g. order no. CA 1000-0101) can be ordered.

Performance of the test requires reagent trays TRAY, which are not provided in the test kits. They are available from EUROIMMUN under the following order no.:

- ZZ 9999-0110 Reagent trays for slides containing up to 10 fields

Storage and stability: The slides and the reagents should be stored at a temperature between +2°C and +8°C. Unopened, all test kit components are stable until the indicated expiry date.

Note: Specifics on the glycine urea buffer can be found on page 4.

Modifications to the former version are marked in grey.

Performing the test (reaction fields 5 x 5 mm)

The **TITERPLANE Technique** was developed by EUROIMMUN in order to standardise immunological analyses: Samples or labelled antibodies are applied to the reaction fields of a reagent tray. The BIOCHIP slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. Position and height of the droplets are exactly defined by the geometry of the system. As the fluids are confined to a closed space, there is no need to use a conventional “humidity chamber”. It is possible to incubate any number of samples next to each other and simultaneously under identical conditions.

Prepare: The preparation of the reagents and of the serum and plasma samples is described on **page 4** of this test instruction.

Pipette: Apply **25 µl of glycine urea buffer** to each reaction field of the reagent tray, avoiding air bubbles. Transfer the glycine urea buffer according to the number of samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.

Incubate (1): Start reactions by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Wash: Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least **15 minutes**. Shake with a rotary shaker if available. Wash max. 16 slides per cuvette, then replace PBS-Tween with new buffer.

Pipette: Apply **30 µl of diluted sample** to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.

Incubate (2): Start reactions by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Wash: Fill cuvette with new PBS-Tween. Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards into the cuvette filled with the new PBS-Tween for at least **5 minutes**. Shake with a rotary shaker if available. Wash max. 16 slides per cuvette, then replace PBS-Tween with new buffer.

Pipette: Apply **25 µl of conjugate** to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The conjugate should be mixed thoroughly before use. To save time, conjugate can be pipetted onto separate reagent trays during the incubation with the diluted sample.

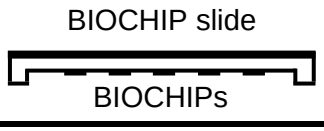
Incubate (3): Remove one BIOCHIP slide from the cuvette. Within five seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs and liquids. Then continue with the next BIOCHIP slide. From now on, protect the slides from direct sunlight. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

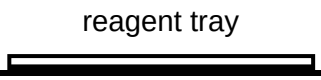
Wash: Fill cuvette with new PBS-Tween. Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and put them into the cuvette filled with the new PBS-Tween for at least **5 minutes**. Shake with a rotary shaker if available. Wash max. 16 slides per cuvette, then replace PBS-Tween with new buffer.

Mount: Place mounting medium onto a cover glass – drops of **max**. **10 µl per reaction field**. Use a polystyrene mounting tray. Remove one BIOCHIP slide from PBS-Tween and dry the back and all four sides with a paper towel. Put the BIOCHIP slide, with the BIOCHIPs facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary.

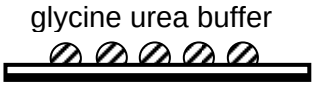
Evaluate: Read the fluorescence with the microscope.
General recommendation: objective 20x (tissue sections, infected and transfected cells), 40x (cell substrates).
Excitation filter: 450-490 nm, colour separator: 510 nm, blocking filter: 515 nm.
Light source: mercury vapour lamp, 100 W, EUROIMMUN LED, EUROStar Bluelight.

