Guide to Autoimmune Testing

Wieslab

The Autoimmune Laboratory

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Preface

Wieslab in Malmö, Sweden, is Scandinavia's biggest private special laboratory for the diagnosis of autoimmune diseases. Wieslab was founded in 1991 and has been an independent division of Euro-Diagnostica since 2004. In 2010 Wieslab opened a new laboratory in Nijmegen, The Netherlands. The laboratory has established a high level of expertise in kidney and lung related autoimmune diseases, e.g. vasculitis. Autoimmune diseases is a complex and demanding field of medicine in constant development. Wieslab strives actively to be at the front of this development to be able to offer new and sometimes unusual analyses for the diagnosis of rare autoimmune diseases.

In 1996 the first *Guide to Autoimmune Testing* was published. The initiative to publish a brief summary of analyses in the autoimmune area came as a result of the great interest in this exciting and rapidly growing area. From the very beginning our *Guide to Autoimmune Testing* attracted a lot of interest among both laboratory personnel and personnel in clinical care. Due to the continued great interest we now also have the pleasure of presenting the first *Apple iPhone* application on diagnosing autoimmune diseases.

Jörgen Wieslander, PhD, Professor, Research Director
Martin Olsson, MD, Senior Clinical Immunologist, Medical Director

Practical Information

When do I order an autoantibody analysis?
On suspicion of chronic or acute inflammatory diseases with the presence of autoantibodies, in particular to confirm or exclude the diagnosis, and to assess disease activity, prognosis and effect of treatment of certain diseases.

Remember!
On suspicion of an autoimmune disease it should be noted that findings of autoantibodies can only be used to support the diagnosis, as autoantibodies may occur without a disease or as a transient phenomenon during infection. A positive or negative result of a test can therefore not be used for a diagnosis, if there are no defined clinical disease criteria. In some diseases it may be appropriate to monitor the concentration of autoantibodies with regard to the development of manifest disease, on other occasions with regard to the assessment of disease activity, prognosis or effect of the treatment.

What should I send?
Take a venous or capillary blood sample in a tube without additive, let the sample clot and centrifuge it. The sample can be kept in a refrigerator while waiting for transport. Send 1-3 ml serum by ordinary mail. This will be enough for one or several analyses. For complement factor C3d, EDTA plasma is also required. Acute analyses should be sent by taxi or courier.

What acute analyses are offered?
Acute analyses for anti-GBM and ANCA are performed all days of the year. For analyses on evenings and weekends, please call the laboratory to discuss means of transportation.

Contact us!
E-mail: info@wieslab.com, web page: www.wieslab.com
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Panels of Analyses Based on Clinical Suspicion

The analysis panels are based on clinical suspicion or symptoms. They are presented with analyses, indications and clinical background. For several of them you will find flow charts of recommended investigation strategies. There are also often other individual analyses than the ones that are included in the panel and that may complement the investigation.

Antiphospholipid syndrome (APS)

Analyses
ANA screen (HEp-2 cells), anti-dsDNA, anti-cardiolipin and anti-b2 glycoprotein 1.

Indication
Suspicion of antiphospholipid syndrome. Thromboembolism in patients without other risk factors. Unexplained prolonged APT time. Repeated spontaneous abortions.

Clinical background
Antiphospholipid syndrome (APS) is characterized in the patient by thromboses in the larger arteries and veins and thrombotic microangiopathy. Common manifestations are deep vein thrombosis, thrombocytopeny and neurological symptoms or repeated spontaneous abortions. APS occurs in up to a third of patients with lupus erythematosus. Diagnostic criteria suggested for APS are at least one clinical manifestation of thromboembolism or pregnancy complication and at least one laboratory criterion, see below.

The increased tendency to thrombosis formation is due to the presence of antibodies against phospholipids, which can inhibit both pro- and anticoagulative components of the coagulation system. In vitro the antibodies inhibit the phospholipid-dependent coagulation factors, which leads to a prolonged APT time.

Laboratory tests for antiphospholipid syndrome
Lupus anticoagulans: (Performed in clinical chemistry laboratories with different methods.) Detection of prolonged APT time that is not due to lack of individual coagulation factors and that is restored by adding phospholipids to the sample.

Anti-cardiolipin: Cardiolipin is a phospholipid. Antibodies against cardiolipin occur in APS and in up to 30% of patients with SLE. Anti-cardiolipin occurs in some percentage of healthy controls and it is not clear what the clinical significance is. In SLE, however, more than half the patients with anti-cardiolipin develop APS. Anti-cardiolipin also occurs in certain infections, like borreliosis, syphilis and malaria and then is not associated with APS.

Anti-b2-glycoprotein 1: b-2glycoprotein 1 is a cofactor of cardiolipin in the coagulation cascade. Anti-b2-glycoprotein is more specific than anti-cardiolipin, as it does not occur in infectious conditions (38-40).

Autoimmune hepatitis

The panel contains tests for a basic investigation. Suggestions for an expanded investigation are given in the text and in the flow chart.

Analyses
ANA screen (HEp-2 cells), smooth muscle antibodies (SMA), SLA/LP, liver/kidney microsomes (LKM) and mitochondria (AMA). Expanded analysis: LKM-1, LC-1, Mitochondria type M2.

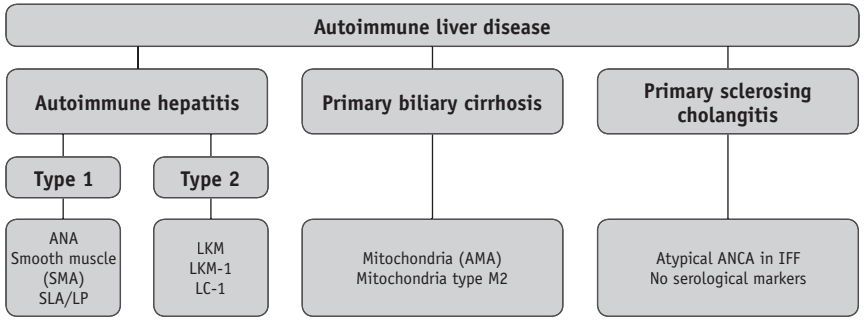
Indication
Suspicion of autoimmune liver disease.

Clinical background
Different types of autoimmune hepatitis can affect both sexes of all ages. The clinical picture may vary from a mild or even subclinical disease to acute relapses of liver failure. The onset of the disease may also be cryptogenic cirrhosis. In autoimmune hepatitis type 1 70% of the patients have antibodies against smooth muscle, 10% have antibodies against SLA/LP. Isolated occurrence of anti-SLA/LP has been reported. ANA is often positive in autoimmune hepatitis type 1 with varying patterns.

Autoimmune hepatitis type 2 is not common in northern Europe and mainly affects young women. The disease is defined by the presence of antibodies against LKM, LKM1 or LC1.

Most patients with primary biliary cirrhosis have antibodies against mitochondria (AMA and M2).

There is no specific serological marker for primary sclerosing cholangitis, even if these patients in 20-30% of the cases show a positive ANA with a pattern of nuclear dots. Some patients have atypical ANCA with immunofluorescence investigation (54-58).



Serological investigation of autoimmune liver disease.

Coeliac disease

Analyses
Anti-transglutaminase IgA, anti-enodomysium IgA, anti-deamidated gliadin (DGP) IgG, IgA quantification.

Indication
Suspicion of coeliac disease or dermatitis herpetiformis. Control of effect of gluten-free diet.

Clinical background
Coeliac disease (gluten intolerance) is defined as a typical mucosal change of the small intestine, caused by gluten in the diet. In ”classical” coeliac disease the patient has diarrhea, weight loss and various deficiencies due to inadequate nutrient uptake. Today milder forms of the disease are often diagnosed with less serious symptoms, e.g. abdominal pains, stomach gas, tiredness, anemia and osteoporosis. About 1% of the population is considered to suffer from the disease and it often occurs together with other autoimmune diseases, such as diabetes, autoimmune thyroid disease and psoriasis. About 2-5% of the patients may have IgA deficiency. If this is the case, tests for IgG anti-tTG and IgG anti-gliadin should be performed. In small children anti-gliadin may be the only antibody. The final diagnosis is made through small intestine biopsy.

Coeliac disease has strong genetic links to HLA-DQ2 and HLA-DQ8. If both HLA markers are negative, the diagnosis can almost be excluded.

Anti-tTG and anti-endomysium are considered to have almost 100% sensitivity and specificity in clinical coeliac disease. The level of the antibodies often correlates with mucosal change and therefore a lower sensitivity is observed early in the disease and when there are minor changes of the small intestine. This means that repeated testing has to be done when screening early in the disease. The antibodies normally disappear with gluten-free diet (53).

Congenital heart block

Analyses
SSA/Ro52 and Ro60, SSB/La and SSA p200.

Indication
Pregnant women with known autoimmune disease, mainly SLE or Sjögren’s syndrome. Low heart rate in foetus in routine control. Patients who have given birth to babies with congenital heart block or myocarditis.

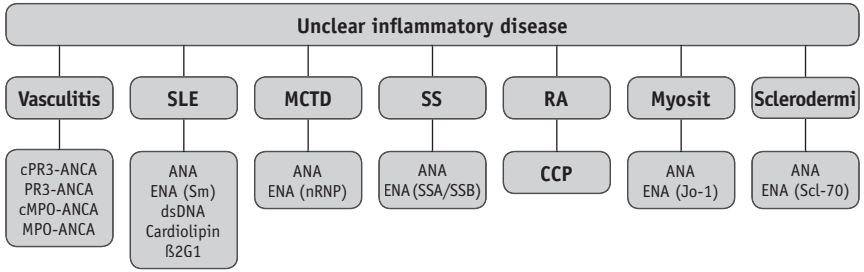
Clinical background
Antibodies against Ro or La can be transferred from mother to child through the placenta. These antibodies may cause neonatal lupus, whose most serious manifestation is congenital heart block. In many cases the mother is asymptomatic and only later, if at all, does she develop a manifest autoimmune disease. In most cases of verified congenital heart block the antibodies are directed against part of the Ro52 antigen called p200. When these antibodies are detected in pregnant women, the heart of the foetus is examined repeatedly by ultrasound till the 24th week of pregnancy, when the risk of developing a heart block decreases (37, 42-45).

Connective tissue disease – undifferentiated

Analyses
PR3-ANCA, capture PR3-ANCA, MPO-ANCA, capture MPO-ANCA, ANA screen (HEp-2 cells), ENA screen, anti-dsDNA, anti-cardiolipin, anti-b2-glycoprotein 1, anti-CCP.

Indication
Unclear inflammatory conditions.

Clinical background
The panel may give some indication of a possible diagnosis in accordance with the flow chart. A negative result does not exclude the diagnosis.



Cystic fibrosis

Analyses
IgG-BPI-ANCA and IgA-BPI-ANCA.

Indication
Control of disease activity or assessment of prognosis of cystic fibrosis.

Clinical background
Cystic fibrosis is a genetically conditioned disease, where a defective ion channel in the airway epithelium gives rise to mucus. The viscous mucus gets colonized by bacteria. In a young child staphylococci and other Gram-positive bacteria are predominating, but in older patients Pseudomonas aeruginosa becomes the dominating pathogen. The prognosis of cystic fibrosis varies. Some patients lose lung function early on, whereas others remain relatively unaffected high up into adulthood despite chronic bacterial colonization. Colonization with P aeruginosa is usually established by cultivation but may be detected earlier by means of serology (antibodies against the bacteria). The level of antibodies correlates with the quantity of bacteria in the airways.

For reasons unknown many patients have autoantibodies against BPI, i.e. BPI-ANCA. IgG-BPI-ANCA is most common and may occur in up to 90% of the patients. About 40% have IgA-BPI-ANCA. There is a strong correlation between BPI-ANCA and colonization with P aeruginosa. Within the group of patients who are colonized by P aeruginosa a high level of BPI-ANCA is associated with a serious lung damage. It has been shown that positive IgA-BPI-ANCA is a risk factor for developing respiratory insufficiency within 5 years.

IgA-BPI-ANCA has a stronger correlation to lung function impairment and P aeruginosa colonization than IgG-BPI-ANCA (14, 15, 85-91).

Diabetes

Analyses
Islet cell antibodies, anti GAD-65, anti IA-2, anti-insulin.

Indication
Suspicion of type 1 diabetes (insulin dependent diabetes mellitus, IDDM).

Clinical background
Type 1 diabetes is due to an autoimmune destruction of the insulin producing b-cells in the Langerhans islets in the pancreas. The destruction is probably triggered by one or more unknown environmental factors which in genetically predisposed individuals starts a slowly progressive process. Only when the number of viable b-cells is below a critical threshold, does hyperglycaemia with the accompanying symptoms arise. Islet cell antibodies are detected with IIF on monkey pancreas and the analysis is positive in 70-90% of IDDM patients at the time of the diagnosis. The antibodies react with glutamic acid decarboxylase (GAD), insulinom-associated protein 2 (IA-2), insulin and probably other unknown antigens. Specific antibodies against GAD 65, IA2 and insulin can be detected with immunoprecipitation. So far all prospective studies of relatives of type 1 diabetes patients have shown that the combination of two or more antibodies give a higher positive predictive value than only one antibody. Up to 90% of the asymptomatic antibody carriers will be insulin-dependent within 6 years (59, 60).

Drug induced lupus

Analyses
ANA screen (HEp-2 cells), anti-histones, ANCA – IIF, anti-cardiolipin.

Indication
Suspicion of drug induced lupus or vasculitis.

Clinical background
Through various mechanisms some drugs may give lupus like syndromes as a side effect. They may activate a latent lupus or cause a separate syndrome called drug induced lupus. The symptom comprises a constellation of systemic symptoms like fever, myalgia, arthritis and rashes. Renal effects and CNS symptoms are unusual. Differences and similarities between drug-induced lupus and spontaneous lupus are shown in the table below. The medicines that are mainly associated with drug induced lupus are procainamide, hydralazine, diltiazem and anti-TNF (tumour necrosis factor) preparations.

Patients with renal complications often have necrotizing glomerulonephritis that may be associated with P-ANCA, where the antibodies are usually directed against MPO or lactoferrine (52).

Phenomenon	SLE	Drug induced lupus
Age	20-40	50
Women:men	9:1	1:1
Onset	Gradual	Abrupt
Arthralgia	90%	95%
Rashes	74%	10-20%
Renal effects	53%	5%
CNS effects	32%	0%
ANA	95%	95%
Anti-Sm	20-30%	Unusual
Anti-histones	80%	90%
Complement	Activated	Normal

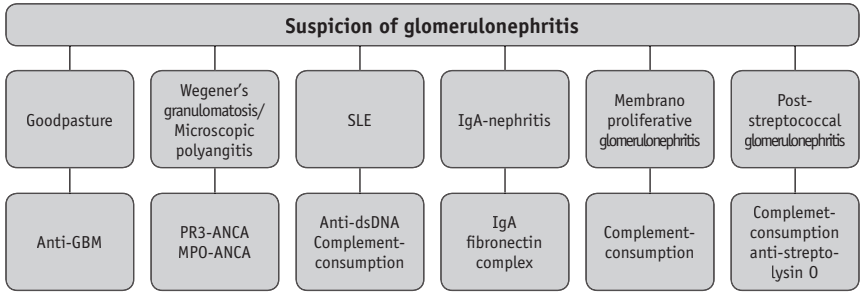
Organ symptoms in SLE and drug induced lupus.

Glomerulonephritis

Analyses
Anti-GBM, PR3-ANCA, capture PR3-ANCA, MPO-ANCA, capture MPO-ANCA, ANA screen (HEp-2 cells), anti-dsDNA, IgA-fibronectin complex, antistreptolysin O. Expanded analysis: C3, C4, C1q, complement function, C3d.

Indication
Renal failure of unknown cause.

Clinical background
Suspicion of glomerulonephritis arises as a rule when finding haematuria and/or proteinuria. The onset symptoms may also arise due to renal impairment. When investigating the urine sediment you usually see cylinders. The genesis of glomerulonephritis decides the treatment strategy and prognosis. The laboratory findings are a guide to diagnosis, even though it is generally believed that biopsy verification is necessary. The diagnosis of glomerulonephritis is described in detail in the appendix on page 143 (24-33).



Inflammatory bowel diseases (IBD)

Analyses
ANCA – IIF and ASCA. If ANCA – IIF is positive, a follow-up with ANCA expanded analysis is recommended to determine the specificity of ANCA.

Indication
Suspicion of and differentiation between inflammatory bowel diseases.

Clinical background
The clinical entities ulcerative colitis (UC) and Crohn's disease, the former with a predilection for rectum and colon, the latter for the ileocecal region and other bowel sections, are traditionally combined under the term inflammatory bowel diseases.

IgA antibodies against Saccharomyces cerevisiae (ASCA), occur in 60-75% of patients with Crohn's disease but are rare in patients with ulcerative colitis. Patients with UC have ANCA of the so-called P-ANCA type (also called atypical ANCA) in 40-50%. The combination of ASCA and ANCA is therefore used to distinguish UC from Crohn's disease, as a typical UC patient has ANCA whereas a typical Crohn's disease patient has ASCA.

The typical onset of ulcerative colitis occurs in rectum and propagates upwards in colon and engages the mucous membrane inflammatorily with ulcerations and bloody diarrhea as a result. A number of extraintestinal manifestations (sacroiliitis, uveitis, pyodermitis, erythema nodosum and sclerosing cholangitis) also occur. Crohn's disease is characterized pathologically anatomically by segmental extension and more profound inflammatory changes, comprising both the mucous membrane and the intestinal wall with granuloma formation, perforations and fistula formation in the surrounding tissues as a result. Diarrhea and abdominal pains are typical and can be followed by arthritis, uveitis, sclerosing cholangitis and cobalamine deficiency (in case of ileocecal engagement). The diagnosis is mainly based on the findings of sigmoideoscopy and coloscopy and what is found in biopsies from inflamed intestinal mucous membrane. Contrast radiography of the small intestine is also valuable. Fulminant ulcerative colitis (pancolitis, toxic mega colon) is usually preceded by bloody diarrhea but clinically it can resemble angry infectious and toxic colitis and acute diverticulitis and the intestinal involvement in acute systemic vasculitis, above all WG and polyartheritis nodosa (PAN). In WG necrotising small vessel vasculites dominate but also larger blood vessels are involved and when local multiple aneurysms develop that are detectable in contrast radiography, PAN can be suspected. In order to ensure the diagnosis and follow-up in case of less acute forms of proctitis, colitis and suspected Crohn's disease and of intestinal diseases with an atypical distribution and course, a referral to a gastroenterological unit with endoscopic competence and experience is recommended (20, 34, 35).

