Anti-Phospholipase A2 Receptor IIFT (IgG)

- **High sensitivity and maximal specificity for primary membranous nephropathy (MN)**
- **Ideally suited for differentiation of primary and secondary MN**
- **Reliable screening test for qualitative and semiquantitative autoantibody determination**

Technical data

Antigen substrate	Transfected cells and control-transfected cells (EU 90)
Sample material	Serum or plasma
Sample dilution	Qualitative 1:10; semiquantitative: 1:10, 1:100, 1:1000 etc.
Reagents	Ready for use, with the exception of the PBS Tween buffer
Test procedure	30 min (sample) / 30 min (conjugate), room temperature
Microscopy	Objective: 20x, light source: EUROIMMUN LED, EUROStar Bluelight or mercury vapour lamp, 100 W Excitation filter: 450-490 nm, colour separator: 510 nm, blocking filter: 515 nm
Stability	18 months from the date of manufacture when stored at +2 °C to +8 °C
Test kit format	10 slides, each containing 3, 5 or 10 test fields
Order number	**FA 1254-####-50 G**
Related products	FC 1254-####-50 G Anti-Phospholipase A2 receptor (PLA2R) IIFT EUROPattern

Clinical significance

Primary membranous nephropathy (MN) is a chronic inflammatory disease of the glomeruli, which is accompanied by an increasing restriction of the kidney function. The underlying autoimmune mechanism is based on the reaction of autoantibodies directed against the glycoproteins phospholipase A_2 receptor (PLA_2R) and thrombospondin type-1 domain-containing protein 7A (THSD7A). These transmembrane proteins are expressed on the surface of podocytes in human glomeruli. As a result of the binding of antibodies, the podocytes are damaged and protein enters the primary urine (proteinuria). MN is the most frequent kidney disorder with nephrotic syndrome in adults. The disease is prevalent in all ethnic groups and genders, with Caucasian men over 40 years of age being more frequently affected. In young women with suspected primary MN, lupus nephritis should also be considered. Primary MN occurs very rarely in children. The primary form should be discriminated from the secondary form, which is a secondary disease that can occur in infections, in drug therapy or abuse or intake of toxins, in collagenoses and other autoimmune diseases and in tumours, and which improves with treatment of the underlying disease. The treatment of primary MN improves prognosis, particularly with respect to nephrotic syndrome and hypertonicity. It is known since 2014 that circulating autoantibodies against THSD7A are mainly detected in patients with primary MN who are negative for anti-PLA_2R antibodies. In rare cases, autoantibodies against PLA_2R and THSD7A may also occur together. Whereas autoantibodies against PLA_2R are found in the serum of up to 75 % of patients with primary MN, the prevalence of anti-THSD7A varies from 2 % to up to 14 %, depending on the primary MN cohort.

Diagnostic application

The Anti-Phospholipase A_2 Receptor (PLA_2R) IIFT is a well-established screening test for qualitative and semiquantitative serological detection of anti-PLA_2R antibodies. Autoantibodies of class IgG against PLA_2R are highly specific for the diagnosis of primary MN and can be detected in the serum of up to 75 % of patients. In healthy persons and patients with secondary MN anti-PLA_2R autoantibodies are only very rarely found. Therefore, the detection of these antibodies is helpful in the differentiation of primary and secondary MN. The serological detection rate is increased by using a two-step screening strategy, additionally investigating patients with a seronegative anti-PLA_2R result for anti-THSD7A antibodies.

EUROIMMUN AG · Seekamp 31 · 23560 Luebeck (Germany) · Tel +49 451 5855 0 · Fax +49 451 5855 591 · euroimmun@euroimmun.de · www.euroimmun.com

Evaluation

Fluorescence pattern (positive reaction): Antibodies against phospholipase A_2 receptor (PLA_2R) react with the transfected cells of the substrate. They cause a fluorescence of the cytoplasm, partly including the cell membrane. The cell nuclei are only weakly stained.

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
PLA_2R-transfected cells	**PLA_2R**	IgG	0%	1:10	178

Sensitivity and specificity

A total of 560 clinically characterised samples (275 from patients with primary membranous nephropathy (MN), 285 from control groups) were investigated for anti-PLA_2R antibodies (IgG) in different clinical studies. Primary MN diagnosis was based on kidney biopsy. The disease was considered as idiopathic/primary when no secondary cause of MN was suspected on the basis of clinical and laboratory criteria. The samples were drawn eight weeks after biopsy, before treatment. Patients who had been or were being treated with immunosuppressive drugs at that time were excluded, as were patients with a history of medication and neoplasia. With the Anti-PLA_2R IIFT using the cut-off dilution of 1:10, a sensitivity of 77.1% was found in MN, which is the expected value of approx. 75% reported in scientific literature. The specificity was 100%.

Cohort (n = 560)	n	Anti-PLA_2R IIFT positive
Primary MN	275	212
Clinical sensitivity	**275**	**77.1%**
Secondary MN	68	0
Non-membranous MN	63	0
Systemic lupus erythematosus	30	0
Systemic sclerosis	30	0
Psoriasis arthritis	30	0
Rheumatoid arthritis	14	0
Thyroiditis	50	0
Clinical specificity	**285**	**100%**

Literature

1. Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009 Jul 2;361(1):11-21.
2. Dähnrich C, Komorowski L, Probst C, Seitz-Polski B, Esnault V, Wetzels JF, Hofstra JM, Hoxha E, Stahl RA, Lambeau G, Stöcker W, Schlumberger W. Development of a standardized ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. Clin Chim Acta. 2013 Jun 5;421:213-8.
3. Hoxha E, Harendza S, Zahner G, Panzer U, Steinmetz O, Fechner K, Helmchen U, Stahl RAK. An Immunofluorescence Test for Phospholipase-A2-Receptor Antibodies and its Clinical Usefulness in Patients with Membranous Glomerulonephritis. Nephrol Dial Transplant. 2011, 26: 2526-2532.
4. Hoxha E, Kneißler U, Stege G, Zahner G, Thiele I, Panzer U, Harendza S, Helmchen UM, Stahl RA. Enhanced expression of the M-type phospholipase A2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy Kidney Int. 2012 Oct;82(7):797-804.
5. Hoxha E, Thiele I, Zahner G, Panzer U, Harendza S, Stahl RA. Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. J Am Soc Nephrol. 2014 Jun;25(6):1357-66.
6. Larsen CP, Cossey LN, Beck LH. THSD7A staining of membranous glomerulopathy in clinical practice reveals cases with dual autoantibody positivity. Mod Pathol. 2016 Apr;29(4):421-6.
7. Stahl R, Hoxha E, Fechner K. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. N Engl J Med. 2010 Jul 29;363(5):496-8.
8. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, Dolla G, Hoxha E, Helmchen U, Dabert-Gay A-S, Debayle D, Merchant M, Klein J, Salant DJ, Stahl RAK, Lambeau G. Thrombospondin Type-1 Domain-Containing 7A in Idiopathic Membranous Nephropathy. N Engl J Med. 2014, 371(24): 2277-2287.

EUROIMMUN AG · Seekamp 31 · 23560 Luebeck (Germany) · Tel +49 451 5855 0 · Fax +49 451 5855 591 · euroimmun@euroimmun.de · www.euroimmun.com

FA_1254_D_UK_A06, 07/2016