TITERPLANE Technique





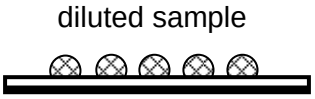
Pipette: 25 µl per field



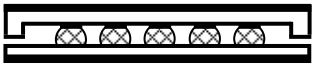
Incubate (1): 30 min

Wash: 1 s flush
15 min cuvette

Pipette: 30 µl per field

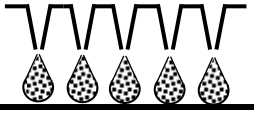


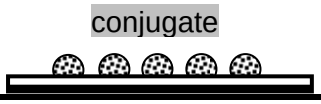
Incubate (2): 30 min



Wash: 1 s flush
5 min cuvette

Pipette: 25 µl per field

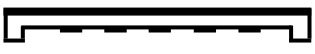


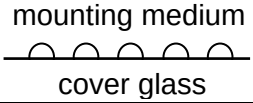


Incubate (3): 30 min

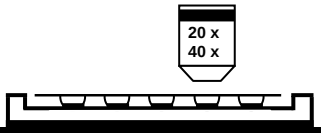
Wash: 1 s flush
5 min cuvette

Mount: max. 10 µl per field





Evaluate: fluorescence microscopy



Automated Incubation: The test kit can be incubated by using automated devices, e.g. IF Sprinter, Sprinter XL, EUROLabLiquidHandler or others. The incubation and washing conditions programmed should be the same as described in the manual procedure. The test settings for EUROIMMUN devices are validated in combination with the kit. Any other combination has to be validated by the user. For details please refer to the device manual.

Preparation and stability of reagents

Note: After initial opening, the reagents are stable until the expiry date when stored between +2°C and +8°C and protected from contamination, unless stated otherwise below.

- **Slides:** Ready for use. Remove the protective cover only when the slides have reached room temperature (+18°C up to +25°C; condensed water can damage the substrate). Do not touch the BIOCHIPs. If the protective cover is damaged, the slide must not be used for diagnostics.

- **FITC-labelled secondary antibody:** Ready for use. Before using for the first time, mix thoroughly. The conjugate is sensitive to light. Protect from sunlight.

- **Positive and negative controls:** Ready for use. Before using for the first time, mix thoroughly.

- **PBS-Tween:** 1 pack of “Salt for PBS” should be dissolved in 1 liter of distilled water (optimal: aqua pro infusione, aqua ad injectabilia) and mixed with 2 ml of Tween 20 (stir for 20 minutes until homogeneous). The prepared PBS-Tween can be stored at +2°C to +8°C, generally for 1 week. PBS-Tween should not be used if the solution becomes cloudy or contamination appears.

- **Mounting medium:** Ready for use.

- **Glycine urea buffer:** Ready for use. Before using mix glycine urea solution thoroughly. The buffer should be light yellow and may not be used if the colour changes to green or blue. Glycine urea solution is heat sensitive. Store at +2°C to +8°C. The buffer can also be portioned and stored at -20°C. Any flocking occurring during thawing can be broken up by heavily shaking the solution.

- **Reagent trays:** Reaction fields of the reagent tray must be hydrophilic and surrounding area hydrophobic. If necessary, leave in 2% Deconex 11 universal (EUROIMMUN order number: ZZ 9912-0101) for 12 hours. Afterwards rinse generously with water and dry. Cleaning: Rub reagent trays with 5% Extran MA 01 (EUROIMMUN order number: ZZ 9911-0130) and rinse generously with plenty of water. To disinfect: Spray reagent trays generously with Mikrozid AF (EUROIMMUN order number: ZZ 9921-0125), turn over and leave for 5 minutes. Afterwards, rinse generously with water and dry.

Waste disposal: Patient samples, controls and slides are to be handled as potentially infectious materials. All reagents are to be disposed of in accordance with official disposal regulations.

Warning: The BIOCHIPs coated with antigen substrates have been treated with a disinfecting fixing agent. Neither HBsAg nor antibodies against HIV-1, HIV-2, and HCV could be detected in the control sera using appropriate ELISA or indirect immunofluorescence tests. **Nevertheless, all test system components should be handled as potentially infectious materials.** Some of the reagents also contain sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of serum and plasma samples

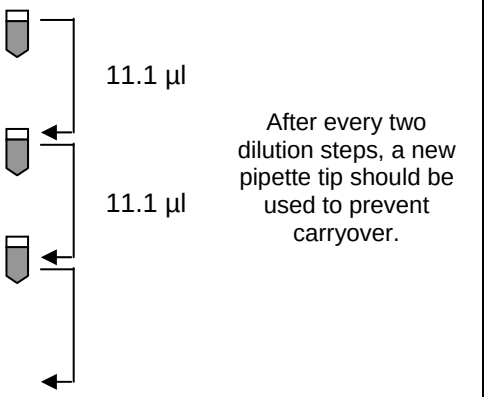
Samples: Human sera or EDTA, heparin or citrate plasma.