Multiple Sclerosis/Neuromyelitis optica (Devic's syndrome)

Analyses
Anti-aquaporin 4, anti-MOG, anti-MBP.

Indication
Suspicion of multiple sclerosis (MS) or neuromyelitis optica (NMO).

Clinical background
MS and NMO are inflammatory, demyelinating diseases that affect the central nervous system (CNS). In MS the inflammation leads to the patchy destruction of the myelin and the nerve impulses are stopped. Certain data indicate that it is myelinspecific T lymphocytes that cause the disease. Autoantibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) also occur. MOG is a small part of the myelin (0.01-0.05%) and is only found in the the central nervous system. MBP constitutes about 30% of the myelin protein and is located on the cytoplasmic side. The antibodies may occur in up to half the patients, more often in men than women. It is, however, not clear if they constitute a specific part of MS or are only a marker for CNS injury.

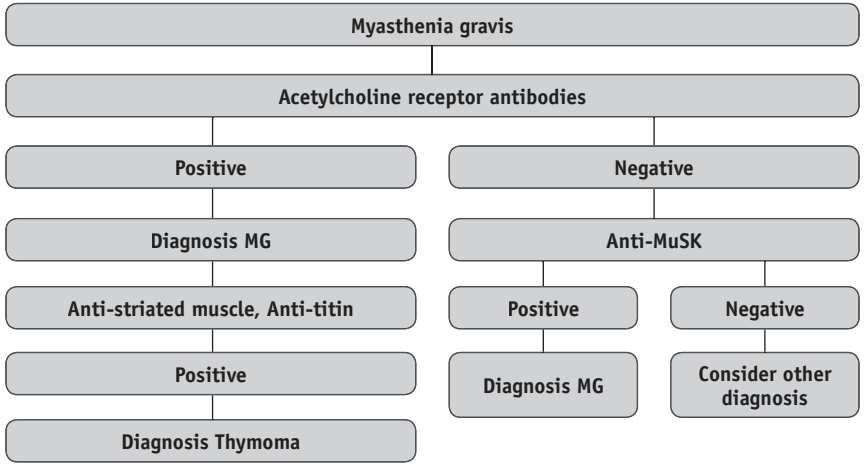
Neuromyelitis optica (NMO) used to be regarded as a variant of MS but is now regarded as a separate disease. With the support of, among other things, detected autoantibodies against the ion channel aquaporin 4 it has now been possible to define the condition as a separate entity. The differential diagnosis of MS is important, as the diseases require different treatments. The test is of interest in myelitis and optic neuritis, individually or in combination (79, 80, 81).

Myasthenia gravis

Analyses
Striated muscle antibodies and acetylcholine receptor (anti-AChR) antibodies. Supplementary addition in case of negative result of anti-AChR: Anti-MuSK. Supplementary addition in case of positive result of anti-AChR: Anti-titin.

Indication
Suspicion of myasthenia gravis or thymoma.

Clinical background
Myasthenia gravis is an autoimmune disease that is characterized by weakness and tiredness in the skeletal muscles. Antibodies against the acetylcholine receptor are crucial for the pathogenesis and prevent the neuromuscular transmission. About 90% of the patients with myasthenia gravis are positive for anti-AChR. Among the seronegative patients some exhibit antibodies against muscle-specific tyrosine kinase receptor (MuSK), which supports the diagnosis. The clinical picture is the same for seropositive as for seronegative patients. Antibodies against titin and striated muscle may sometimes be detected in patients with myasthenia gravis and is associated with an increased incidence of thymoma (74-78).



Serological investigation of Myasthenia gravis.

Neuropathy – unclear/Guillain-Barré syndrome

Analyses
Antibodies against gangliosides (GM1, asialo GM1, GQ1b, GD1a, and Gd1b) and anti-MAG and anti-SGPG.

Indication
Polyneuropathy where the cause is unknown or is suspected to be autoimmune.

Clinical background
Gangliosides are a group of glycolipids that contain sialic acid and that occur in the nervous system. They are integrated in cell membranes, where they have a ceramide tail in the lipid bilayer and an oligosaccharide part, which protrudes and is accessible for antibodies. Of the many gangliosides that are known it is mainly GM1, asialo GM1, GQ1b, GD1a, and Gd1b that are of immunological interest in peripheral nerve diseases. Antibodies against these antigens, which often crossreact, are in some cases suspected of having a pathogenic role.

IgM antibodies against GM1 and asialo GM1 are found with high frequency in multifocal motor neuropathy with conduction block (MMN), an important differential diagnosis of ALS. IgG antibodies against these gangliosides are found in patients with Guillain-Barré syndrome, mostly in the axonal form (AMAN) that can be triggered by Campylobacter jejuni.

Miller Fisher syndrome, with various degrees of ophthalmoplegia, ataxia and areflexia, is a variant of Guillain-Barré syndrome, where IgG antibodies against the ganglioside GQ1b occur with a very high frequency. IgM antibodies against this antigen are often detected in a special form of sensory atactic polyneuropathy with an M component of IgM class. (CANOMAD)

IgG antibodies against GD1a occur in Guillain-Barré syndrome, in particular in the motor axonal form. IgM antibodies against GD1b are found in MMN and CANOMAD.

Antibodies against myelin-associated glycoprotein (MAG) also bind to the carbohydrate part of the myelin protein P0 and SPGP. High titres of IgM antibodies against this antigen are very often associated with sensory polyneuropathy. Anti-MAG activity is detected in half the patients with an M component of IgM class (61, 62, 63, 64, 65, 66, 67).

Paraneoplastic neurological diseases

Analyses
Anti-Hu, anti-Yo, anti-Ri, anti-Tr, anti-Ta, anti-amphiphysin 1.

Clinical background
Paraneoplastic neurological symptoms are immunologically mediated phenomena, which occur secondary to malignant diseases. All parts of the central and the peripheral nervous system may be affected and give subacute and chronic disease, e.g. encephalopathy, cerebellar degeneration and polyneuropathy. Small cell lung cancer (SCLC) is the most common underlying cause, followed by breast and ovarian tumours, but all cancers may be connected with paramalignant phenomena. The onset of the symptoms is often subacute and can precede cancer diagnosis.

Lambert-Eaton myasthenic syndrome is caused by antibodies against a voltage-dependent presynaptic calcium channel (anti-VGCC). Small cell lung cancer is the most common underlying cause but the syndrome may appear without any underlying malignancy. Antibodies against potassium channels (anti-VGKC) are associated with among other things limbic encephalitis. Antibodies against the acetylcholine receptor in myasthenia gravis are described separately (68-73).

Antibody	Syndrome	Associated cancers
Anti-Hu (ANNA-1)	Encephalomyelitis, sensory neuropathy, cerebellar degeneration	SCLC
Anti-Yo (Purkinje cell antibodies, PCA-1)	Cerebellar degeneration	Gynecologic cancers, breast cancer
Anti-Ri (ANNA-2, Nova 1)	Opsoclonus, ataxia	Gynecologic cancers, breast cancer, SCLC
Anti-Tr	Cerebellar degeneration	SCLC, thymoma, lymphoma
Anti-CV2	Encephalomyelitis, cerebellar degeneration,	Testicular cancer, lung cancer.
Anti-Ta (Ma2)	Limbic, hypothalamic and brainstem encephalitis.	Testicular cancer
Anti-amphiphysin	Stiff person syndrome	SCLC, breast cancer
Purkinje cells, PCA-2	Encephalomyelitis, cerebellar degeneration	Various cancers
Antibodies that occur both with and without association to malignant diseases		
Anti-VGCC	Lambert-Eaton myasthenic syndrome, cerebellar dysfunction	SCLC
Anti-acetylcholine receptor	Myastenia gravis	Thymoma
Anti-VGKC	Neuromyotonia, limbic encephalitis	Thymoma.

Antibodies, paraneoplastic syndromes and associated forms of cancer.

Pemphigus/Pemphigoid (Bullous dermatosis)

Analysis
IIF on monkey oesophagus, anti-BP180, anti-BP230, desmoglein 1 and desmoglein 3.

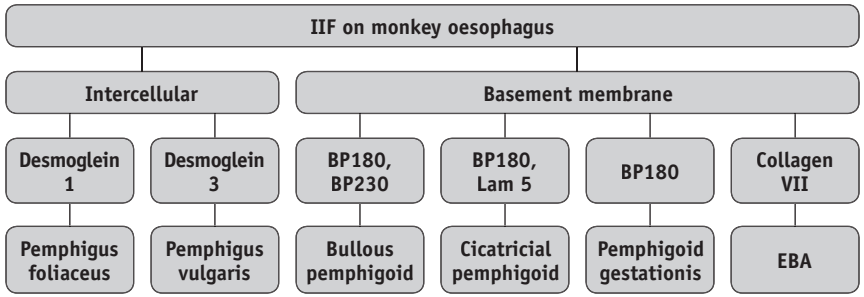
Indication
Suspicion of autoimmune skin disease.

Clinical background
Autoimmune bullous skin diseases include a group of serious diseases with blisters and erosions on skin and mucous membranes. The skin has adhesion molecules that stick the keratinocytes to each other and to the underlying basement membrane. Several of its molecules are the targets in the autoimmune skin diseases. An important factor is circulating antibodies directed against adhesion structures in the epidermis, the basement membrane or anchoring fibrils of the dermis. Pemphigus and pemphigoid are the two most common and most serious bullous diseases and the autoantibodies are considered an important part of the pathogenesis. In pemphigus the antibodies are directed against desmosomal adhesion proteins in the epidermis, whereas hemidesmosomal proteins are the targets of the antibodies in pemphigoid and similar diseases, such as IgA bullous dermatosis. In epidermolysis bullosa acquisita (EBA), anchoring fibrils (type VII collagen) are the antigen.

Pemphigus
Pemphigus is a group of life threatening autoimmune bullous skin diseases with intraepithelial blisters. The molecular background of the blistering is the interference of the adhesion between the keratinocytes (acantholysis) by the autoantibodies against intercellular adhesion structures. Various forms of pemphigus have been identified and they have the intraepidermal division on different levels of the skin. In pemphigus vulgaris (PV) the blisters are located in the deeper layers of the epidermis, whereas in pemphigus foliaceus (PF), a less serious form, they are located in the upper layers of the epidermis. In PV there are autoantibodies against the extracellular domain of the desmoglein 3, an adhesion molecule that is found on the epidermal keratinocytes. Even though desmoglein 3 is the most important antigen in PV, many patients may also have antibodies against desmoglein 1, which is the most important antigen in PF. The autoantibodies in pemphigus are polyclonal and most of them are of the IgG4 subclass in active disease, whereas in remission they are usually IgG1.

Pemphigoid
Bullous pemphigoid (BP) is an autoimmune bullous skin disease that is recognised by subepidermal bullae and circulating antibodies against the basement membrane zone in epithelium. Earlier studies have shown that these autoantibodies primarily react

with two components of hemidesmosomes, BP180 (type XVII collagen) and BP230. Hemidesmosomes are multiple protein complexes, which participate in the epithelial cell adhesion to the underlying basement membranes and bind the cytoskeleton to the extracellular matrix. BP230 is cytoplasmic and binds to intermediary filaments. BP180 is a transmembrane protein that can bind to receptors in the extracellular matrix. Antibodies against BP180 induce blisters if injected in mice. This shows that the autoantibodies against BP180 are pathogenic and are responsible for producing the blisters in BP. It has been shown that the NC16 domain, which is closest to the cell membranes, contains most of the epithopes of the molecule. There are several variants of BP that have antibodies against BP180 but that are clinically distinct from BP. Pemphigoid gestationis (PG) is a bullous disease that occurs in the last trimester of pregnancy and that also has antibodies against BP180. Cicatricial pemphigoid (CP) is a subepidermal autoimmune bullous disease, which, in contrast to BP, mainly affects mucous membranes in the conjunctive and oral, perianal and genital mucous membranes and leads to scarring in the affected tissue. In CP it has been shown that the antibodies react with several antigens besides B180, e.g. laminin 5 (92-95, 98-100).



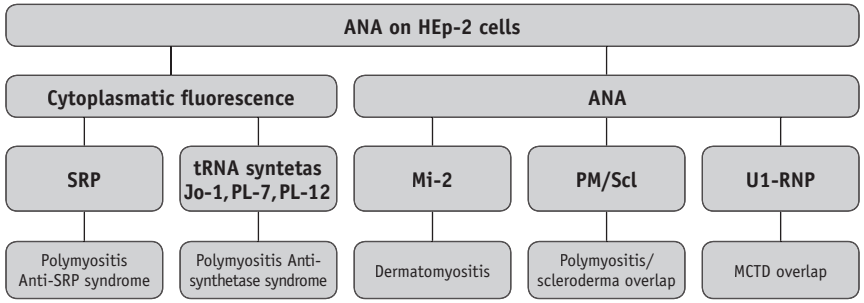
Serological investigation of pemphigus/pemphigoid.

Polymyositis/Dermatomyositis

Analyses
ANA screen (HEp-2 cells), ENA screen (Jo-1, nRNP), SRP, EJ, PL-7, PL-12, Mi-2, PM/Scl.

Indication
Suspicion of polymyositis (PM), dermatomyositis (DM) or overlap syndrome with myositis.

Clinical background
An unusual systemic inflammatory disease that mainly affects the muscles. Skin and muscle symptoms occur in dermatomyositis. PM and DM have the same clinical symptoms but different pathogenesis. DM has complement-dependent membranolysis of intramuscular capillaries, whereas PM is associated with cytotoxic T lymphocytes. Many organs may be involved and the diagnosis is established by means of histological tests of muscle biopsy, EMG, leakage of muscle enzymes (105-106).



Serological investigation of polymyositis/dermatomyositis.

Rheumatoid arthritis (RA)

Analyses
ANA screen (HEp-2 cells), anti-CCP and rheumatoid factor IgM and IgA.

Indication
Suspicion of rheumatoid arthritis.

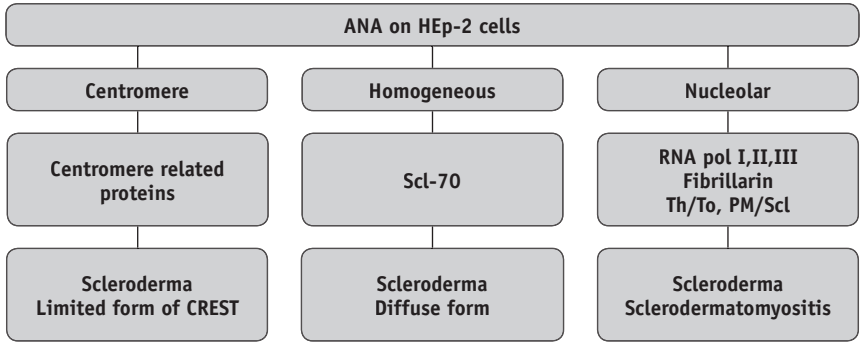
Clinical background
The diagnosis of RA is primarily based on clinical, radiological and immunological parameters. A widely used serological parameter is rheumatoid factor (RF), and IgM RF occurs in about 60-80% of the patients. Although the analysis has a good sensitivity, it has a bad specificity and occurs in healthy people and in patients with other autoimmune diseases (e.g Sjögren's syndrome) and in chronic infections. Even though the analysis has a bad specificity, a positive RF test is considered an important prognostic factor. The presence of RF is one of the tests included in the ACR (the American College of Rheumatism) criteria for RA. Studies have shown that anti-CCP occurs in about 75% of patients with RA with a specificity of 96%. The antibodies are rare in healthy people and also rare in other inflammatory diseases. The antibodies against CCP are mainly of IgG class with a high affinity. They emerge several years before the first symptoms. The correlation between anti-CCP and early RA is good but not between anti-CCP and age or sex. Anti-CCP seems to be of prognostic value and has a good ability to distinguish between erosive and non-erosive RA (46-51, 101-102).

Scleroderma/systemic sclerosis

Analyses
ANA screen (HEp-2 cells), ENA screen (Scl-70), In-depth analysis: Antibodies against Fibrillarin, Th/To, RNA polymerase, I, II, III, PM/Scl.

Indication
Suspicion of scleroderma or systemic sclerosis. In-depth analysis is ordered when diagnosis has been established or if there is a strong suspicion of scleroderma/systemic sclerosis and when it is desired to differentiate between subentities.

Clinical background
A disease with an unclear etiology, which affects many organ systems, mainly the skin and the blood vessels but the lungs and the gastrointestinal tract may also be affected. Kidney, heart and muscle symptoms are less common. The most common clinical characteristics are dermatofibrosis of varying intensity and extent and circulation disorders in fingers and toes (Raynaud's phenomenon). The disease is usually divided into different forms, such as: 1. Limited form (usually in the face and distally toward the elbow and knee) fits in with CREST syndrome. 2. Diffuse forms of skin symptoms, proximally to the elbow and on the body. 3. Overlap syndrome (106).



Serological investigation of scleroderma.

Sjögren's syndrome

Analyses
ANA screen (HEp-2 cells), ENA screen (SSA/Ro60, SSB/La).

Indication
Suspicion of primary Sjögren's syndrome.

Interpretation
Primary Sjögren's syndrome is an inflammatory disease, where the symptoms are due to reduced function of salivary and lachrymal glands. The patient suffers from dry mouth, a feeling of gravel in the eyes (Keratoconjunctivitis sicca). Extraglandular symptoms occur and usually from skin, joints, muscles and thyroidea. 2.5% of the patients develop non-Hodgkin's lymphoma.

ANA is positive in about 70% of patients with Sjögren's syndrome, usually with speckled fluorescence. In about 80% of the cases the patients have antibodies directed against Ro/SSA and about 70% have antibodies directed against La/SSB. In vasculitis in connection with Sjögren's syndrome and systemic lupus erythematosus, anti-SSA and anti-SSB rise at the same time until some year before these manifestations emerge.

