



Anti-PLA₂R ELISA (IgG)



- High sensitivity and maximal specificity for primary membranous nephropathy (MN)
- Ideally suited for differentiation of primary and secondary MN
- Antibody titer allows assessment of therapy success, disease course and risk of relapse

Technische Daten

Antigen	Recombinant phospholipase A ₂ receptor			
Calibration	Quantitative, in relative units per millilitre (RU/ml)			
	Calibration serum 1:	2 IU/ml	Calibration serum 4:	500 RU/ml
	Calibration serum 2:	20 IU/ml	Calibration serum 5:	1500 RU/ml
	Calibration serum 3:	100 RU/ml		
	Recommended upper threshold of the normal range (cut-off value): 20 RU/ml			
Sample dilution	Serum or plasma, 1:101 in sample buffer			
Reagents	Ready for use, with the exception of the wash buffer (10x)			
Test procedure	30 min / 30 min / 10 min, room temperature, fully automatable			
Measurement	450 nm, reference wavelength between 620 nm and 650 nm			
Test kit format	96 break-off wells; kit includes all necessary reagents			
Order no.	EA 1254-9601 G			

Clinical significance

Primary MN is a chronic inflammatory disease of the glomeruli which is accompanied by a progressive impairment of the kidney function. The underlying autoimmune mechanism, which was first discovered and described in 2009, is the result of autoantibodies reacting with phospholipase A₂ receptors (PLA₂R), which are expressed in human glomeruli on the surface of podocytes. As a result, the podocytes are damaged and protein enters the primary urine (proteinuria). Primary MN 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. Primary MN 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 primary MN, lupus nephritis should be considered. Primary MN is rare in children (only 2% to 3% of kidney disorders in children). Primary MN should be discriminated from secondary membranous nephropathy, 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.

Diagnostic application

Autoantibodies of class IgG against phospholipase A₂ receptors (PLA₂R) are highly specific for the diagnosis of primary MGN. They can be detected in the serum of up to 70% to 75% patients. The ELISA allows qualitative and quantitative determination of human autoantibodies of class IgG against PLA₂R. The anti-PLA₂R antibody titer is suited for assessing the therapy success. A titer increase, decrease or disappearance precedes a change in the clinical status. Thus, the determination of the antibody titer has a high predictive value with respect to clinical remission or relapse and risk estimation after kidney transplantation.



Reference range

The levels of anti-PLA₂R antibodies were investigated in 191 sera from healthy blood donors using the EUROIMMUN Anti-PLA₂R ELISA (IgG). The mean concentration of antibodies against PLA₂R was 0.4 RU/ml and the values ranged from 0.0 to 5.0 RU/ml. With a cut-off of 20 RU/ml no blood donor was anti-PLA₂R positive.

Blood donors (n = 191)		
Percentile	99 th	100 th
Cut-off	3.8 RU/ml	5.0 RU/ml

Reproducibility

The reproducibility was investigated by determining the intra- and inter-assay coefficients of variation using three sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on three determinations performed in ten different test runs.

Serum	Intra-assay variation, n = 20		Inter-assay variation, n = 3 x 10	
	Mean value (RU/ml)	CV (%)	Mean value (RU/ml)	CV (%)
1	26	3.4	28	4.2
2	97	1.7	99	6.4
3	861	5.7	878	10.3

Sensitivity and specificity

The anti-PLA₂R antibody concentrations were determined in 198 sera from patients with primary membranous nephropathy (MN), 545 sera from a control panel with other diseases (e.g. lupus type V, ANCA-associated vasculitis, systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome) and in 291 sera from healthy blood donors using the EUROIMMUN Anti-PLA₂R ELISA (IgG). The ELISA achieved a sensitivity of 96% and a specificity of 99.9%. Borderline samples were excluded from the calculation.

Cohort (n = 1034)	n	Anti-PLA ₂ R ELISA (IgG) positive/borderline
Primary MN ^{1,2,3}	198*	190
Sensitivity	198	96%
Other nephritides ^{1,2}	230	0
Other autoimmune diseases	315	0
Healthy blood donors	291	1
Specificity	836	99.9%

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*Anti-PLA₂R-positive sera from patients with primary MN

	Results for discrepant sera from the pMN panel							
	1	2	3	4	5	6	7	8
Anti-PLA ₂ R IgG positive IIFT	1:100	1:100	1:100	1:32	1:32	1:32	1:32	1:10
Anti-PLA ₂ R IgG negative ELISA (RU/ml)	10	6	5	13	7	3	3	6

Primary MN panel (n = 198)	IIFT		
	positive	borderline	negative
EUROIMMUN Anti-PLA ₂ R ELISA (IgG)			
positive	188	0	0
borderline	2	0	0
negative	8	0	0

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. 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.
3. 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.
4. Hoxha E, Harendza S, Pinnschmidt H, Panzer U, Stahl RA. PLA₂R antibody levels and clinical outcome in patients with membranous nephropathy and non-nephrotic range proteinuria under treatment with inhibitors of the renin-angiotensin system. *PLoS One.* 2014 Oct 14;9(10):e110681.
5. Ruggenti P, Debiec H, Ruggiero B, Chianca A, Pellé T, Gaspari F, Suardi F, Gagliardini E, Orisio S, Benigni A, Ronco P, Remuzzi G. Anti-Phospholipase A2 Receptor Antibody Titer Predicts Post-Rituximab Outcome of Membranous Nephropathy. *J Am Soc Nephrol.* 2015 Oct;26(10):2545-58.
6. Hoxha E, Harendza S, Pinnschmidt HO, Tomas NM, Helmchen U, Panzer U, Stahl RA. Spontaneous remission of proteinuria is a frequent event in phospholipase A2 receptor antibody-negative patients with membranous nephropathy. *Nephrol Dial Transplant.* 2015 Nov;30(11):1862-9.