Stability: The patient samples to be investigated can generally be stored up to 14 days at a temperature between +2°C and +8°C. Diluted samples must be incubated within one working day.

Recommended sample dilution for qualitative evaluation: The sample to be investigated is diluted 1:10 in PBS-Tween. For example, dilute 11.1 µl sample in 100 µl PBS-Tween and mix thoroughly, e.g. vortex for 4 seconds.

Recommended sample dilution for semiquantitative evaluation: The dilution of samples to be investigated is performed using PBS-Tween. Add 100 µl of PBS-Tween to each tube and mix with 11.1 µl of the next highest concentration, e.g. vortex for 2 seconds. EUROIMMUN recommends incubating samples from a dilution of 1:10.

Dilution	Dilution scheme
1:10	100 µl PBS-Tween + 11.1 µl undiluted sample
1:100	100 µl PBS-Tween + 11.1 µl 1:10 diluted sample
1:1000	100 µl PBS-Tween + 11.1 µl 1:100 diluted sample
⋮	⋮

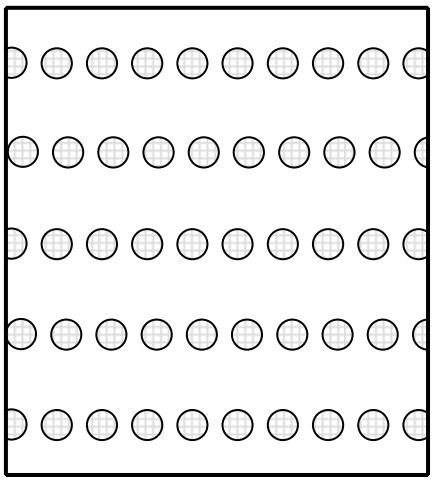


Evaluation

Fluorescence pattern (positive reaction): Antibodies against **renal glomeruli** react with the basement membrane of the glomerulus capillaries (GBM). A fine linear fluorescence becomes visible in the glomeruli. The pattern obtained is essentially the same as for the positive control serum. A linear fluorescence in the **renal tubuli** is an indication of autoantibodies against antigens of the tubular basement membrane. The glomeruli remain negative.
Antibodies against alveolar basement membrane react with the primate lung. A fine linear fluorescence of the basement membrane of the **lung alveoli** becomes visible.

The **GBM EUROPLUS BIOCHIPs** were coated with highly purified antigen in the form of microscopically small droplets. If GMB-specific antibodies are present in the serum, the circular areas show a green fluorescence in front of a dark background.

In a **negative reaction** the entire GMB BIOCHIP remains dark, the described circular areas can hardly be detected or not detected at all. For a secure differentiation between positive and negative results the positive and negative control sera and, if necessary, several normal sera must be compared with the patient samples.



BIOCHIP coated with GBM antigen

If the positive control shows no specific fluorescence pattern or the negative control shows a clear specific fluorescence the results are not to be used and the test is to be repeated.

A large range of fluorescence images can be found on the EUROIMMUN website (www.euroimmun.com).

Recommended qualitative evaluation:

Anti-glomerular, alveolar basement membrane and GBM BIOCHIP reactivity (IgG)	**Evaluation**
No reaction at 1:10	Negative. No antibodies against glomerular and alveolar basement membrane and GBM detected in the patient sample.
Positive reaction at 1:10	Positive. Indication of glomerulonephritis, Goodpasture's syndrome.

Recommended semiquantitative evaluation: The titer is defined as the sample dilution factor for which specific fluorescence is just identifiable. This should be compared to the reaction obtained with an equivalently diluted negative serum.

Antibody titers can be determined according to the following table from the fluorescence of the different sample dilutions.