Sjögren's syndrome also occurs secondarily to other rheumatic diseases, in particular rheumatoid arthritis. In secondary Sjögren's syndrome SSA and SSB are positive in 10-15% of the cases (37, 41, 42).

Sudden deafness

Analyses
Anti-HSP-70, ANA screen (HEp-2 cells), anti-cardiolipin, ANCA – IIF.

Indication
Suspicion of autoimmune cause of sudden hearing loss.

Clinical background
Sudden hearing loss affects primarily middle-aged people and develops as a rule unilaterally in the course of one to three days. Autoimmune inner ear disease is regarded as a subgroup, where cortisone has positive effects. In these patients the hearing loss as a rule becomes bilateral after some time. Indirect immunofluorescence on bone tissue from the ear has been used to find out if an autoimmune process is involved. In 1990 Harris et al. reported that 35% of patients with progressive neurogenic hearing loss had a serum antibody against cochlea. In a patient material with unexplained progressive hearing loss there was a 1/3 chance that an immunological etiology was behind the hearing loss. In a study with 279 patients with idiopathic bilateral hearing loss 32% had antibodies that were observed with western blotting. There have also been reports that antibodies against the 68kD band were detected in the very patients whose condition improved with immunosuppressive treatment. 89% of the patients with an active bilateral neurogenic hearing loss had antibodies against the 68kD antigen but patients whose disease was inactive were negative. 75% of the positive patients responded to steroid treatment, whereas only 18% of the negative patients responded to the treatment. In a report it was shown that the 68kD band is heat shock protein 70 or a protein associated with it. Therefore HSP-70 is either the target antigen of the immune defence, shares epitopes with the real antigen or anti-HSP 70 is an epiphenomenon that arises as a result of inner ear damage. Whatever the cause may be, the presence of this antibody in patients with progressive hearing loss is a tool for diagnosing an immune-mediated hearing loss and predicts steroid sensitivity. A large number of the patients have antibodies against the collagen-like part of C1q. Sudden hearing loss may also occur as a part of system diseases, such as Wegener's granulomatosis, antiphospholipid syndrome or SLE (24, 82).

Systemic lupus erythematosus (SLE)

Analyses
ANA screen (HEp-2 cells), anti-dsDNA, ENA screen (Sm), anti-cardiolipin.

Indication
Diagnosis or follow-up of systemic lupus erythematosus (SLE).

Clinical background
The age of onset of SLE is 20-40 years of age and it affects women nine times as often as men. The prevalence is about 40 cases per 100 000 people. The disease is chronic but there are relapses with relatively symptomfree intervals. Inflammation and organ damage occur in different parts of the body as a result of deposition of immune complexes with accompanying complement activation. The link to MHC is strong and there are indications of disorders of the apoptosis and the removal of apoptic material. This may result in immunogenic nucleosomes that can activate B and T lymphocytes. The disease may present as arthritis, hypersensitivity to the sun, discoid rashes, butterfly exanthema, cold sores, serositis, renal failure, neurological symptoms, cytopenias, myalgia, myositis or other clinical symptoms like Raynaud's phenomenon, alopecia, vasculitis, lung and heart symptoms.The etiology remains unknown.

The American College of Rheumatology has published diagnostic criteria for SLE. Four out of eleven must be met. Two criteria include autoantibodies: 1. Anti-dsDNA of an elevated level or anti-Sm or anti-cardiolipin IgG or IgM; 2. Elevated level of ANA with IIF without the patient taking medicine that may cause syndromes similar to those of lupus.

If there is a clinical suspicion of SLE, the investigation usually starts with ANA. This test has a sensitivity of around 95% but with a low specificity. This means that the clinical significance of a positive ANA in patients with few symptoms is low.

Anti-dsDNA seldom occurs in healthy people and is practically pathognomonic for SLE. It occurs in 50-80% of untreated patients with SLE. It is interesting to find that in several studies the level of the antibody has been shown to vary with disease activity. A high level often correlates with lupus nephritis. An increasing level may also predict relapses of SLE nephritis, whereas a declining level is consistent with reduced disease activity. It is therefore important to follow the level during the treatment of lupus nephritis

Anti-Sm antibodies have a very high diagnostic value, as they have a high specificity but a low sensitivity.

A number of other antibodies occur in SLE and among the more common ones is SSA/B, which may be important to analyze if there is a risk of congenital heart block, especially the 52kD protein and the SSA p200 peptide, see p. 23. Anti-histone antibodies are also common, especially in drug induced lupus (32, 36, 37).

Systemic vasculitis – acute screening

Indication
In cases of suspected renopulmonary syndrome, rapidly growing progressive glomerulonephritis, rapidly rising creatinin, Goodpasture syndrome, Wegener's granulomatosis or microscopic polyangitis. The analysis is not suitable for monitoring patients or for discovering relapses in known patients, as the analysis is not quantitative.

Method
ELISA with the purified antigens of the alpha 3 chain from type IV collagen, proteinase 3 and myeloperoxidas from granulocytes.

Result
The result is given as the ratio between the absorption of patient serum and normal serum.

Reference range
<3 with borderline <4.

Interpretation
Patients with hemoptysis or lung infiltrate together with glomerulonephritis, in particular rapidly progressing, belong to the group of renopulmonary syndrome. The patients have often initially been investigated for infections or malignities. The relation between lung and kidney engagements may vary and the symptoms may come from either of the organs. The most frequent diagnoses are Goodpasture syndrome, ANCA-associated systemic vasculitis (Wegener's granulomatosis, microscopic polyangitis), SLE or drug induced glomerulonephritis. A quick diagnosis is important, as this will result in a quick start of the therapy to manage the lung and kidney function. In case of renopulmonary syndrome or rapidly progressive glomerulonephritis it has been shown that up to 80% of the patients have anti-GBM, PR3-ANCA and MPO-ANCA.

The method is a screening method of high sensitivity and therefore the percentage of false-positive cases is about 1%. These will be negative in a quantitative analysis (1-3).

Systemic vasculitis – ANCA quantification

Analyses
PR3-ANCA, capture PR3-ANCA, MPO-ANCA, capture MPO-ANCA.

Indication
Diagnosis and follow-up of ANCA associated systemic vasculites (such as Wegener's granulomatosis and microscopic polyangiitis).

Clinical background
ANCA- associated systemic vasculites are described in more detail in the appendix. Patients with Wegener's granulomatos (WG) have, with few exceptions, ANCA. About 90% have PR3-ANCA and 5-10% have MPO-ANCA. In patients with a limited disease and in patients in remission ANCA is positive in half the cases.

Patients with microscopic polyangiitis (MPA) are as a rule positive for MPO-ANCA, but cases with positive PR3-ANCA occur. Even patients with Churg-Strauss disease and Goodpasture syndrome may be positive for MPO-ANCA.

Both in the case of WG and of MPA an increase in the ANCA level may precede a relapse. The Capture method has proved superior for follow-up, as it predicts relapses with a greater certainty (1-5).

Systemic vasculitis – follow-up

Analyses
PR3-ANCA and capture PR3-ANCA or MPO-ANCA and capture MPO-ANCA.

Indication
Follow-up of a patient with a known vasculitic disease. Control of disease activity and treatment effect.

Clinical background
Patients with Wegener's granulomatos have ANCA in 90% of the cases. The majority has PR3-ANCA but about 10% of the ANCA-positive patients have MPO-ANCA. In microscopic polyangiitis MPO-ANCA is the predominant antibody. Both diseases progress to relapses, which as a rule are preceded by a rise in the ANCA level. At the time of the onset of clinical disease the ANCA level is usually high. Successful treatment is usually followed by a reduction in the ANCA level (3, 5, 8-13).

It is possible to obtain the patient's ANCA level on a graph. Tick the box "Titer curve/Graph" on the back of the referral form.

Systemic vasculitis – special analysis

Analyses
Subclass analysis of specific IgG and PiZ identification of alpha 1 antitrypsin.

Indication
In Goodpasture syndrome and Wegener's granulomatosis and other renopulmonary syndromes.

May indicate patients who should be followed with extra care and may predict relapses.

Clinical background
The most frequent subclasses of IgG in these diseases are IgG1 and IgG4. There is only a low number of IgG2 and IgG3. Patients with MPO-ANCA have more often IgG2 and patients with PR3-ANCA have more often IgG3. An unusual subclass distribution may indicate a different clinical course. Changes in the subclass distribution can be used to study relapses of the disease.

A heterozygous form of alpha-1-antitrypsine occurs in about 4.7% of the normal population and a homozygous form occurs in about 6 out of 10000 individuals. Heterozygotes have a somewhat lower level of alpha-1AT in the blood, whereas homozygotes only have 5-10% of the normal concentration of 0.9-1.7 g/L. Patients with PR3-ANCA have an overrepresentation of PiZ. About 20% can have the gene and in the group of biopsi-verified granulomae only 1/3 had the Piz gene. These patients often have a more widespread disease (more organ engagements) and a shorter survival.

Thyroid disease

Analyses
Antibodies against thyroglobulin (TG), thyroid peroxidase (TPO) and TSH receptor.

Indication
Suspicion of autoimmune disease in the thyroid. Goitre. Hypo- and hyperthyreosis.

Clinical background
Chronic autoimmune thyroiditis (Hashimoto's disease) affects primarily women between 30 and 50 years of age. Antibodies against thyroid antigens induce inflammation and tissue damage. Histologically lymphocyte infiltrate is observed and the patient often exhibits goitre. The function of the thyroid is reduced, which leads to hypothyreosis with elevated values of the thyroid stimulating hormone (TSH). 90 % of the patients have antibodies against thyroid peroxidase. Most of them also have antibodies against thyroglobulin.

Diffuse toxic goitre (Graves' disease) is caused by antibodies against the receptor of the thyroid stimulating hormone. As a rule the antibodies have a stimulating effect on the receptor, which results in hyperthyreosis. The patient is also often affected by an eye reaction, known as endocrine ophthalmopathy. The antibodies may also be of an inhibiting type, where the clinical effect is hypothyreosis. A patient may change the type of antibody during the disease. The content of anti-TSH receptor antibodies in serum is usually monitored during the course of the disease and gives some information about disease activity and treatment effect.

Both the stimulating and inhibiting antibodies can be transferred from mother to foetus, if the mother has high levels of antibodies during the third trimester, and may interfere with the thyroid function of the foetus (83, 84).

Individual Analyses

The analyses are presented in alphabetical order. Here you will find indication for analyses, what method is used, how the result is given, reference ranges and interpretation of positive answers. In connection with each analysis there are also references to panels, where more can be read about the clinical background and to analyses that share indication.

Our range of analyses increases all the time, in accordance with enquiries and wishes of our customers. If you can't find the autoantibody analysis you are looking for, call us for a discussion of possible options.

Acetylcholine receptor antibodies

Indication
Suspicion of Myasthenia gravis and thymoma.

Method
Immunoprecipitation with human native antigen.

Answer
The result is given as negative or positive with a value.

Reference range
0.4 nmol bungarotoxin binding sites/L.

Interpretation
Antibodies against acetylcholine receptor (ACh-R) are found in 85-90% of the patients with Myasthenia gravis. About 15% of the patients with MG lack acetylcholine receptor antibodies. The antibodies remain in 60% of the patients even if they are in remission.

Panels and other analyses
See also panel *Myasthenia gravis* as well as analyses *Striated muscle antibodies*, *MuSK antibodies* and *Titin antibodies*.

Adrenal cortex antibodies

Indication
Suspicion of primary Addison's disease (idiopathic adrenal cortex insufficiency).

Method
Indirect immunofluorescence with sections of adrenal cortex from monkeys. The antibodies are directed against microsomal antigens in adrenal cortex cells.

Answer
The result is given as negative or weak, medium or strong positive after evaluation of the intensity of the immunofluorescence.

Reference range
Negative.

Interpretation
Positive in about 67% of patients with idiopathic adrenal cortex insufficiency. More common in women than men and most common in patients who have also had clinical or serological signs of other autoimmune diseases, e.g. Hashimoto's thyroiditis, juvenile diabetes mellitus or pernicious anemia. Negative in healthy people.

Amphiphysin 1 antibodies

Indication
Suspicion of paraneoplastic syndromes.

Method
Immunoblot.

Answer
The result is given as negative or positive with a titer.

Reference range
Negative.

Interpretation
Occurs in patients with the paraneoplastic variant of Stiff person syndrome that is sometimes observed in persons with breast cancer and small cell lung cancer. Some of these later develop paraneoplastic encephalitis. Patients who exhibit Stiff person syndrome but not cancer often have antibodies against GAD.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Hu antibodies*, *Yo antibodies*, *Ri/Nova 1 antibodies*, *Ta/Ma 2 antibodies* and *Purkinje cell antibodies (Tr)*, *Purkinje cell antibodies (PCA 2)*, *GAD 65 antibodies*.

ANA screen (HEp-2 cells)

Indication
Suspicion of collagenosis diseases, systemic lupus erythematosus, scleroderma, mixed connective tissue disease, primary Sjögren's syndrome, chronic active hepatitis, hypergammaglobulinemic purpura, juvenile rheumatoid arthritis, drug induced lupus.

Method
Indirect immunofluorescence with human epithelial cells (HEp-2 cells) as antigen. Serum is diluted 160 times at a screening investigation. At the titration the sample is diluted up to 1280 times.

Answer
The result is given as negative, + (weak positive), ++ (positive), +++ (strong positive) depending on the intensity of the fluorescence and is divided into 8 patterns. Weak positive fluorescence is observed in 5% of healthy men and women. It occurs more often in women in the menopause and in persons older than 65.

Reference range
Negative.

Interpretation
Through the detailed nuclear morphology and many mitoses of the HEp-2 cell, a number of specific patterns can be distinguished at the fluorescence investigation. Positive ANA, patterns 7 and 8, is supplemented automatically with ANA titration, ENA screen (Sm, nRNP, SSA, SSB, Scl-70, Jo-1), anti-dsDNA and anti-histones. High titers (1/640) are particularly observed in systemic lupus erythematosus, mixed connective tissue disease, scleroderma, primary Sjögren's syndrome, chronic active hepatitis and hypergammaglobulinemic purpura. When the reactivity is weak, it is often difficult to distinguish between the nuclear antibody patterns 7 and 8.

1. Centromere. (Anti-centromere) Occurs in CREST syndrome (also called limited progressive systemic sclerosis). In scleroderma about 50% of the patients are positive.

2. Nucleoli. (Anti-nucleoli) Occurs in systemic scleroderma (diffuse progressive systemic sclerosis). In scleroderma about 15% of the patients are positive. The antibodies may be directed against RNA polymerases, against fibrillarin and other nucleolar proteins.

3. Proliferating cell nuclear antigen (PCNA). (Anti-proliferating cell nuclear antigen). Occurs in 10% of patients with SLE. The antigen is a co-factor of DNA polymerase delta.

4. Mitotic spindle apparatus. (Anti-mitotic spindle apparatus). Occurs in about 50% of patients with Mycoplasma pneumoniae infections. Other disease associations are less clear.

5. Nuclear dots. (Anti-nuclear dots). The disease association of nuclear dots is unclear but many seropositive patients are found to have chronic liver disease/biliary disease (PBC).

6. Nuclear envelope. (Anti-nuclear envelope). Associated with seronegative polyarthritis, primary antiphospholipid syndrome, systemic lupus erythematosus and chronic liver disease.

7. Homogeneous and/or peripheral nuclear staining. Antibodies against dsDNA, histones, Scl 70(100) and other less known antigens.

8. Speckled nuclear staining. Antibodies against ENA, SSA, SSB, Mi 2 and other less known antigens.

Cytoplasma staining. One diffuse and one mitochondrial-like staining. The diffuse one is caused by antibodies against Jo-1 and other tRNA synthetases and rRNP. Anti Jo-1 occurs mainly in polymyositis, anti rRNP mainly in SLE, and the mitochondrial-like staining occurs mainly in primary biliary cirrhosis.

Panels and other analyses

See also panels *SLE*, *Connective tissue disease – undifferentiated*, *Antiphospholipid syndrome*, *Sjögren's syndrome*, *Congenital heart block*, *Polymyositis/dermatomyositis*, *Rheumatoid arthritis*, *Scleroderma/systemic sclerosis*, *Drug induced lupus* and *Autoimmune hepatitis* as well as analyses *ENA screen*, *dsDNA antibodies* and *Histone antibodies*.

ANCA – expanded analysis

Indication

Suspicion of autoimmune hepatitis, ulcerative colitis, rheumatoid arthritis, cystic fibrosis or for the follow-up of positive samples of indirect immunofluorescence that are negative for PR3- and MPO-ANCA.

Method

ELISA with the purified proteins azurocidin, BPI (CAP57), cathepsin G, elastase, lactoferrin and lysozyme as antigen.

These proteins are mainly found in granules in myeloid cells or as granulocytes and monocytes and participate in the body's inflammation defence by killing bacteria through the enzymatic function and the formation of oxygen radicals.

Answer

The result is given as a ratio between patient serum and normal serum.

Reference range

3 with a bordeline value of 4.

Interpretation

These antibodies rarely occur in systemic vasculitis but more often in ulcerative colitis, Crohn's disease, primary sclerosing cholangitis, autoimmune hepatitis, rheumatoid arthritis, Felty's syndrome and cystic fibrosis. The pathogenic importance is unknown. However, the antibodies also occur in systemic lupus erythematosus and therefore cannot be regarded as diagnostically very interesting except in particular patients. In a few patients with systemic vasculitis these antibodies occur at varying degrees, but they are not diagnostic whereas PR3- and MPO-ANCA are the most common and are of great diagnostic importance.

The ANCA expanded analysis is important for the follow-up of samples that are positive at indirect immunofluorescence analysis and that at the same time are negative for PR3- and MPO-ANCA. The reason is that the same pattern may arise from several different antibodies. Anti-MPO can, for instance, sometimes give a C-ANCA pattern. Anti-BPI can give both C- and P-ANCA staining, whereas anti-PR3 usually gives a C-ANCA pattern. With a positive IIF result it is therefore always necessary to verify it with a specific analysis, such as ELISA, as a pattern may arise from different antibodies but only PR3-ANCA and MPO-ANCA are of interest for the diagnosis of systemic vasculitis.

Patients with RA have ANCA and particularly those with Felty's syndrome but the antigens are unknown and the IIF pattern is often called GS-ANA because of the dominant nuclear staining. About half the patients with ulcerative colitis have

P-ANCA with mostly unknown antigens. These ANCA also occur in Crohn's disease at lower frequencies. In sclerosing cholangitis lactoferrin ANCA in particular has been reported. P-ANCA also occurs in autoimmune hepatitis, again with unknown antigens. BPI-ANCA is of interest in cystic fibrosis investigations, as BPI-ANCA is a marker for pseudomonas infection and the level of the antibody correlates with the lung function. ANCA with a specificity for MPO, elastase, lactoferrin etc also occurs in association with drug induced conditions, e g hydralazine and propylthiouracil. Anti-elastase has been reported in connection with cocaine abuse. (14-23).

Panels and other analyses
See also panels *Inflammatory bowel disease* and *Cystic fibrosis* as well as analyses *ANCA – IIF* and *BPI-ANCA IgG and IgA*.

ANCA – IIF

Indication
Suspicion of systemic vasculitis and inflammatory diseases.

Method
Indirect immunofluorescence (IIF) method with human granulocytes as substrate.

Answer
The result is given as negative, weak, medium or strong positive after evaluation of the intensity of the immunofluorescence.

Reference range
Negative.

Interpretation
The classical ANCA in systemic vasculitis is a reaction of IgG antibodies against granulocytes and is observed as a grainy staining of the granulocyte cytoplasm (C-ANCA) at the IIF investigation. C-ANCA occurs mainly in Wegener's granulomatosis and other systemic vasculites, e.g. microscopic polyangiitis, Churg-Strauss syndrome, focal and extracapillary necrotizing glomerulonephritis. In active phases of the disease 90% of Wegener patients have C-ANCA. Another ANCA pattern is the perinuclear staining of granulocytes, the so-called P-ANCA, which also occurs in systemic vasculitis, more often in microscopic polyangiitis than in Wegener's granulomatosis and above all when the disease is mainly located in the kidney. P-ANCA often occurs in inflammatory bowel disease, rheumatoid arthritis and drug induced SLE. It is worth noting that C- and P-ANCA are IIF patterns and may be caused by antibodies with different specificities against granule proteins. In case of positive IF it is therefore advisable to follow up with antigen specific ELISA analyses.

The IIF pattern can also be called atypical and is then more related to inflammatory diseases, such as UC, RA and not to vasculitis. If nuclear staining of other cells is observed in the investigation, it is called uncertain ANCA due to organ unspecific nuclear staining, since it is then not possible to know whether or not there is ANCA.

Panels and other analyses
See also panels *Systemic vasculitis*, *Inflammatory bowel disease*, *Sudden deafness*, *Drug induced lupus*, *Connective tissue disease – undifferentiated* and analysis *ANCA expanded analysis*.

Aquaporin 4 antibodies

Indication
Suspicion of neuromyelitis optica (NMO).

Method
Radioimmunoprecipitation.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value of 15.

Interpretation
These antibodies are directed against Aquaporin 4 water channels that are located in astrocyte membranes and occur in NMO, also called Devic's syndrome. This disease has been regarded as a subtype of multiple sclerosis (MS) but has several unique features. Anti-aquaporin 4 has 75% sensitivity and 91% specificity for NMO.

Panels and other analyses
See also panel *Multiple sclerosis/Neuromyelitis optica*.

ASCA (anti-Saccharomyces cerevisiae) – IgA and IgG

Indication
Suspicion of Crohn's disease.

Method
ELISA with mannan from Saccharomyces cerevisiae as antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
IgA 1.2 with a borderline value of 1.4 and IgG 0.8 with a borderline value of 0.9.

Interpretation
IgA antibodies against Saccharomyces cerevisiae (ASCA) occur in patients with Crohn's disease in 60-75% but are rare in patients with ulcerative colitis. IgG ASCA also often occurs together with IgA ASCA but some patients have only one Ig class. The antigen is oligomannos epitopes from the cell wall of ordinary yeast (Saccharomyces cerevisiae). Patients with ulcerative colitis (UC) have ANCA of the so-called P-ANCA type (also called atypical ANCA). The antigens are unknown but they may be elastase, lactoferrin, BPI etc. The combination of ASCA and ANCA is therefore used to distinguish UC from Crohn's disease, as a typical UC patient has ANCA, whereas a typical Crohn's patient has ASCA.

Panels and other analyses
See also panel *Inflammatory bowel disease* and analyses *ANCA – IIF* and *ANCA expanded analysis*.

ß2 glycoprotein 1 antibodies – IgG and IgM

Indication
Suspicion of autoimmune arterial and/or venous thromboses, thrombocytopenia and repeated abortions. This is observed in primary antiphospholipid syndrome and systemic lupus erythematosus with secondary antiphospholipid syndrome.

Method
The analysis is performed with ELISA with beta 2 glycoprotein 1 as antigen. Results are given both for IgG and IgM antibodies.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
1.0 with a borderline value of 1.4.

Interpretation
Beta 2 glycoprotein 1 is the protein that has been found to bind to cardiolipin and in most cases is the cause of the cardiolipin reactivity. These antibodies correlate better with thrombosis risk than anti-cardiolipin. It is positive in 30-40% of patients with systemic lupus erythematosus with thromboses and in patients with primary antiphospholipid syndrome. This is a lupus-like syndrome, which often has negative autoimmune serology and presents with recurrent arterial and venous thromboses, repeated abortions and in some cases livido reticularis, unstable hypertension, migraine and neurological signs of disease.

Panels and other analyses
See also panels *Antiphospholipid syndrome* and *Connective tissue disease – undifferentiated* as well as analysis *Cardiolipin antibodies IgG and IgM*.

BP180 and BP230 antibodies – IgG and IgA

Indication
Suspicion of autoimmune skin disease, e.g. pemphigoid and differential diagnosis of pemphigus, EBA etc.

Method
Immunoblot with recombinant BP180 and BP230. BP180 has a molecular weight of 180kD and is also called BPAG2. BP230 has a molecular weight of 230kD and is also called BPAG1.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Antibodies against BP180 and BP230 occur in patients with the bullous disease bullous pemphigoid (BP). The antibodies are directed against two components of the basement membrane zone in epithelium, BP180 (type XVII collagen) and BP230. Anti-BP180 occurs in about 65% of the patients with BP, whereas anti-BP230 occurs in almost all patients. The serum level of anti-BP180 correlates with disease activity. It seems, however, as if anti-BP230 does not cause blistering but is rather a consequence of damage of the keratinocytes.

Panels and other analyses
See also panel *Pemphigus/Pemphigoid* and analyses *Skin antibodies (intercellular substance and basement membrane)*, *Desmoglein 1 and 3 antibodies*, *Collagen VII antibodies*, *Laminin 5 antibodies IgG and IgA* as well as *Desmoplakin I and II antibodies*.

BPI-ANCA – IgG and IgA

Indication
Follow-up of patients with cystic fibrosis. Suspicion of inflammatory diseases with ANCA or follow-up of positive IIF-ANCA.

Method
ELISA with purified BPI (bactericidal/permeability-increasing protein) from human granulocytes. BPI is a 55kD protein from azurophilic granules in granulocytes and is one of the most important proteins in these cells.

Answer
The result is given as arbitrary units after comparison with a standard serum. The analysis measures IgG antibodies and IgA antibodies.

Reference range
BPI-ANCA IgG: 38 with a borderline value 50. BPI-ANCA IgA: 53 with a borderline value 67.

Interpretation
Cystic fibrosis is a genetically conditioned disease, where a defect ion channel in the airway epithelium causes tough mucus. The tough mucus is colonized by bacteria. In children staphylococci and other Gram-positive bacteria dominate, but at older ages Pseudomonas aeruginosa becomes the dominant pathogen. The prognosis for cystic fibrosis is varying. Some patients lose lung function rapidly, whereas others remain relatively unaffected high up in adulthood despite chronic bacterial colonization. As a rule the colonization by P aeruginosa is established with cultivation but can be detected earlier using serology (antibodies against the bacteria). The antibody level correlates with the quantity of bacteria in the airways.

For unknown reasons many patients have autoantibodies against BPI, i.e. BPI-ANCA. IgG-BPI-ANCA is the most common and may occur in up to 90% of the patients. IgA-BPI-ANCA is found in about 40%. There is a very strong correlation between BPI-ANCA and the colonization by P aeruginosa. Within the group of patients colonized by P aeruginosa, a high level of BPI-ANCA is associated with serious lung damage. It has been found that positive IgA-BPI-ANCA is a risk factor for respiratory insufficiency within five years.

IgA-BPI-ANCA has a stronger correlation with lung function impairment and P aeruginosa colonization than IgG-BPI-ANCA. BPI-ANCA also occurs in inflammatory bowel diseases and in some patients with vasculitis.

Panels and other analyses
See also panel *Cystic fibrosis*.

Cardiolipin antibodies – IgG and IgM

Indication
Suspicion of autoimmune arterial and/or venous thromboses, thrombocytopenia and repeated abortions. This is observed in primary anti-phospholipid syndrome in anti-phospholipid syndrome and systemic lupus erythematosus.

Method
The analysis is performed with ELISA with cardiolipin, incubated with bovine serum, since the thrombosis-related antigen is -glycoprotein 1 bound in complex with cardiolipin. IgG and IgM anti-cardiolipin analysis are performed.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
1.0 with a borderline value 1.4.

Interpretation
Positive in patients with primary anti-cardiolipin syndrome (APS). This is a lupus-like syndrome that otherwise has a negative autoimmune serology and is characterized by recurrent arterial and venous thromboses, repeated abortions and, in some cases, livido reticularis, unstable hypertension, migraine and neurological disease signs. Thrombocytopenia and elevated APTT often occur. Positive in some cases of pulmonary hypertension in connection with vasculopathy and thromboses. Positive in 30-40% of patients with systemic lupus erythematosus, half of whom have clinical APS. In men 45 years of age with acute myocardial infarction the risk of developing arterial and venous complications may increase, if they are anti-cardiolopin positive.

Panels and other analyses
See also panels *Antiphospholipid syndrome*, *SLE, Connective tissue disease – undifferentiated* and *Sudden deafness* and analysis *ß2 glycoprotein 1 antibodies IgG and IgM*.

CCP antibodies

Indication
Suspicion of rheumatoid arthritis (RA).

Method
ELISA with cyclic citrullinated peptide (CCP2) as antigen.

Answer
The result is given in arbitrary units after comparison with a standard serum.

Reference range
25 units/ml.

Interpretation
Studies have shown that anti-CCP occurs in about 75% of RA sera with a specificity of 96%. The antibodies are rare in healthy people and also rare in other inflammatory diseases. The antibodies against CCP are mainly of IgG class with high affinity. The antibodies are present several years before the first symptoms. There is a good correlation between anti-CCP and early RA but not between anti-CCP and age or sex. Anti-CCP seems to have a prognostic value and an ability to distinguish between erosive and non-erosive RA. The presence of anti-CCP is connected with the tissue antigen HLA-DRB1 and it is now considered that there are two subpopulations of RA, one with anti-CCP and HLA-DRB1, which constitutes about 75%, and another anti-CCP negative, where there is no correlation with DRB1. It is also debated whether various environmental factors may trigger the presence of anti-CCP, e.g. smoking and bacterial infections.

Panels and other analyses
See also panels *Rheumatoid arthritis* and *Connective tissue disease – undifferentiated* as well as analyses *Rheumatoid factor IgA* and *Rheumatoid factor IgM*.

Collagen type VII antibodies

Indication
Suspicion of autoimmune skin disease, such as pemphigoid and differential diagnosis of pemphigus, EBA etc.

Method
Immunoblot.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
These antibodies occur in epidermolysis bullosa acquisita (EBA).

Panels and other analyses
See also panel *Pemphigus/Pemphigoid* and analyses *Skin antibodies (intercellular substance and basement membranes)*, *BP180 and BP230 antibodies IgG and IgA*, *Desmoglein 1 and 3 antibodies*, *Desmoplakin I and II antibodies* and *Laminin 5 antibodies IgG and IgA*.

Complement analysis

Indication
Suspicion of congenital or acquired complement deficiency or complement activation.

Method
Nephelometry (C3 and C4), immuno electrophoresis (C1q and C3d) and complement function by ELISA.

Answer
The result of C3 and C4 is given in g/L, C3d in mg/L and C1q as a percentage of normal sera. Results of complement function is also given as percentages of normal sera.

Reference range
C3: 0.77-1.38 g/L. C4: 0.12-0.33 g/L. C1q: 78-131% of normal sera. C3d: 5 mg/L. Complement function: classical pathway: 69-129%, alternative pathway: 30-113%, MBL pathway: 0-125%.

Interpretation
The complement system is a part of the immune defence. It strengthens the function of the antibodies and contributes to the killing of bacteria. Three pathways of activating the complement system are known: the classical, the alternative and the MBL pathway. (MBL=Mannan binding lectin). The classical pathway is activated by immune complexes, which is the case in SLE. C1q then binds to C1r and C1s and this triggers a cascade where C2 and C4 form a complex which cleaves C3. The alternative pathway is activated by formation of a complex between C3 and factor B through a reaction that especially occurs on the surface of bacteria. The MBL pathway is activated by a reaction between MBL and certain carbohydrates, which are found only in certain bacteria.

When measuring complement activation in vitro, a measure is obtained of the ability of the serum to activate the complement system through a certain pathway. The activation rate is a measure of the serum concentration of the components that are contained in the activating pathway. If the patient has a total lack of a component, the activation of that pathway becomes zero. In a patient with an active SLE disease, an activation of the classical pathway often occurs. This leads to a depletion of the components (C1q, C1s, C1r, C2, C4, C3 and C5-9). Serum from this patient is likely to have a reduced ability to activate the classical pathway in vitro. As some components are shared with the other pathways, their ability to be activated may be reduced because of the depletion of the components of the classical pathway.

Panels and other analyses
See also panels *Glomerulonephritis* and *SLE*.

CV2 antibodies

Indication
Suspicion of paraneoplastic syndrome.

Method
Immunoblot

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Autoantibodies against CV2 are of interest when investigating paraneoplastic neuropathies. Associated tumours are testis cancer and lung cancer.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Hu antibodies*, *Yo antibodies*, *Ri/Nova 1 antibodies*, *Ta/Ma 2 antibodies*, *Amphiphysin 1 antibodies*, *Purkinje cell antibodies (Tr)* and *Purkinje cell antibodies (PCA 2)*.

Desmoglein 1 and 3 antibodies

Indication
Suspicion of autoimmune skin disease, e.g. pemphigus and differential diagnosis of pemphigoid, EBA etc.

Method
Radioimmunoprecipitation with the recombinant antigens desmoglein 1 and 3. Desmoglein 1 is a desmosomal cadherin with a molecular weight of 160kD, whereas desmoglein 3 has a molecular weight of 130kD.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Pemphigus is a group of life threatening autoimmune bullous diseases that present with intraepithelial blisters. The molecular background of the blistering is that the adhesion between keratinocytes is disturbed (acantholysis) by the autoantibodies against intercellular adhesive structures. In pemphigus foliaceus the antibodies are mainly directed against desmoglein 1. In pemphigus vulgaris the antibodies are mainly directed against the extracellular domain of desmoglein 3 but some patients may also have antibodies against desmoglein 1.

Panels and other analyses
See also panel *Pemphigus/Pemphigoid* and analyses *Skin antibodies (intercellular substance and basement membrane)*, *BP180 and BP230 antibodies IgG and IgA*, *Collagen VII antibodies*, *Laminin 5 antibodies IgG and IgA* as well as *Desmoplakin I and II antibodies*.

Desmoplakin I and II antibodies

Indication
Suspicion of autoimmune skin disease, e.g. pemphigus and differential diagnosis of pemphigoid, EBA etc.

Method
Immunoblot.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Desmoplakin I and II are two pemphigus antigens, 250kDa and 210kDa in size. Antibodies against Desmoplakin I and II mainly occur in paraneoplastic pemphigus but also in pemphigus foliaceus.

Panels and other analyses
See also panel *Pemphigus/Pemphigoid* and analyses *Skin antibodies (intercellular substance and basement membranes)*, *BP180 and BP230 antibodies IgG and IgA*, *Desmoglein 1 and 3 antibodies*, *Laminin 5 antibodies IgG and IgA* as well as *Collagen VII antibodies*.

dsDNA antibodies

Indication
Suspicion of systemic lupus erythematosus or incipient exacerbation of this disease, chronic aggressive hepatitis of the "lupoid hepatitis" type.

Method
ELISA method with circular plasmid DNA as antigen. Samples that are positive in ELISA are followed up with IIF with the flagellate Crithidia luciliae as a substrate on a slide.

Answer
The result is given as international units after comparison with reference serum Wo80. For IIF the result is given as negative, weak, medium or strong positive after evaluation of the intensity of the immunofluorescence.

Reference range
ELISA 25 with a borderline value 39 IU/ml. IIF negative.

Interpretation
The presence in human serum of IgG antibodies against double-stranded DNA is characteristic of systemic lupus erythematosus (SLE) and is included in the ACR criteria. Positive findings of these antibodies have a great diagnostic specificity for SLE (90%) and can be used when evaluating the treatment effect as an activity marker. In active SLE with kidney involvement up to 95% can have anti-dsDNA, in inactive SLE without kidney involvement fewer than 40% have these antibodies. A rapid increase in the level of anti-dsDNA often indicates a worsening of the disease. In remission the values can be normal. ELISA has a higher sensitivity (about 75%) than Crithidia (sensitivity 60%) but a lower specificity. Therefore there may be patients who are ELISA positive but Crithidia negative. Our laboratory has found, when analyzing patient material, that a positive result with Crithidia has a diagnostic specificity of 99-100% for SLE. Titration is usually not done, as it has little prognostic/diagnostic value.

Some patients who have positive anti-dsDNA but do not meet the criteria for SLE may develop SLE within four years. Weak positive findings may occur in various chronic inflammatory rheumatoid diseases (RA, SS, scleroderma, drug induced conditions) and in chronic aggressive hepatitis of the "lupoid" type, but in fewer than 10% of these cases.

Panels and other analyses
See also panels *Antiphospholipid syndrome*, *SLE* and *Glomerulonephritis* as well as analysis *ANA screen (HEp-2 cells)*.

EJ antibodies

Indication
Suspicion of dermatomyositis/polymyositis.