Fluorescence at				Antibody titer
1:10	1:100	1:1000	1:10,000	
weak	negative	negative	negative	1:10
moderate	negative	negative	negative	1:32
strong	weak	negative	negative	1:100
strong	moderate	negative	negative	1:320
strong	strong	weak	negative	1:1000
strong	strong	moderate	negative	1:3200
strong	strong	strong	weak	1:10,000
⋮	⋮	⋮	⋮	⋮

Limitations of the procedure

1. A diagnosis should not be made based on a single test result. The clinical symptoms of the patient should always be taken into account along with the serological results by the physician.
2. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
3. Mishandling of slides during the staining procedure, especially allowing slides to dry between steps, may result in a “washed out” pattern appearance and/or a high level of background staining.
4. Coplin jars used for slide washing should be free from all residues. Use of coplin jars containing residues may cause staining artefacts.
5. The light source, filters and optical unit of the fluorescence microscope can influence the sensitivity of the assay. Using traditional mercury vapour lamp systems, the performance of the microscope depends on correct maintenance, especially alignment of the lamp and replacement of the lamp after the recommended period of time. The EUROIMMUN fluorescence microscopes with LED Bluelight as the light source offer many advantages. Contact EUROIMMUN for details.

Test characteristics

Antigen: For the detection of autoantibodies against glomerular basement membrane (GBM) by indirect immunofluorescence, the standard substrate used are frozen sections of primate kidney. Antibodies against alveolar basement membrane are detected using frozen sections of primate lung.

GBM BIOCHIPs are coated with recombinant GBM antigen, which corresponds to the shortened alpha-3 chain of type IV collagen (NC-1 domain). By the use of specific GBM BIOCHIPs, positive results can be confirmed in one and the same test.
In case of unclear fluorescence patterns, e.g. caused by unspecific antibody or serum reactions, they help to facilitate evaluation.

Measurement range: The dilution starting point for this measurement system is 1:10. Samples can be further diluted by a factor of 10 so that the dilution series is 1:100, 1:1000, 1:10,000 etc. There is no upper limit to the measurement range.

Reproducibility: The intensity of the specific fluorescence as a numeric value is called fluorescence intensity level by EUROIMMUN. These values can reach from “0” (no specific fluorescence) to “5” (extremely strong specific fluorescence).

Reproducibility	Inter-lot	Intra-assay	Inter-assay
Minimum requirement	3 lots x 3 samples x 1 run x single determination: max. ± 1 intensity level	1 lot x 3 samples x 1 run x tenfold determination: max. ± 1 intensity level	1 lot x 3 samples x 2 runs x double determination: max. ± 1 intensity level
Kidney (monkey)	Maximum deviation ± 1 intensity level	Is assured since inter-lot reproducibility was investigated with more than 10 lots.	Is assured since inter-lot reproducibility was investigated with more than 10 lots.
GBM BIOCHIP	No deviation	No deviation	Maximum deviation ± 1 intensity level

Cross reactivity: There is no data known to EUROIMMUN in which cross reactivity is described.

Interference: Haemolytic, lipaemic and icteric samples showed no influences on analysis results with one exception: lipaemic samples may increase background staining on lung tissue which can interfere with the specific fluorescence of weak positive samples leading to higher titers.

Reference range: Titer 1: < 10

The following antibody prevalences were determined using a panel of samples from healthy blood donors (origin: Germany):

Substrate	Antibodies against	Conjugate	Prevalence	Cut-off	Number of samples
Kidney (monkey)	GBM	IgG	0%	1:10	137
	TBM		0.7%		
GBM BIOCHIPs	GBM		0%		198
Lung (monkey)	GBM		0%		137

Specificity and sensitivity:

Substrate	Ig class	Reference (number and origin of samples)	Specificity	Sensitivity
Kidney (monkey): Anti-GBM	IgG	Reference centres (n = 128, origin: Europa)	100%	100%
		Patients with Goodpasture´s syndrome (n = 14, origin: Germany)	-	100%
GBM BIOCHIPs: Anti-GBM		Panel of healthy blood donors (n = 100, origin: Germany)	100%	-
		Anti-GBM ELISA (n = 44, origin: Germany)	-	100%

Clinical significance

Autoimmune diseases of the kidneys and lungs are generally life-threatening. They include primary membranous glomerulonephritis (pMGN), an autoimmune glomerulonephritis which is also known by the incorrect term idiopathic membranous glomerulonephritis (idiopathic membranous nephropathy, IMN), and Goodpasture's syndrome. Secondary membranous glomerulonephritis is caused by non-renal diseases such as infections, intoxications, diabetes mellitus, amyloidosis, etc.

Glomerulonephritis (actually glomerulitis), primary or secondary, is an inflammation of the glomeruli (kidney filters, part of the 1.2 million nephrons of the kidney). A chronic course leads to glomerulosclerosis, which is the main cause of dialysis-dependent kidney failure.