Method
Radioimmunoprecipitation with a recombinant antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Antibodies against EJ are directed against glycyl-tRNA synthetase. These antibodies are observed in polymyositis and anti-synthetase syndrome together with antibodies against SRP and Mi 2. Patients with anti-tRNA synthetase manifest similar symptoms as those with anti-Jo-1.

Panels and other analyses
See also panel *Polymyositis/Dermatomyositis* and analyses *tRNA synthetase antibodies*, *SRP antibodies*, *Mi 2 antibodies* and *PM/Scl antibodies*.

ENA screen (Extractable nuclear antigens)

Indication
ENA are analyzed in order to determine the specificity of antibodies that have produced a positive ANA (pattern 7 - homogeneous and pattern 8 - speckled) on HEp-2 cells. Included antigens are Ro60 (SSA), La (SSB), Sm, nRNP, Scl-70 and Jo-1. Answers to the result of antibodies against Ro52 are given after ticking in a box on the referral form.

Method
Qualitative immunoblot, where positive results are confirmed with ELISA.

Answer
The result is given as positive or negative.

Interpretation
SSA/Ro60: Positive in about 70% of patients with primary Sjögren's syndrome and in 10-15% of patients with secondary Sjögren's syndrome. 40% of patients with SLE are positive for SSA. Other disease associations, e.g. scleroderma and MCTD, have also been described. Positive anti-SSA also occurs in malaria, bilharzia and leishmaniasis.

SSB/La: Positive in about 70% of patients with primary Sjögren's syndrome, 5-30% of patients with SLE. It occurs most frequently together with SSA in Sjögren's and SLE but sometimes occurs in scleroderma and RA. Isolated occurrence of SSB has been described in primary biliary cirrhosis and autoimmune hepatitis.

Sm: Positive in about 10-30% of patients with SLE in Western Europe and the specificity in this disease is high. The frequency is considerably higher in Afro-Americans and Asians with SLE than in Caucasians. Sm is a nuclear non-histone protein. Patients with SLE often remain positive for Sm in remission and therefore detection of Sm can be valuable, when anti-dsDNA cannot be measured.

nRNP: Occurs in almost all patients with MCTD, in 5-50% of patients with SLE and is most common in patients with lung manifestations or signs of myositis, Raynaud's phenomenon and positive RF-IgM. The antibodies may also occur in RA and polymyositis/dermatomyositis as well as scleroderma. Some healthy people and patients with malaria, bilharzia and leishmaniasis have antibodies against nRNP.

Scl-70: Positive in about 15% of patients with scleroderma, especially of the systemic progressive form (diffuse progressive systemic sclerosis). Patients with Raynaud's syndrome and positive anti Scl-70(100) often develop a typical diffuse progressive systemic scleroderma later.

Jo-1: Positive in about 25% of patients with polymyositis/dermatomyositis and largely pathognomonic for the disease. Antibodies against Jo-1 are directed against histidyl-tRNA synthetase. Other anti-synthetase antibodies include antigens OJ, EJ, PL-7, PL-12, KS and Zo. Patients with other anti-tRNA-synthetase antibodies manifest similar symptoms as those with anti-Jo-1.

SSA/Ro 52: Antibodies against Ro 52 may play a role in the development of congenital heart block. The antibodies can be a tool for the identification of mothers with a risk of giving birth to children with congenital heart block. It often occurs in variants of myositis together with Jo-1 antibodies. When detected isolated it has no role in the diagnosis of SS.

Panels and other analyses
See also panels *SLE, Sjögren's syndrome*, *Congenital heart block*, *Drug induced lupus*, *Polymyositis/dermatomyositis*, and *Scleroderma/Systemic sclerosis* as well as analysis *ANA screen (HEp-2 cells)*.

Endomysium antibodies – IgA

Indication
Suspicion of coeliac disease (CD) or dermatitis herpetiformis.

Method
Indirect immunofluorescence with sections of monkey oesophagus as substrate.

Answer
The result is given as negative, weak, medium or strong positive after evaluation of the intensity of the fluorescence.

Reference range
Negative.

Interpretation
Almost 100 % specificity in clinical CD. Antibodies disappear on a gluten-free diet (GFD). The test is negative in healthy people. Together with anti-DGP (anti-gliadin) it is one of the recommended tests when there is a suspicion of gluten-induced enteropathy. It has been shown that tissue transglutaminase (tTG) is the antigen of anti-endomysium. A positive result with IgA antibodies (IgA deficient patients should be tested for IgG tTG instead) confirms the celiac disease diagnosis, but the final diagnosis must still be based on a small intestine biopsy and clinical remission on a GFD. However, new diagnostic criteria are being discussed, which may make the biopsy redundant given symptoms suggesting CD, high titers of anti-endomysial (or anti-tTG) antibodies and/or anti-DGP antibodies, predisposing HLA genotype (DQ2/8) and improvement on a GFD.

Panels and other analyses
See also panel *Coeliac disease* and analyses *Gliadin antibodies IgG*, *Transglutaminase IgA antibodies* and *IgA quantification*.

Entactin antibodies – IgG and IgM

Indication
Suspicion of autoimmune glomerulonephritis.

Method
ELISA with purified entactin as antigen.

Answer
The result is given in arbitrary units after comparison with a reference serum.

Reference range
14 units with a borderline value 30 units/ml.

Interpretation
Anti-entactin is an autoantibody that is directed against a cell-attachment protein in basement membranes. The antibody occurs mainly in young patients, who soon after an infection have developed proteinuria or nephrotic syndrome without extra-renal symptoms. Morphologically they belong to the group of primary proliferative glomerulonephritis. A smaller group is made up of patients with glomerulonephritis secondary to systemic lupus erythematosus. Anti-entactin may also occur in systemic lupus erythematosus without kidney involvement.

Panels and other analyses
See also panel *Glomerulonephritis*.

Fibrillarin antibodies

Indication
Suspicion of scleroderma, differential diagnosis for Raynaud's phenomenon.

Method
Immunoblot with nuclear extract. Fibrillarin is a 34kD protein and is the most important protein in the U3-RNP complex that participates in pre-rRNA processing.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Occur in about 4% of patients with systemic scleroderma, often together with antibodies against centromeres, RNA-polymerase and Scl-70. It has also been described in SLE and primary Raynaud's phenomenon. Anti-fibrillarin is more common in patients of African descent than Caucasians. Anti-fibrillarin is more specific for the diffuse form of scleroderma than the limited one.

Panels and other analyses
See also panel *Sclerodorma/Systemic sclerosis* and anlysis *ANA screen (HEp-2 cells)*.

GAD-65 antibodies

Indication
Suspicion of risk of development of insulin-dependent diabetes mellitus (IDDM) and for early diagnosis of IDDM in both young and old people.

Method
Radioimmunoprecipitation with a recombinant antigen. The antigen is an isoform of glutamic acid decarboxylase GAD-65 with a molecular weight of 65 kD. GAD-65 participates in the transformation of glutamic acid into GABA, an inhibitory neurotransmitter.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Antibodies against GAD-65 of the IgG class occur in IDDM. About 70% of young newly diagnosed people and 25% of people with adult-onset diabetes have this antibody. If, at the same time, there are antibodies against islet cells, this confirms the diagnosis of IDDM. The antibodies can often be observed before clinical signs of IDDM. Slightly elevated values may occur in autoimmune endocrine diseases, especially autoimmune thyroiditis.

Anti-GAD also occurs in 60% of patients with Stiff person syndrome associated with IDDM.

Panels and other analyses
See also panel *Diabetes* and analyses *Islet cell antibodies*, *Insulin antibodies* and *IA-2 antibodies*.

Ganglioside antibodies – IgG and IgM

Indication
Suspicion of autoimmune neuropathies.

Method
ELISA with gangliosides GM1, asialo GM1, GQ1b, GD1a and GD1b as antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Gangliosides is a group of sialic acid containing glycolipids, which occur widely in the nervous system. They are found on cell membranes and have a ceramide tail in the lipid bilayer and an oligosaccharide part extending outwardly. There are many gangliosides but GM1, asialo GM1, GQ1b, GD1a and GD1b are those that are usually associated with autoantibodies. These autoantibodies occur most frequently in motor and sensory neuropathies. High titers of antibodies against GM1 are typical of multifocal and peripheral motor neuropathies but antibodies against asialo GM1 and GD1b also occur. Anti-GM1 and -asialo GM1 antibodies are most common in Guillain-Barré's syndrome and amyotrophic lateral sclerosis. Anti-GQ1b is considered common in the Miller Fisher variant. Patients with motor and sensory neuropathies may have antibodies against one or several of the gangliosides and therefore it is essential to analyze them all.

Panels and other analyses
See also panel *Neuropathy – unclear/Guillain-Barré syndrome* and analysis *Myelin-associated glycoprotein (MAG) antibodies* and *SGPG antibodies*.

GBM antibodies (the Goodpasture antigen)

Indication
Suspicion of Goodpasture's syndrome.

Method
ELISA with the purified antigen alpha 3 chain from type IV collagen.

Answer
The result is given as arbitrary units after comparison with a standard serum.

Reference range
11 units and a borderline value 20 units/ml.

Interpretation
In Goodpasture's syndrome (rapidly progressive GN with or without pulmonary bleeding) the anti-GBM antibodies are directed against the alpha 3 chain of type IV collagen. The molecule is specific for the basement membranes of kidney and lung, which explains the limited organ involvement in classical Goodpasture's syndrome, i e kidneys and lungs. The level of the antibodies is high in most cases of Goodpasture's syndrome and drops during treatment to a normal level. The antibody does not occur in healthy people or in other diseases, e.g. the majority of systemic vasculites.

Panels and other analyses
See also panels *Glomerulonephritis*, *Systemic vasculitis – acute screening of anti-GBM, PR3-ANCA and MPO-ANCA*, *Systemic vasculitis – specific analysis* and analyses *IgG subclasses of ANCA and anti-GBM* and *PiZ determination of alpha 1 antitrypsin*.

Gliadin antibodies – IgG

Indication
Suspicion of coeliac disease (CD) or dermatitis herpetiformis.

Method
ELISA with deamidated gliadin peptides (DGP) as antigen.

Reference range
1.0 with a borderline value 1.4.

Interpretation
The IgG DGP test performs about as well as the IgA tissue transglutaminase (tTG) test, i.e. has a sensitivity around 85 % and a specificity of around 99 %. The test can pick up individuals with CD even if they are IgA deficient (10 times more common in CD patients than in the general population) and is better for detection of CD in infants (<2 years of age).

The antibodies disappear on a gluten-free diet (GFD) and do not occur in healthy people.

A positive result in these tests confirms the CD diagnosis but the final diagnosis must still be based on a small intestine biopsy and clinical remission on a GFD. However, new diagnostic criteria are being discussed, which may make the biopsy redundant given symptoms suggesting CD, high titers of anti-DGP antibodies and/or anti-transglutaminase (or anti-endomysial) antibodies, predisposing HLA genotype (DQ 2/8) and clinical improvement on a GFD.

Panels and other analyses
See also panel *Coeliac disease* and analyses *Endomysium antibodies IgA*, *Transglutaminase antibodies IgA* and *IgA quantification*.

Histone antibodies

Indication
Suspicion of drug induced lupus syndrome.

Method
ELISA with a mixture of histones as antigen. The antibodies are directed against strongly basic proteins that are closely associated with DNA in the chromatin of the cell nucleus and are called H1, H2A, H2B, H3 and H4.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
1.0 with a borderline value 1.4.

Interpretation
Positive in about 95% of patients with drug induced LE syndrome, usually after long-term use of procainamide and hydralazin. Also positive in 30-40% of patients with systemic lupus erythematosus, where the antibodies are mainly directed against H1 and H2B and in about 20% of patients with rheumatoid arthritis, in particular in patients who develop rheumatoid vasculitis.

Panels and other analyses
See also panel *Drug induced lupus* and analysis *ANA screen (Hep-2 cells)*.

HSP70 antibodies

Indication
Suspicion of autoimmune hearing loss.

Method
Immunoblot.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Antibodies against a 68 kD protein (heat shock protein 70) occur in up to 40% of patients with idiopathic hearing loss. Sometimes these patients may be treated successfully with immunosuppressive drugs. The majority of patients with active disease may have these antibodies and up to 75% of these patients respond to steroid treatment.

Panels and other analyses
See also panel *Sudden deafness*.

Hu antibodies

Indication
Suspicion of paraneoplastic syndrome, sensory neuropathy.

Method
Immunoblot.

Answer
The result is given as negative or positive with a titer.

Reference range
Negative.

Interpretation
Anti-Hu is also called ANNA-1 (type I antineural nuclear antibody). The antigen is found in all the neurons of the central and peripheral nervous system and in several tumour types, especially in the lung. Anti-Hu occurs in paraneoplastic subacute sensory neuropathy and/or paraneoplastic encephalomyelitis, which in 83% of the patients occur together with small cell lung cancer and sometimes also with other forms of tumours (lung adenocarcinoma, small cell intestinal carcinoma, neuroblastoma, prostate cancer, malignant melanoma etc.). In 50% of the patients neurological symptoms develop before clinical manifestations of the tumour.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Yo antibodies*, *Ri/Nova 1 antibodies*, *Purkinje cell antibodies (Tr)*, *Purkinje cell antibodies (PCA 2)*, *Ta/Ma 2 antibodies* and *Amphiphysin 1 antibodies*.

IA-2 antibodies

Indication
Suspicion of risk of developing insulin-dependent diabetes mellitus (IDDM) and of early diagnosis of IDDM in both young and old people.

Method
Radioimmunoprecipitation with recombinant antigen. IA-2 is also called ICA512 and is an unusual member of the protein tyrosine phosphatase (PTP) family. As an amino acid has been changed in the catalytic part, it has no enzymatic function. The molecular weight is 106kD.

Answer
The result is given as units.

Reference range
70 units.

Interpretation
Antibodies against IA-2 of the IgG class occur in IDDM. About 60-70% of young newly diagnosed people have this antibody. If there are antibodies against islet cells, this confirms the IDDM diagnosis. The antibodies can often be observed before clinical signs of IDDM. At the time of the diagnosis up to 90% can have antibodies against one or several of the antigens GAD-65, IA-2 or insulin. The percentage of positive patients depends on age (young people have a higher percentage), disease duration (the percentage decreases over time) and ethnic background. Slightly elevated values may occur in autoimmune endocrine diseases, in particular autoimmune thyroiditis.

Panels and other analyses
See also panel *Diabetes* and analyses *Islet cell antibodies*, *Insulin antibodies* and *GAD-65 antibodies*.

IgA fibronectin complex

Indication
Suspicion of IgA nephritis.

Method
ELISA with fibronectin binding collagen fragments as antigen.

Answer
The result is given as arbitrary units after comparison with a standard serum.

Reference range
39 units.

Interpretation
In IgA nephritis there are circulating immune complexes containing IgA and fi-bro-nectin. Such complexes can bind to the kidney's collagens via fibronectin. It is not clear whether they can mediate kidney inflammation but their presence in the circulation is a diagnostic marker for IgA nephritis. The complexes occur in about 50-60% of cases with IgA nephritis.

Panels and other analyses
See also panel *Glomerulonephritis*.

IgA quantification

Indication
Exclusion of IgA deficiency in connection with coeliac disease diagnosis.

Method
ELISA

Answer
The result is given as g/L.

Reference range
0.88-4.5 g/L.

Interpretation
Selective IgA deficiency (IgA 0.05 g/L) is relatively common and occurs with a frequency of 1/600 of the population. The condition is more common in patients with coeliac disease and must therefore be excluded, when the serological diagnosis of coeliac disease is based on IgA antibodies.

Panels and other analyses
See also panel *Coeliac disease* and analyses *Endomysium antibodies IgA*, *Gliadin antibodies IgG* and *Transglutaminase antibodies IgA*.

IgG subclasses of ANCA and anti-GBM

Indication
Follow-up analysis of Goodpasture's syndrome and Wegener's granulomatosis and other renopulmonary syndromes. May indicate patients who should be monitored very closely and may predict relapses.

Method
ELISA with a specific antigen (PR3, MPO, GP antigen) and subclass specific monoclonal antibodies .

Answer
The result is given as arbitrary units after comparison with a standard serum.

Reference range

	GP	PR3	MPO
IgG1	3	4	4
IgG2	8	18	16
IgG3	27	8	17
IgG4	7	4	4

Interpretation
The most common subclasses of these diseases are IgG1 and IgG4. IgG2 and IgG3 occur in low quantities. Patients with MPO-ANCA often have more IgG2 and patients with PR3-ANCA more often have IgG3. An unusual subclass distribution may indicate a different clinical course. Changes in the subclass distribution can be used to study relapses in the disease.

Panels and other analyses
See also panels *Systemic vasculitis – ANCA quantification*, *Glomerulonephritis*, and *Systemic vasculitis – specific analysis*.

Insulin antibodies

Indication
Suspicion of risk of developing insulin-dependent diabetes mellitus (IDDM) and for early diagnosis of IDDM in both young and old patients.

Method
Radioimmunoprecipitation with native antigen. Insulin regulates blood glucose levels and consists of 51 amino acids. It cannot be analyzed after the insulin treatment has started as most patients produce antibodies against exogenous insulin.

Answer
The result is given as units.

Reference range
110 with a borderline value 250 nU/ml.

Interpretation
Antibodies against insulin of the IgG class occur in IDDM but do not occur as often as antibodies to GAD-65 or IA-2. This is due to age effect, as anti-insulin is common in newly diagnosed children, whereas the percentage of positive cases decreases in teenagers and in young adults.

Panels and other analyses
See also panel *Diabetes* and analyses *Islet cell antibodies*, *IA-2 antibodies* and *GAD-65 antibodies*.

Intrinsic factor antibodies

Indication
Suspicion of pernicious anemia or Atrophic gastritis (autoimmune metaplastic athrophic gastritis = AMAG).

Method
Radioimmunoprecipitation with recombinant antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Intrinsic factor antibodies occur in 50-80 % of cases with pernicious anemia but also in an inherited form of atrophic gastritis – autoimmune metaplastic atrophic gastritis (AMAG) when the immune response is directed against parietal cells and intrinsic factor. The antibodies can either block the binding of vitamin B12 to intrinsic factor or block the receptor.

Panels and other analyses
See also analysis *Parietal cell antibodies*.

Islet cell antibodies

Indication
Suspicion of development of insulin-dependent diabetes mellitus (IDDM) and of early diagnosis of IDDM in both young and old people.

Method
Indirect immunofluorescence with monkey pancreas as substrate.

Answer
The result is given as negative, weak, medium or strong positive after evaluation of the intensity of the fluorescence.

Reference range
Negative.

Interpretation
Islet cell antibodies are positive in 70-90% of IDDM patients at the time of the diagnosis. The antibody may occur long before clinical signs of disease and is negative in healthy people. The antibodies react with glutamic acid decarboxylase (GAD), tyrosine phosphatase-like molecule IA-2, insulin and probably other unknown antigens. So far, all prospective studies of relatives of type 1 diabetes patients have shown that the combination of two or several antibodies give a higher positive predictive value than just one antibody. Up to 90% of non-insulin-dependent patients with antibodies develop insulin dependency within 6 years.

Panels and other analyses
See also panel *Diabetes* and analyses *Insulin antibodies*, *IA-2 antibodies* and *GAD 65 antibodies*.

Jo-1 antibodies

Indication
Jo-1 is included in the analysis ENA screen. See ENA for indication, method, answer and reference range.

Interpretation
Positive in 25% of patients with polymyositis/dermatomyositis and almost pathognomonic for the disease. Antibodies against Jo-1 are directed against histidyl-tRNA synthetase. Other anti-synthetase antibodies include the antigens OJ, EJ, PL-7, PL-12, KS and Zo. Patients with other anti-tRNA-synthetase antibodies manifest symptoms much the same as those with anti-Jo-1.

Panels and other analyses
See also panel *Polymyositis/dermatomyositis* and analyses *ANA screen (HEp-2 cells)* and *tRNA synthetase antibodies*.

Laminin 5 antibodies – IgG and IgA

Indication
Suspicion of autoimmune skin disease, such as pemphigoid and differential diagnosis of pemphigus, EBA etc.

Method
ELISA.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Laminin 5 is an epidermal antigen. Antibodies against laminin 5 occur in autoimmune skin diseases, such as mucous membrane pemphigoid and bullous SLE.

Panels and other analyses
See also panel *Pemphigus/Pemphigoid* and analyses *Skin antibodies (intercellular substance and basement membranes)*, *BP180 and BP230 antibodies IgG and IgA*, *Desmoglein 1 and 2 antibodies*, *Desmoplakin I and II antibodies* and *Collagen VII antibodies*.

Liver cytosol antigen 1 (LC-1) antibodies

Indication
Suspicion of autoimmune hepatitis (type II).

Method
ELISA.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Antibodies against LC-1 occur mainly in autoimmune hepatitis type II, often together with LKM-1 antibodies.

Panels and other analyses
See also panel *Autoimmune hepatitis* and analyses *Liver/kidney microsomal (LKM) antibodies* and *Liver/kidney microsome type 1 (LKM-1) antibodies*.

Liver/kidney microsomal (LKM) antibodies

Indication
Suspicion of autoimmune hepatitis (type II).

Method
Indirect immunofluorescence with liver and kidney tissues as substrates. The antibodies are mainly directed against cytochrome P-450 IID6.

Answer
The result is given as negative, weak, medium and strong positive after evaluation of the intensity of the fluorescence.

Reference range
Negative.

Interpretation
Antibodies against LKM mainly occur in autoimmune hepatitis and particularly often in a subgroup of girls and young women. Anti-LKM may occur in hepatitis C and delta and these infections should therefore be excluded.

Panels and other analyses
See also panel *Autoimmune hepatitis* and analyses *Liver/kidney microsomal type 1 (LKM-1) antibodies* and *Liver cytosol antigen 1 (LC-1) antibodies*.

Liver/kidney microsomal type 1 antibodies (LKM-1 and cytochrome P450)

Indication
Suspicion of autoimmune hepatitis (type II).

Method
Immunoblot.

Answer
The result is given as negative or positive with a ratio.

Reference range
Negative.

Interpretation
Positive reaction of autoantibodies directed against LKM type 1 (CYP2D6) occurs mainly in autoimmune hepatitis type II.

Panels and other analyses
See also panel *Autoimmune hepatitis* and analyses *Liver/kidney microsomal (LKM) antibodies* and *Liver cytosol antigen 1 (LC-1) antibodies*.

Mi-2 antibodies

Indication
Suspicion of dermatomyositis, unclear myositis.

Method
Immunoblot method. Mi-2 are nuclear proteins with molecular weights between 34 and 240 kD.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Mi-2 antibodies are found in 15-30% of patients with dermatomyositis but rarely occur in patients with polymyositis (1%) and do not occur in other muscle diseases. The diagnostic specificity is 96% in patients with dermatomyositis. In patients with Mi-2-antibodies 95% have dermatomyositis and about 3% polymyositis.

Panels and other analyses
See also panel *Polymyositis/dermatomyositis* and analyses *ANA screen (HEp-2 cells)* and *ENA screen (Extractable nuclear antigens)*.

Mitochondrial antibodies (AMA)

Indication
Suspicion of primary biliary cirrhosis or autoimmune hepatitis.

Method
Indirect immunofluorescence on rat kidney/stomach substrates. In primary biliary cirrhosis the antibodies are directed against pyruvate dehydrogenase, subunit E2 (dihydrolipoamide acetyl-transferase).

Answer
The result is given as negative, weak, medium or strong positive after evaluation of the intensity of the immunofluorescence.

Reference range
Negative.

Interpretation
Positive in about 90% of patients with primary biliary cirrhosis, in 5-10% of patients with autoimmune hepatitis, in 10% of patients with RA (often with clinical or biochemical signs of liver affection). It is also positive in some cases of scleroderma, primary Sjögren's syndrome, systemic lupus erythematosus, pernicious anemia and autoimmune hemolytic anemia. AMA occur in about 1% of healthy people.

Panels and other analyses
See also panel *Autoimmune hepatitis* and analysis *Mitochondrial (type M2) antibodies*.

Mitochondrial (type M2) antibodies

Indication
Suspicion of primary biliary cirrhosis.

Method
Immunoblot.

Answer
The result is given as negative or positive.

Reference range
Negative.

Interpretation
Autoantibodies against mitochondria type M2 are strongly associated with primary biliary cirrhosis (PBC) and occur in more than 95% of the cases. They occur to a lesser degree also in other conditions, such as Sjögren's syndrome, thyroid disease, CREST syndrome, scleroderma and polymyositis. The antigens are subunits in an enzyme complex (2-oxoacid dehydrogenase) in the inner mitochondrial membrane.

Panels and other analyses
See also panel *Autoimmune liver disease* and analysis *Mitochondrial antibodies AMA*.

MPO-ANCA (capture technique)

Indication
Suspicion of systemic vasculitis, especially with kidney involvement.

Method
Capture ELISA with purified myeloperoxidase as antigen.

Answer
The result is given as international units after comparison with a reference serum.

Reference range
5 with a borderline value 7 IU/ml.

Interpretation
Capture MPO-ANCA is an alternative method to determine MPO/ANCA, where a monoclonal antibody is used to catch MPO instead of having the protein bound directly to the plastic. The monoclonal antibody is chosen so that it blocks an epitope of anti-MPO antibodies, which gives C-ANCA that is not related to vasculitis. This makes the analysis more specific for vasculitis. MPO-ANCA occurs in systemic vasculitis, primarily in microscopic polyangiitis, Wegener's granulomatosis, Churg Strauss and focal and extra capillary necrotizing glomerulonephritis, especially when it is mainly located in the kidney. MPO-ANCA may sometimes also occur in drug induced conditions.

Panels and other analyses
See also panels *Systemic vasculitis – ANCA quantification*, *Glomerulonephritis*, *Connective tissue disease – undifferentiated* and *Systemic vasculitis – special analysis* and analyses *MPO-ANCA*, *IgG subclasses of ANCA* and *anti-GBM and PiZ determination of alpha 1 antitrypsine*.

MPO-ANCA (direct technique)

Indication
Suspicion of systemic vasculitis, especially with kidney involvement.

Method
Direct ELISA with purified granulocyte myeloperoxidase as antigen.

Answer
The result is given as international units after comparison with a reference serum.

Reference range
6 with a borderline value 8 IU/ml.

Interpretation
The antigen is the enzyme myeloperoxidase (MPO) in primary granules from neutrophils. MPO-ANCA occurs in systemic vasculitis and primarily in microscopic polyangiitis, Wegener's granulomatosis, Churg Strauss and focal and extra capillary necrotizing glomerulonephritis, especially when it is mainly located in the kidney. MPO-ANCA may sometimes also occur in drug induced conditions and in rare occasions in infections and other inflammatory conditions.

Panels and other analyses
See also panels *Systemic vasculitis – ANCA quantification*, *Glomerulonephritis*, *Connective tissue disease – undifferentiated* and *Systemic vasculitis – special analysis* and analyses *MPO-ANCA (capture technique)*, *IgG subclass determination of ANCA and anti-GBM* and *PiZ determination of alpha 1 antitrypsine*.

MuSK antibodies

Indication
Suspicion of myasthenia gravis.

Method
Radioimmunoprecipitation with a recombinant antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
MuSK stands for muscle-specific receptor tyrosine kinase. These antibodies are observed in sero-negative patients (negative for anti-acetylcholine receptor) with myasthenia gravis and confirm the diagnosis. The clinical picture is similar to that of sero-positive patients.

Panels and other analyses
See also panel *Myasthenia gravis* and analysis *Striated muscle antibodies*, *Acetylcholine receptor antibodies* and *Titin antibodies*.

Myelin associated glycoprotein (MAG) antibodies and SGPG antibodies

Indication
Suspicion of autoimmune neuropathy.

Method
ELISA with a purified antigen.

Answer
The result of MAG is given as units/ml and of SGPG as a ratio between patient serum and normal serum.

Reference range
MAG: 1000 units/ml. SGPG: 10 with a borderline value 15.

Interpretation
Several different forms of motor and sensory neuropathies have been shown to be associated with antibodies against myelin-associated glycoprotein. The antibodies bind to the carbohydrate part of the protein, particularly sulphated glucuronic acid parts. The globoside SGPG is important in adhesion and cell contact and is believed to react with the same type of antibodies as MAG.

High titers against MAG have especially been observed in demyelinating motor and sensory neuropathies. Low titers have been observed in multiple sclerosis, inflammatory neuropathies and motor neuron diseases. About half the patients with IgM monoclonal gammopathies and associated peripheral neuropathies have antibodies against MAG.

Panels and other analyses
See also panel *Neuropathy – unclear/Guillain-Barré syndrome* and analysis *Ganglioside antibodies IgG and IgM*.

Myelin basic protein (MBP) antibodies

Indication
Suspicion of multiple sclerosis (MS).

Method
ELISA with a purified antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Myelin basic protein is a CNS antigen that is expressed in the myelin sheath. Anti-MBP can induce MS-like symptoms in animals. Antibodies against MBP can occur in MS but the clinical value is uncertain.

Panels and other analyses
See also panel *Multiple sclerosis/Neuromyelitis optica* and analyses *Aquaporin 4 antibodies* and *Myelin oligodendrocyte glycoprotein (MOG) antibodies*.

Myelin oligodendrocyte glycoprotein (MOG) antibodies

Indication
Suspicion of multiple sclerosis (MS).

Method
Immunoblot.

Answer
The result is given as negative or positive.

Reference range
Negative.

Interpretation
Myelin oligodendrocyte glycoprotein is a CNS antigen that is expressed on the myelin sheath. In experimental studies antibodies against MOG have been shown to be pathogenic. They occur in MS but both sensitivity and specificity vary widely between different studies and the clinical value is uncertain.

Panels and other analyses
See also panel *Multiple sclerosis/Neuromyelitis optica* and analyses *Aquaporin 4 antibodies* and *Myelin basic protein (MBP) antibodies*.

nRNP (U1-RNP and snRNP) antibodies

Indication
nRNP-antibodies are included in the analysis ENA screen. See ENA screen for indication, method, answer and reference range.

Interpretation
Occur in almost all patients with MCTD, in 5-50% of patients with SLE and is most common in patients with lung manifestations or signs of myositis, Raynaud's phenomenon and positive RF-IgM. Antibodies may also occur in RA and polymyositis/dermatomyositis and scleroderma. A few healthy people, as well as people with malaria, bilharzia and leishmaniasis, may have antibodies against nRNP.

Panels and other analyses
See also panel *Polymyositis/dermatomyositis* and analysis *ANA screen (HEp-2 cells)*.

Parietal cell antibodies

Indication
Suspicion of pernicious anemia and atrophic gastritis.

Method
Indirect immunofluorescence with sections of gastric parietal cells as substrate. The antibodies are directed against microsomal antigens in the parietal cells and react primarily with subunits of H/K-ATPase, (proton pump).

Answer
The result is given as negative or weak, medium or strong positive after evaluation of the intensity of the fluorescence. 6% of healthy people can be positive.

Reference range
Negative.

Interpretation
Positive for pernicious anemia in about 85% of the patients, for Hashimoto's thyroiditis, primary myxoedema and for thyrotoxicosis about 25%. Often positive for atrophic gastritis with achylia without signs of pernicious anemia.

Panels and other analyses
See also analysis *Intrinsic factor antibodies*.

PiZ analysis of alpha 1 antitrypsin

Indication
Follow-up analysis for Goodpasture's syndrome and Wegener's granulomatosis and other renopulmonary syndromes. Indicates patients who should be monitored closely.

Method
ELISA with a monoclonal antibody specific for the PiZ protein. The alpha-1 antitrypsin PiZ gene has a mutation which makes the amino acid 342 become a glycine instead of a glutamic acid. This affects the folding of the protein and the export from the liver and results in a lower concentration of alpha-1 AT.

Answer
The result is given as negative or positive.

Reference range
Negative.

Interpretation
A heterozygous form of alpha-1 AT occurs in about 4.7% of the normal population and a homozygous form occurs in about 6 out of 10,000 individuals. Heterozygotes have a somewhat lower level of alpha-1 AT in the blood, whereas homozygotes only have 5-10% of the normal concentration of 0.9-1.7 g/l. Patients with PR3-ANCA have an overrepresentation of PiZ. About 20% may have the gene and in the group of biopsy verified granulomas 1/3 had the PiZ gene. These patients often have more organs involved in the disease and a significantly shorter survival.

Panels and other analyses
See also panels *Systemic vasculitis – ANCA quantification*, *Glomerulonephritis*, and *Systemic vasculitis – special analysis*.

PL-7 antibodies and PL-12 antibodies

Indication
Suspicion of polymyositis/scleroderma overlap syndrome, scleroderma, unclear myositis.

Method
Radioimmunoprecipitation with recombinant PL-7 and PL-12.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Antibodies against PL-7 and PL-12 are directed against threonyl-tRNA synthetase and alanyl-tRNA synthetase, respectively. These antibodies are observed in polymyositis and anti-synthetase syndrome together with antibodies against SRP and Mi-2. Patients with anti-tRNA synthetase manifest symptoms similar to patients with anti-Jo1.

Panels and other analyses
See also panels *Polymyositis/Dermatomyositis* and *Scleroderma/Systemic sclerosis* and analyses *ANA sceen (HEp-2 cells)*, *ENA screen (Extractable nuclear antigens)*, *tRNA synthetase antibodies*, *SRP antibodies* and *PM/Scl antibodies*.

PM/Scl antibodies (P100 + P75)

Indication
Suspicion of polymyositis/scleroderma overlap syndrome, scleroderma, unclear myositis.

Method
The analysis is performed with ELISA. The PM/Scl antigen is found in the exosome, a complex consisting of 11-16 proteins in the granular part of the nucleoli and in the nucleoplasma. The proteins act as exoribonucleases during the RNA processing.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
The PM/Scl antibodies occur in about 24% of patients with polymyositis/scleroderma overlap, in 8-12% of patients with polymyositis/dermatomyositis and in 1-16% in patients with scleroderma. These antibodies occur almost only in patients with myositis or scleroderma. 50-70% of patients with PM/Scl antibodies have polymyositis/scleroderma overlap syndrome, 20% have idiopathic myositis and 10% have scleroderma. They occur almost exclusively in Caucasians.

Panels and other analyses
See also panels *Polymyositis/Dermatomyositis* and *Scleroderma/Systemic sclerosis* and analyses *ANA screen (HEp-2 cells)* and *ENA screen (Extractable nuclear antigens)*.

PR3-ANCA (capture technique)

Indication
Suspicion of systemic vasculitis and follow-up of patients with vasculitis.

Method
Capture ELISA with purified granulocyte proteinase 3 as antigen,

Answer
The result is given as international units after comparison with a reference serum.

Reference range
5 with a borderline value 7 IU/ml.

Interpretation
Capture ELISA is a complementary method in the analysis of PR3-ANCA, where a monoclonal antibody is used to capture proteinase 3 to avoid having it bound directly to the plastic surface. The advantage is that the protein maintains its structure and the patient's antibodies react better. The capture method has a higher sensitivity and shows much higher values in individual patients. The antigen is a serine protease, proteinase 3 (PR3), in primary granules. PR3-ANCA occurs in systemic vasculitis and especially in Wegener's granulo-matosis and other vasculites, such as microscopic polyangiitis, Churg-Strauss syndrome, focal and extracapillary necrotizing glomerulonephritis. In the active phase of the disease about 90% of Wegener patients have PR3-ANCA. Studies have shown that the antibody level falls and often becomes negative when the disease is in remission. A rising level may indicate a relapse.

Panels and other analyses
See also panels *Systemic vasculitis – ANCA quantification*, *Glomerulonephritis*, *Connective tissue disease – undifferentiated* and *Systemic vasculitis – special analysis* and analyses *PR3-ANCA*, *IgG subclasses of ANCA and anti-GBM* and *PiZ determination of alpha 1 antitrypsin*.

PR3-ANCA (direct technique)

Indication
Suspicion of systemic vasculitis and follow-up of patients with vasculitis.

Method
Direct ELISA with purified granulocyte proteinase 3 as antigen.

Answer
The result is given as international units after comparison with a reference serum.

Reference range
4 units with a borderline value 6 IU/ml.