Clinically, glomerulonephritis usually manifests as nephritic syndrome with haematuria, proteinuria and reduced kidney function or as nephrotic syndrome with protein excretion in the urine (>3.0 to 3.5 g/d in adults), hypoalbuminaemia, hyperlipidaemia, thrombophilia and oedema.

A special form of autoimmune glomerulonephritis is **Goodpasture's syndrome** (pulmorenal syndrome), named after the US-American pathologist Ernest William Goodpasture (1886-1960), who in 1919 described the combination of glomerulonephritis with lung bleeding. Clinically, the simultaneous occurrence of rapid progressive anti-basement membrane glomerulonephritis and lung haemosiderosis is a strong indication of this disease. Lung bleeding is often the first sign of the disease. Both fulminant and abortive forms can occur. Around 70% of affected individuals are men, predominantly young adults. If therapy is commenced at an early stage (immunosuppression, plasmapheresis until remission), renal function is preserved in 60% of patients. However, relapses are possible.

Primary membranous glomerulonephritis (pMGN), which is often called just membranous glomerulonephritis, is a chronic inflammatory disease of the renal corpuscle (glomeruli), which is accompanied by a progressive reduction in kidney function. MGN is the most frequent kidney disorder with nephrotic syndrome. With increasing proteinuria, the long-term risk of kidney failure with major morbidity and mortality rises, particularly in connection with thromboembolic and cardiovascular complications.

MGN is prevalent in all ethnic groups and genders, with men over 40 years of age and of white skin colour being more frequently affected. In young women with suspected MGN, lupus nephritis should also be considered. MGN is much rarer in children (only 2 to 3% of kidney disorders in children).

Symptoms in MGN:
- Around 80% of MGN patients suffer from nephrotic syndrome with sometimes severe oedema in the legs and eye lids, weight gain and reduced urination.
- Around 20% of patients have proteinuria without any additional symptoms. Fatty bodies, droplets or cylinders are frequently found in the urine sediment.
- Around 50% of patients have microscopic haematuria, albuminuria and glucosuria.
- Around 70% of patients show normal blood pressure and kidney function at the onset of the disease.

In MGN there is a strong association with an allele on chromosome 6p21 which encodes an HLA class II antigen (HLA-DQA1). This allele most likely facilitates an autoimmune reaction against self-antigens such as variants of PLA2R (phospholipase A2 receptor).

Autoantibodies against GBM antigens (basement membrane of the glomeruli) are detectable in autoimmune glomerulonephritis. They can target the alveolar basement membrane in addition to the glomerular basement membrane. Anti-GBM glomerulonephritis accounts for 0.5 to 2% of all glomerulonephritides.

Circulating antibodies against antigens of the kidney tubule basal membrane react predominantly in the region of the proximal tubules. They can be found in different forms of nephritis including post-transplantation rejection reactions, and they aid differential diagnosis of tubular interstitial diseases.

In cases without lung involvement GBM antibodies are detected in the serum or plasma of over 60% of patients, in cases with lung involvement in over 90%. They are predominantly of class IgG, more rarely of class IgA and IgM.

The primary target antigen of all anti-GBM glomerulonephritides, including the classic Goodpasture's syndrome, is the NC1 region of the alpha 3 chain of the network-structured type IV collagen of the basal membrane lamina densa. These autoantibodies can be detected by IIFT or ELISA. The antibody concentration correlates with the clinical course of disease. High-titer circulating GBM antibodies indicate an unfavourable progression.

The serological investigation can be supplemented by the determination of autoantibodies against PLA2R and against pANCA or cANCA. A positive result indicates rapid progressive glomerulo-nephritis or Wegener’s granulomatosis. pANCA vasculitis with primarily mild glomerulonephritic symptoms can also develop into severe anti-GBM glomerulonephritis.

With a negative serum result and continuing suspicion of anti-GBM glomerulonephritis, a kidney biopsy should be performed.

Autoantibodies against TBM antigens (basement membrane of the tubuli) can be detected in idiopathic interstitial nephritis, drug-induced interstitial nephritis and anti-basement membrane glomerulonephritis. Since their pathogenetic significance is not yet completely clear, the additional determination of GBM autoantibodies, pANCA and/or cANCA and anti-dsDNA is indicated in the diagnosis of autoimmune kidney diseases.

Autoantibodies against ABM antigens (basement membrane of the alveoli) are found in Goodpasture's syndrome and in some patients with idiopathic pulmonary bleeding. These antibodies are directed against particular epitopes of subunits of the glomerular and/or the alveolar basement membrane. In cases without lung involvement, basement membrane antibodies can be detected in the serum of 60% of patients, in cases with lung involvement in 80 to 90%. These antibodies belong mainly to the IgG class, rarely to the IgA class.

The alveolar and glomerular basement membranes contain biochemically identical target antigens, although in different configurations. The accessibility of the epitopes leads to different antibody reactivities in the lungs and the kidneys. Reactions with components of the basement membrane in other tissues that contain collagen type IV (in monomeric form) have also been described. High anti-ABM serum titers correlate with an unfavourable prognosis.

ABM autoantibodies are detected by IIFT using frozen sections of primate lung as the substrate. In Goodpasture's syndrome the antibodies react with the basal laminae of primate kidney and primate lung. The lung tissue is also attacked by anti-endothelial cell antibodies.

Autoantibodies against PLA2R are detected in membranous glomerulonephritis (MGN). In 2002 neutral endopeptidase (NEP) was identified as the antigen responsible in a subgroup of infants with prenatal membranous glomerulonephritis. This discovery provided evidence that an antigen on the podocytes of the basement membrane was the target for podocytopathic antibodies. In 2009 the M type phospholipase A2 receptor (PLA2R) was identified as the most important podocyte antigen involved in MGN.

Autoantibodies against PLA2R can be detected using immunohistochemical procedures. If autoantibodies of class IgG against PLA2R are present in a sample, a protein band of around 185 kDa can be found in western blot analysis based on extracts from human kidney tissue or recombinant PLA2R. The antigen used in the standardised ELISA is the purified protein of the extracellular domain of PLA2R1 expressed in HEK293. The Anti-PLA2R1 IgG ELISA is characterised by very high specificity and sensitivity. A similarly high sensitivity is provided by the Anti-PLA2R1 IgG4 ELISA. The test of choice (gold standard) for serological screening is the Anti-PLA2R IIFT using transfected cells as the standard substrate. This method is suited for qualitative and quantitative determination of human autoantibodies of class IgG against PLA2R. The sensitivity of the test system with respect to patients with MGN is up to 70%; the specificity with respect to apparently healthy blood donors is 100%. The Anti-PLA2R IIFT can be used to assess the activity of MGN (severity and duration) and the success of therapeutic measures. This can be achieved by determining the increase, decrease or disappearance of autoantibodies, whereby the serological result always precedes the clinical picture (e.g. with respect to the severity of proteinuria).

Autoantibodies against laminin (next to type IV collagen a further essential component of the basement membrane, of which 15 laminin isoforms are currently known), can bind to antigens of the basement membrane of various tissues (e.g. lung, kidney and epidermis). Little is known at present about the pathogenetic significance of these glycoproteins. Antibodies against laminin A, B or C are also found in patients with suspected SLE. Furthermore, laminin-5 (epiligrin) antibodies are detectable in IgA nephropathy, bullous dermatosis, pemphigoid and Chagas myocarditis.

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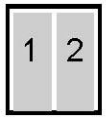

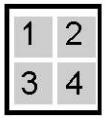

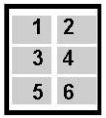

This test instruction is valid for the following test kits (#### is a place holder for different test formats, e.g. 1005 = 10 slides with 5 fields):

Order no.	Description	BIOCHIPs per field						Field size (mm)
		1	2	3	4	5	6	
FA 1250-####	IIFT: Kidney (Monkey)	Kidney, monkey						5 x 5
FA 1250-####-1	EUROPLUS: Kidney (monkey)/GBM	Kidney, monkey	GBM BIOCHIPs					5 x 5
FA 1271-####-1	IIFT Mosaic: Kidney (Monkey)/Lung (Monkey)	Kidney, monkey	Lung, monkey*					5 x 5

* For the clinical evaluation the results obtained must be confirmed with a CE marked test system.