Interpretation
The antigen is a serine protease, Proteinase 3 (PR3), in primary granules. PR3-ANCA occurs in systemic vasculitis and especially in Wegener's granulomatosis and other systemic vasculites, such as microscopic polyangiitis, focal and extracapillary necrotizing glomerulo-nephritis. In the active phase of the disease about 90% of Wegener patients have PR3-ANCA. Studies have shown that the antibody level falls and often becomes negative when the disease is in remission. A rising level may indicate a relapse. As a rule it does not occur in healthy people and is rare in infections and other inflammatory diseases.

Panels and other analyses
See also panels *Systemic vasculitis – ANCA quantification*, *Glomerulonephritis*, *Connective tissue disease – undifferentiated* and *Systemic vasculitis – special analysis* and analyses *PR3-ANCA (capture technique)*, *IgG subclasses of ANCA and anti-GBM* and *PiZ determination of alpha 1 antitrypsin*.

Purkinje cell antibodies (Tr)

Indication
Suspicion of paraneoplastic syndrome.

Method
Indirect immunofluorescence.

Answer
The result is given as negative or positive with a titer.

Reference range
Negative.

Interpretation
The antigen Tr (Trotter antigen) is found in purkinje cells, most major neurons and several tumour types, e.g. breast, ovary and SCLC, thymoma and in Hodgkin's lymphoma. They often present as encephalomyelitis or cerebellar degeneration.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Hu antibodies*, *Yo antibodies*, *Ri/Nova 1 antibodies*, *Purkinje cell antibodies (PCA 2)*, *Ta/Ma 2 antibodies* and *Amphiphysin 1 antibodies*.

Purkinje cell antibodies (PCA 2)

Indication
Suspicion of paraneoplastic syndrome.

Method
Indirect immunofluorescence.

Answer
The result is given as negative or positive with titer.

Reference range
Negative.

Interpretation
The antigen PCA-2 (Purkinje cell antigen 2) is found in Purkinje cells, most major neurons and several tumour types, e.g. breast, ovary and SCLC. They often present as encephalomyelitis or cerebellar degeneration.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Hu antibodies*, *Yo antibodies*, *Ri/Nova 1 antibodies*, *Purkinje cell antibodies (Tr)*, *Ta/Ma 2 antibodies* and *Amphiphysin 1 antibodies*.

Rheumatoid factor – IgA

Indication
Suspicion of rheumatoid arthritis or primary Sjögren's syndrome.

Method
ELISA with affinity purified human IgG as antigen. Autoantibody of IgA class reacts with areas of the Fc part of the IgG molecule.

Answer
The result is given as international units after comparison with reference serum.

Reference range
20 with a borderline value 30 IU/ml.

Interpretation
Positive in about 70% of patients with rheumatoid arthritis, somewhat more common in patients who are IgM-RF positive than in those who are IgM-RF negative. Positive in about 50% of patients with Sjögren's syndrome. Positive IgA-RF in rheumatoid arthritis is especially observed in patients who develop bone and joint erosions. Positive in 2% of healthy people.

Panels and other analyses
See also panel *Rheumatoid arthritis* and analyses *CCP antibodies* and *Rheumatoid factor IgM*.

Rheumatoid factor – IgM

Indication
Suspicion of rheumatoid arthritis, primary Sjögren's syndrome, cryoglobulinemia, hypergammaglobulinemic purpura, subacute cutaneous LE etc.

Method
ELISA with affinity purified human IgG as antigen. Autoantibody of IgM class reacts with areas of the Fc part of the IgG molecule.

Answer
The result is given as international units after comparison with reference serum.

Reference range
16 with a borderline value 24 IU/ml.

Interpretation
Positive in about 80% of patients with rheumatoid arthritis, in about 70% of patients with primary Sjögren's syndrome, in about 70% of patients with subacute cutaneous LE, in about 22% of patients with systemic lupus erythematosus and in about 35% of patients with primary biliary cirrhosis. Often positive in chronic inflammation of the lungs, liver and bile ducts, subacute bacterial endocarditis, infected shunts and a number of tropical parasitic and bacterial infections. Often transiently positive in infections with Epstein-Barr-virus, cytomegalovirus, varicella zoster virus, hepatitis B virus and Mycoplasma pneumoniae. Positive in 5% of healthy people but the incidence increases with age and 10% of people over 60 may be positive.

Panels and other analyses
See also panel *Rheumatoid arthritis* and analyses *CCP antibodies* and *Rheumatoid factor IgA*.

Ri/Nova 1 antibodies

Indication
Suspicion of paraneoplastic syndrome.

Method
Immunoblot.

Answer
The result is given as negative or positive with titer.

Reference range
Negative.

Interpretation
The antibodies against Ri are also called Nova 1 or ANNA-2 (type II anti-neural nuclear antibody) and are directed against antigens in neurons in the central nervous system. The antigen is also found in tumours, e.g. in breast, lungs and ovary. It is less common than Hu or Yo. The neurological presentation includes, for instance, spinal marrow, brain stem or cerebral dysfunction but is sometimes presented as motor or sensory neuropathy.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Hu antibodies*, *Yo antibodies*, *Ri/Nova 1 antibodies*, *Purkinje cell antibodies (Tr)*, *Purkinje cell antibodies (PCA 2)*, *Ta/Ma 2 antibodies* and *Amphiphysin 1 antibodies*.

Ribosomal RNP (rRNP and P-protein) antibodies

Indication
Suspicion of systemic lupus erythematosus, especially with CNS involvement.

Method
ELISA with purified proteins. The antibodies react with three phosphoproteins of 38, 19 and 17 kD, which are all bound to ribosomal RNA.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Positive in about 10% of patients with systemic lupus erythematosus, especially in lupus manifestations in the central nervous system.

Panels and other analyses
See also panel *SLE* and analysis *ANA screen (Hep-2 cells)*.

RNA Polymerase (RNAP I, II, III) antibodies

Indication
Suspicion of scleroderma or Raynaud's phenomenon.

Method
Radioimmunoprecipitation with cell extract. RNA polymerase is a multiprotein complex which consists of 8-14 proteins. RNAP I 190 and 126 kD, RNAP II 220, 180 and 145 kD, RNAP III 155 and 138 kD.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
RNAP I antibodies are positive in 4-11% of patients with scleroderma but very rare in other autoimmune diseases. RNAP II often occurs together with RNAP I and III. It has been reported in SLE and overlap syndrome. RNAP III antibodies are positive in 12-23% of patients with scleroderma, often together with RNAP I and II. RNAP III antibodies seem to be specific to scleroderma, as they have not been reported in other diseases.

Panels and other analyses
See also panel *Scleroderma/Systemic sclerosis* and analysis *ANA screen (Hep-2 cells)*.

Scl-70 (100) antibodies

Indication
Scl-70 is included in analysis ENA screen. See therefore "ENA screen" for indication, method, answer and reference range.

Interpretation
Positive in about 15% of patients with scleroderma, especially with the systemic progressive form (diffuse progressive systemic sclerosis). Patients with Raynaud's syndrome and positive anti Scl-70(100) often later develop typical diffuse progressive systemic scleroderma.

Panels and other analyses
See also panel *Scleroderma/Systemic sclerosis* and analyses *ANA screen (Hep-2 cells)*, *PM/Scl antibodies*, *Th/To antibodies*, *RNA Polymerase (RNAP I, II, III) antibodies* and *Fibrillarin antibodies*.

Skin antibodies (intercellular substance and basement membranes)

Indication
Suspicion of autoimmune skin disease, e.g. pemphigus, pemphigoid and EBA etc.

Method
Indirect immunofluorescence of monkey oesophagus.

Answer
The result is given as negative, weak, medium or strong positive after evaluation of the intensity of the immunofluorescence.

Reference range
Negative.

Interpretation
Autoantibodies against skin proteins are an important tool when diagnosing chronic blister forming skin diseases, such as pemphigus, pemphigoid, cicatrial pemphigoid and epidermolysis bullosa acquisita (EBA). Antibodies against antigens in the intercellular substance of the skin are diagnostic for pemphigus and occur in more than 90% of patients during active disease. Antibodies against basement membrane antigens in the skin occur in 70% of patients with bullous pemphigoid, in 50% of patients with EBA and in 10% in patients with cicatrial pemphigoid. A ”split skin” test by IIF can be made to distinguish EBA from bullous pemphigoid. A direct immunofluorescence investigation of a biopsy is more sensitive than the indirect one which is made on monkey substrates. With a negative result a skin biopsy should therefore be made, if the suspicion remains.

Panels and other analyses
See also panel *Pemphigus/Pemphigoid* and analyses *BP180 and BP230 antibodies IgG and IgA*, *Desmoglein 1 and 3 antibodies*, *Desmoplakin I and II antibodies*, *Collagen VII antibodies* and *Laminin 5 antibodies IgG and IgA*.

SLA/LP antibodies

Indication
Suspicion of autoimmune hepatitis type 1.

Method
Radioimmunoprecipitation with a recombinant antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
SLA/LP means soluble liver antigen/liver pancreas. Antibodies against SLA/LP occur in 10% of patients with autoimmune hepatitis type 1.

Panels and other analyses
See also panel *Autoimmune hepatitis* and analysis *Smooth muscle antibodies (SMA)*.

Sm antibodies

Indication
Sm antibodies are included in the analysis ENA screen. See therefore “ENA screen” for indication, method, answer and reference range.

Interpretation
Positive in 10-30% of patients with SLE in Western Europe. The specificity is high for this disease. The frequency is considerably higher in Afro-Americans and Asians with SLE than in Caucasians. Sm is a nuclear non-histone protein. SLE patients often remain positive for Sm in remission and therefore detection of Sm may be valuable, when anti-dsDNA cannot be measured.

Panels and other analyses
See also panel *SLE* and analyses *ANA screen (Hep-2 cells)* and *dsDNA antibodies*.

Smooth muscle antibodies (SMA)

Indication
Suspicion of autoimmune hepatitis (type I).

Method
Indirect immunofluorescence with sections of rat ventricle containing smooth muscles. In autoimmune hepatitis the antibodies are often directed against actin.

Answer
The result is given as negative, weak, medium or strong positive after evaluation of the intensity of the fluorescence.

Reference range
Negative.

Interpretation
A strong positive result occurs particularly in autoimmune hepatitis but also in primary biliary cirrhosis and chronic inflammatory diseases. A weak positive result occurs occasionally in acute hepatitis and mononucleosis and in occasional patients with malignant diseases. It may be weakly positive in 7% of healthy people.

Panels and other analyses
See also panel *Autoimmune hepatitis* and analyses *ANA screen (HEp-2-cells)* and *SLA/LP antibodies*.

SRP antibodies

Indication
Suspicion of polymyositis.

Method
Immunoblot. Proteins in the cytoplasmatic Signal Recognition Particle, which is a ribonuclein complex consisting of one 7SL RNA and 6 proteins.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
A diagnostic marker for polymyositis and almost 100% specific with a sensitivity of 4%. The antibodies select a rather homogeneous group of patients, who often do not have either joint, lung or skin involvement but with a bad prognosis.

Panels and other analyses
See also panel *Polymyositis/Dermatomyositis* and analyses *ANA screen (Hep-2 cells)*, *ENA screen (Extractable nuclear antigens)*, *tRNA synthetase antibodies* and *PM/Scl antibodies*.

SSA p200 antibodies

Indication
Suspicion of congenital heart block.

Method
ELISA with purified SSA p200 as antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
0.7.

Interpretation
Autoantibodies against SSA p200 are a marker of an increased risk of developing congenital heart block in foetuses. Mothers with rheumatic diseases and with antibodies against SSA/Ro have a heightened risk of bearing children with neonatal lupus erythematosus. More specifically it has been observed that maternal antibodies against a subregion of SSA/Ro52, amino acids 200-239 (p200), are linked to an increased risk. Congenital complete atrio-ventricular heart block, third degree, is a serious and potentially fatal complication. Positive findings in this test are therefore a support for further investigations of the heart status of the foetus.

Panels and other analyses
Se also panels *Congenital heart block*, *SLE* and *Sjögren's syndrome* as well as analysis *ENA screen (Extractable nuclear antigens)*.

SSA/Ro52 antibodies

Indication
SSA/Ro52 antibodies are included in the analysis ENA screen. See therefore “ENA screen” for indication, method, answer and reference range.

Interpretation
Antibodies against Ro52 may play an important role in the development of congenital heart block. The antibodies may serve as a tool to identify mothers at risk of bearing children with a congenital heart block. They often occur in myositis together with Jo-1 antibodies. When isolated they play no part in the diagnosis of SS.

The result of antibodies against Ro52 is given, if requested in a special box on the referral form, in case of positive ANA screen.

Panels and other analyses
See also panel *Congenital heart block* and analysis *SSA p200 antibodies*.

SSA/Ro60 antibodies

Indication
SSA/Ro60 antibodies are included in the analysis ENA screen. See therefore “ENA screen” for indication, method, answer and reference range.

Interpretation
Positive in about 70% of patients with primary Sjögren’s syndrome and in 10-15% of patients with secondary Sjögren’s syndrome. 40% of patients with SLE are positive for SSA. Other disease associations are also described, e.g. scleroderma and MCTD. Positive anti-SSA also occurs in malaria, bilharzia and leishmaniasis.

Panels and other analyses
See also panels *Sjögren’s syndrome* and *Congenital heart block* and analysis *ANA screen (HEp-2 cells)*.

SSB/La antibodies

Indication
SSB/La antibodies are included in the analysis ENA screen. See therefore "ENA screen" for indication, method, answer and reference range.

Interpretation
Positive in about 70% of patients with primary Sjögren's syndrome and in 5-30% of patients with SLE. It usually occurs together with SSA in Sjögren's syndrome and SLE but may sometimes occur in scleroderma and RA. Isolated occurrence of SSB has been described in primary biliary cirrhosis and autoimmune hepatitis.

Panels and other analyses
See also panels *Sjögren's syndrome* and *Congenital heart block* as well as analysis *ANA screen (HEp-2 cells)*.

Steroid 21 hydroxylase (S21HY) antibodies

Indication
Suspicion of Addison's disease.

Method
Radioimmunoprecipitation with a recombinant antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
Steroid 21-hydroxylase is the principal antigen in autoimmune adrenal cortex insufficiency (Addison's disease). The antibody has been observed in 93% of patients with the diagnosis of Addison's disease.

Panels and other analyses
See also analysis *Adrenal cortex antibodies*.

Streptolysin O antibodies

Indication
Suspicion of glomerulonephritis.

Method
Nephelometry.

Answer
The result is given in international units after comparison with a reference serum.

Reference range
200 IU/ml.

Interpretation
Detection of antibodies against streptococci can give a retrospective diagnosis of infections, caused by beta-haemolytic streptococci group A, when there is a suspicion of complications, e.g. rheumatic fever, acute post-streptococcal glomerulonephritis or erythema nodosum. The antibody levels begin to rise about a week after infection with beta-haemolytic streptococci group A and reach their highest value after 3-5 weeks. Two samples at an interval of at least 4 weeks are recommended to determine the seroconversion. Positive antistreptolysin-O is observed in 80% of patients with rheumatic fever or acute post-streptococcal glomerulo-nephritis (APGN).

Panels and other analyses
See also panel *Glomerulonephritis*.

Striated muscle antibodies

Indication
Suspicion of myasthenia gravis and thymoma.

Method
Indirect immunofluorescence with sections of striated muscles from a rat heart as antigen.

Answer
The result is given as negative or weak, medium or strong positive after evaluation of the intensityt of the fluorescence.

Reference range
Negative.

Interpretation
This occurs in myasthenia gravis and almost all patients who also have thymoma are positive. It is an important test to detect thymoma, where the antigen is considered to be titin, a myocyte protein of the I-band. It may be weakly positive in chronic muscle diseases, such as polymyositic muscle dystrophy and fibromyalgia. It may be weakly positive in 10% of healthy people.

Panels and other analyses
See also panel *Myasthenia gravis* and analyses *Acetylcholine receptor antibodies*, *MuSK antibodies* and *Titin antibodies*.

Ta/Ma 2 antibodies

Indication
Suspicion of paraneoplastic syndrome.

Method
Immunoblot.

Answer
The result is given as negative or positive.

Reference range
Negative.

Interpretation
Antibodies that may occur in, for instance, breast cancer or testicular cancer and cause limbic, hypothalamic or brainstem encephalitis.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Hu antibodies*, *Yo antibodies*, *Ri/Nova 1 antibodies*, *Purkinje cell antibodies (Tr)*, *Purkinje cell antibodies (PCA 2)* and *Amphiphysin 1 antibodies*.

Th/To antibodies

Indication
Suspicion of scleroderma and differential diagnosis of Raynaud's syndrome.

Method
Immunoblot. A 40 kD protein of 7-2/MRP RNP complex, a nucleolar endoribonuclease.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Antibodies are markers for scleroderma and occur in 4-10% of the patients. They may also occur in Raynaud's syndrome and may then be an early marker for scleroderma.

Panels and other analyses
See also panel *Scleroderma/Systemic sclerosis* and analyses *ANA screen (HEp-2 cells)*, *RNA Polymerase (RNAP I, II, III) antibodies*, *Fibrillarin antibodies*, *PM/Scl antibodies* and *ENA screen (Extractable nuclear antigens)*.

Thyroglobulin antibodies

Indication
Suspicion of chronic thyroid diseases with hyper- or hypofunction or subacute thyroiditis.

Method
Luminescence.

Answer
The result is given as units/ml.

Reference range
60 with a borderline value 100 U/ml.

Interpretation
Often positive together with antibodies against thyroid peroxidase in Hashimoto's disease. 15-20% of thyroid cancer patients have antibodies to TG.

Panels and other analyses
See also panel *Thyroid disease* and analyses *Thyroid peroxidase (TPO) antibodies* and *TSH receptor antibodies*.

Thyroid peroxidase (TPO) antibodies

Indication
Suspicion of chronic thyroid disease with hyper- or hypofunction or subacute thyroiditis.

Method
ELISA with purified TPO as antigen.

Answer
The result is given as a ratio.

Reference range
1.0 with a borderline value 1.4.

Interpretation
Positive in 90% of patients with Hashimoto's thyroiditis and primary myxoedema. Positive in 40-70% of patients with other autoimmune thyroid diseases (Grave's disease and postpartum thyroiditis). Weak positive in some patients with non-autoimmune thyroid diseases. Positive in other autoimmune diseases, e.g. pernicious anemia about 60%, insulin-dependent diabetes mellitus about 20%, rare in RA, primary Sjögren's syndrome and primary biliary cirrhosis.

Panels and other analyses
See also panel *Thyroid disease* and analyses *Thyroglobulin antibodies* and *TSH receptor antibodies*.

Titin antibodies

Indication
Suspicion of myasthenia gravis and thymoma.

Method
Immunoblot.

Answer
The result is given as positive or negative.

Reference range
Negative.

Interpretation
Antibodies against titin may sometimes be observed in patients with myasthenia gravis and this is associated with an increased incidence of thymoma.

Panels and other analyses
See also panel *Myasthenia gravis* and analyses *Striated muscle antibodies*, *Acetylcholine receptor antibodies* and *MuSK antibodies*.

Transglutaminase antibodies – IgA and IgG

Indication
Suspicion of coeliac disease (CD) or dermatitis herpetiformis.

Method
ELISA with human transglutaminase as antigen.

Answer
The result is given as a ratio.

Reference range
1.0 with a borderline value 1.4.

Interpretation
Tissue transglutaminase (tTG) is an enzyme that crosslinks proteins in tissues. The antigen of endomysium in CD serology is transglutaminase. IgA anti-transglutaminase antibodies, which are analyzed with ELISA, correlate very well with anti-endomysium, which is analyzed with indirect immunofluorescence. Anti-tTG and anti-endomysium have almost 100 % specificity in clinical celiac disease. A positive result with IgA-antibodies (IgG in IgA deficient patients) confirms the CD diagnosis, but the final diagnosis must still be based on a small intestine biopsy and clinical remission on a GFD. However, new diagnostic criteria are being discussed, which may make the the biopsy redundant given classical symptoms suggesting CD, high titers of anti-DGP antibodies and/or anti-transglutaminase (or anti-endomysial) antibodies, predisposing HLA genotype (DQ 2/8) and improvement on a GFD.

Panels and other analyses
See also panel *Coeliac disease* and analyses *Endomysium antibodies IgA*, *Gliadin antibodies IgG* and *IgA quantification*.

tRNA synthetase antibodies

Interpretation
Antibodies against tRNA synthetase are observed in polymyositis and anti-synthetase syndrome together with antibodies against SRP and Mi2.

Jo-1 (histidyl-tRNA synthetase), see ENA screen.
PL-7 (threonyl-tRNA synthetase), PL-12 (alanyl-tRNA synthetase), see PL-7 and PL-12.
EJ (glycyl-tRNA synthetase), see EJ.

Panels and other analyses
See also panel *Polymyositis/Dermatomyositis*.

TSH receptor antibodies

Indication
Suspicion of chronic thyroid disease with hyper- or hypofunction or subacute thyroiditis.

Method
Radio receptor method.

Answer
The result is given in international units after comparison with a reference serum.

Reference range
1.0 with a borderline value 1.5 IU/ml.

Interpretation
Antibodies against TSH receptors occur in most cases of Graves' disease but may also occur in autoimmune thyroiditis in up to 20% of the cases. The antibodies can be of two different kinds, those that stimulate the TSH receptor and those that inhibit it. The method cannot distinguish between these two antibodies. If the antibodies disappear during therapy, it is a sign of inactive disease. IgG anti-TSH receptor antibodies can be transmitted from mother to fetus and might lead to neonatal hyperthyroidism.

Panels and other analyses
See also panel *Thyroid disease* and analyses *Thyroglobulin antibodies* and *Thyroid peroxidase (TPO) antibodies*.

Tubular basement membrane (TBM) antibodies

Indication
Suspicion of autoimmune tubulointerstitial nephritis.

Method
ELISA with a purified antigen from tubular basement membranes. The antigen is a glycoprotein with a molecular weight of about 60 kD, whose function is not known in detail.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
3 with a borderline value 4.

Interpretation
In some forms of tubulointerstitial nephritis there are autoantibodies against glycoproteins that are specific for the tubular basement membrane. The classical form with linear depositions of antibodies along the TBM and high levels of circulating antibodies is rare but low levels of these antibodies may occur in interstitial nephritis.

Panels and other analyses
See also panel *Glomerulonephritis*.

VGCC (voltage gated calcium channel) antibodies

Indication
Suspicion of autoimmune neuropathy, paraneoplastic syndrome.

Method
Radioimmunoprecipitation.

Answer
The result of the P/Q type is given in pmol/L and the result of the N-type is given as a ratio between patient serum and normal serum.

Reference range
P/Q type 25 pmol/L. N-type 10 with a borderline value 15.

Interpretation
These are antibodies which occur in small cell lung cancer but also without this association and can give rise to Lambert-Eaton myastenic syndrome and cerebellar dysfunction, among other diseases.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analysis *VGKC (voltage gated potassium channel) antibodies*.

VGKC (voltage gated potassium channel) antibodies

Indication
Suspicion of autoimmune neuropathy, paraneoplastic syndrome.

Method
Radioimmunoprecipitation with a recombinant antigen.

Answer
The result is given as a ratio between patient serum and normal serum.

Reference range
10 with a borderline value 15.

Interpretation
These are antibodies which occur in thymoma but also without this association. May give rise to neuromyotonia and limbic encephalitis, among other diseases.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analysis *VGCC (voltage gated calcium channel) antibodies*.

Yo antibodies (Purkinje cells, CDR 62, PCA 1)

Indication
Suspicion of paraneoplastic syndrome.

Method
Immunoblot.

Answer
The result is given as negative or positive with a titer.

Reference range
Negative.

Interpretation
The antibodies against Yo are also called anti-PCA 1 (Purkinje cell antigen 1). The antigen is found in Purkinje cells, most large neurons and several tumour types, such as tumours of the breast and the ovary. The paraneoplastic syndromes often present as subacute cerebral ataxia and often with sensory or motor neuropathy.

Panels and other analyses
See also panel *Paraneoplastic neurological diseases* and analyses *Hu antibodies*, *Ri/Nova 1 antibodies*, *Amphiphysin 1 antibodies*, *Purkinje cell antibodies (PCA 2)* and *Purkinje cell antibodies (Tr)*.

Key Disease Areas

Glomerulonephritis

The glomeruli in the kidney may be damaged by many different mechanisms and exhibit extremely varying clinical courses due to the cause of the damage. Extensive clinical pathological investigations are therefore often necessary to make a correct diagnosis. Glomerulonephritis (GN) is an important but heterogeneous subpopulation.

Proteinuria is a cardinal symptom for most forms of GN, which means that about half the cases of glomerulonephritis today are detected, at various kinds of health check-ups. It is important to identify at an early stage the comparatively large group of patients with proteinuria is due to incipient or manifest diabetic glomerulosclerosis and minor groups with dominant tubular proteinuria and/or findings of light chains in the urine (B-J protein). Concomitant microscopic haematuria and the presence of so-called pathological cylinders in urinary sediment support the suspicion of GN. Generally, isolated haematuria, visible to the eye, has other causes but may be dominant in focal necrotizing damage of glomerular capillaries ("pinpoint lesions"), e.g. in IgA nephropathy, acute post-streptococcal nephritis and systemic vasculites with renal involvement.

Traditionally, the GN diagnosis is based on light, immunoflourescence and electron microscopy of tissue biopsies from the kidney. Often, however, biopsy findings cannot provide information on the degree of disease activity, which makes clinical parameters important. Is the disease acute, subacute (Rapidly Progressive GN, RPGN), chronically progressive, flaring up during relapses or quite sedate? Is the picture dominated by massive proteinuria with nephrotic syndrome or final uraemic syndrome and their consequences? Are secondary events in the progression most important, e.g. increased scarring (glomerulosclerosis) and hypertension? Do other prerenal, renal or postrenal complications cause the deterioration? Is the damage already irreversible, i.e. non-treatable with other than conservative therapy?

GN is roughly divided into "primary" forms and forms secondary to systemic diseases, such as SLE and systemic vasculites (WG, MPA, GP, PAN, HS purpura), each with a different progression, prognosis and response to the therapy administered. A subdiagnosis is therefore important. In many cases GN is associated with the presence of circulating autoantibodies, which can be used for serological-immunological diagnosis.

The analysis panel "Glomerulonephritis" therefore contains: Anti-GBM (Goodpasture's syndrome, GP), PR3-ANCA and capture PR3-ANCA (Wegener's granulomatosis, WG), MPO-ANCA and capture MPO (Microscopic polyangiitis/arthritis, MPA),

anti-dsDNA, ANA (SLE), IgA-Fibronectin complex (IgA nephropathy), complement function and anti-streptolysin. Anti-entactin antibodies will be analysed on request.

A serological diagnosis is often crucial for the diagnosis, treatment and prognosis of the group of patients with clinical RPGN and proliferative crescent formations and necrotizing vasculites in tissue samples. Most of them have specific antibodies and clinically either WG, MPA, GP or SLE. Patients with HS and IgA nephropathy do not always have immune complexes.

More unusual causes of RPGN are post-streptococcal nephritis, chronic hepatitis B and C infection and essential mixed cryoglobulinemia associated with gammopathies, all of which are seronegative. In a post-streptococcal investigation it is important that two measurements of antistreptolysin are made and that a change of the titer is observed, since most of the patients have been exposed to infection. The result of a complement analysis may also support the diagnosis.

A declining renal function may be due, for instance, to sepsis, serum disease and other hypersensitivity inflammations, toxic reactions and circulation disorders, caused by malignant hypertonia, scleroderma or thrombotization of small vessels, such as HUS, TTP and TMA, all of which are antibody-negative. This also applies to patients with underlying metabolic disorders, e.g. rhabdomyolysis, cholesterol embolism and hypocalcaemia secondary to sarcoidosis, malignancy or myeloma, where, however, non-glomerular injury mechanisms may be more important.

Complement analyses are valuable. Activation and consumption occurs chiefly in the following categories of patients with GN: SLE (classical and alternative pathways), primary membrane-proliferative GN, sometimes associated with so-called nephritic factor (the alternative pathway - constant C3 reduction) or as a consequence of mixed cryoglobulinemia with M-component or “shunt nephritis” as a consequence of the infection of shunts for relief of hydrocephalus (classical and alternative pathways), post-streptococcal nephritis (transient activation of the alternative pathway - transient C3 reduction) and, finally, sepsis.

Current therapies are mainly based on the known effect in a certain subgroup but controlled studies are often lacking, mainly due to often large individual variations of clinical status and progression within each group. Thus, the evaluation of prognosis and therapy must often be individual, objectified by repeated measurements of s-creatinine levels, glomerular filtration rate (GFR), quantification of the glomerular protein leakage into the urine, preferably supplemented with qualitative characterization of the latter using serum urine electrophoresis. As s-creatinine is a highly individual-specific variable, depending on current muscle mass, age and renal function, comparative GFR-determinations should be made on and off. Today this is most easily done as a single point iohexol clearance, while measuring plasma levels of cystatin C may be an alternative. In case of iodine hypersensitivity Cr EDTA can be used.

Systemic vasculitis – ANCA testing

Background

ANCA (Anti-Neutrophil Cytoplasmic Antibodies) is a family of antibodies related to vasculitis and inflammatory diseases. The interest in these antibodies started in 1985, when a study was published, which proved that ANCA was present in Wegener’s granulomatosis and that the titer followed the disease activity.

Antigens

It was shown at an early stage that the antigens were found in granules of neutrophils. These contain many different proteins that attack and kill bacteria, for instance. The principal reactivity in vasculitis is against proteinase 3 (PR3), a serinprotease with a molecular weight of 29kD. This protein is very similar to elastase and cathepsin G, other important proteases in granules. The other important antigen is myeloperoxidase (MPO) with a molecular weight of 140kD. MPO generates oxygen radicals during the inflammation process. Antibodies against a number of other granular proteins have been observed, e.g. elastase, cathepsin G, lactoferrin, BPI and azurocidin. These autoantibodies are not as important with regard to systemic vasculitis as to other types of inflammatory diseases, like cystic fibrosis, ulcerative colitis, autoimmune hepatitis and RA.

Antigen in the c-ANCA pattern

Lysozyme
Enolase
BPI
PR3
MPO
Elastase

Antigen in the p-ANCA pattern

BPI
MPO
Elastase
Azurocidin
Lactoferrin
Cathepsin
b-Glucoronidase

ANCA ELISA specificity – IIF pattern. PR3-ANCA and MPO-ANCA are the main auto-antibodies in systemic vasculitis (Wegener’s granulomatosis and Microscopic polyangiitis respectively).

Methods of analysis

The first method to be established for analyzing ANCA was indirect immunofluorescence (IIF) on ethanol-fixed granulocytes. With this method it is possible to see two main types of patterns, one a cytoplasmic pattern called C-ANCA, the other a perinuclear pattern called P-ANCA. The C-ANCA pattern is often caused by anti-

bodies against PR3. The P-ANCA pattern is caused by a fixation artifact that occurs when positively charged molecules, like MPO or elastase, move to the negatively charged nucleus. When the antigens became known, specific methods of analysis could be developed with ELISA. Antibodies against PR3 are called PR3-ANCA and antibodies against MPO are called MPO-ANCA. Sometimes it is possible to see other patterns on the neutrophil, which are called atypical ANCA. The antigens of atypical ANCA are today practically unknown. Problems arise when ANA is present, because it is then impossible to tell if it is ANCA or ANA or both. This is called uncertain ANCA due to the presence of non- organ specific reactions.

There are a couple of questions to consider when interpreting ANCA results:

1. The pattern of IIF is not consistent with specific ELISA, which is due to the fact that the same pattern may appear for a number of different antibodies. For instance, anti-MPO can sometimes give a C-ANCA pattern. Anti-BPI can give both C- and P-ANCA staining, whereas anti-PR3 usually gives a C-ANCA pattern. It is therefore always necessary in case of a positive IIF result to verify it with a specific analysis like ELISA, as a pattern may arise from different antibodies. However, only PR3-ANCA and MPO-ANCA are of interest in the diagnosis of systemic vasculitis.

2. The titer determined with IIF does not correlate with the ELISA level. The reason is that the antigen presents itself in various ways and is more or less denatured in different methods. This is evident when alternative methods for ELISA are used, like the capture method, where a monoclonal antibody presents PR3 in a native form with a higher sensitivity as a result or with MPO-ANCA with capture technique, where the result is a somewhat higher specificity for vasculitis diseases.

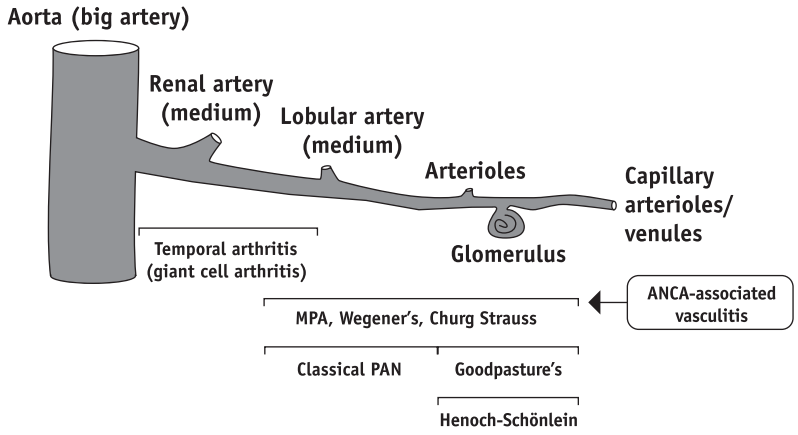
There is nowadays an International Standard for ANCA and therefore the results are given in International Units.

ANCA in the diagnosis of vasculitis diseases

The main use of ANCA is in the diagnosis of vasculitis diseases. A positive test result increases the likelihood that the patient suffers from a disease that can be treated with immunosuppression.

With few exceptions patients with Wegener's granulomatosis (WG) have ANCA. About 90% have PR3-ANCA and 5-10% have MPO-ANCA. Half the patients with limited disease and those in remission have ANCA.The presence of the PiZ form of alpha 1-antitrypsin is an important marker for patients with PR3-ANCA who have a more severe disease, as alpha 1-antitrypsin is the main inhibitor of PR3.

Patients with microscopic polyangiitis (MPA) often have positive ANCA but more often MPO-ANCA than PR3-ANCA. MPO-ANCA also occurs in half the cases of Churg-Strauss syndrome and in about 30% of the cases of Goodpasture's syndrome.



Classification of systemic vasculitis with regard to vessel size.

ANCA in the follow-up of vasculitis diseases

In patients who were ANCA positive at the onset, the PR3- and MPO-ANCA levels can be used to monitor disease activity, as ANCA negative relapses are rare in these patients. Relapses occur more frequently in patients with residual levels of PR3- and MPO-ANCA than in those without. Changes in the level may predict relapses and treatment based on ANCA level has shown that the patient needs lower doses of immunosuppression but this view is controversial. The capture method is more suitable for the follow-up of patients, since it detects relapses better. Changes in IgG subclass distribution could be a means of monitoring the patients and catch relapses.

ANCA in other diseases

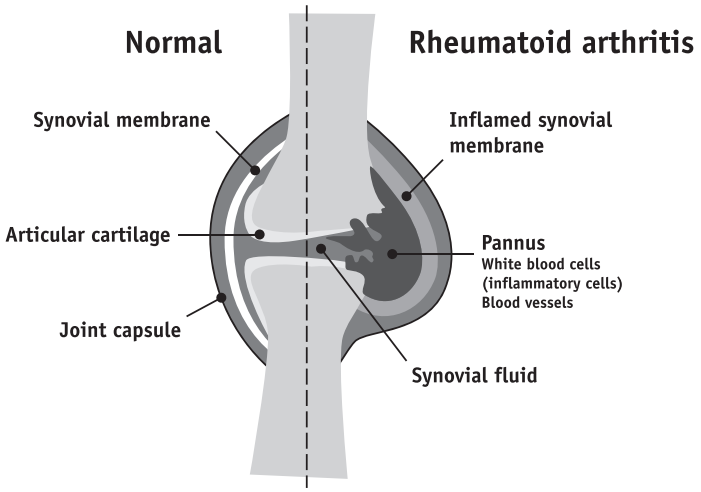
About half the patients with ulcerative colitis have P-ANCA (atypical ANCA) with mostly unknown antigens. Lactoferrin ANCA, in particular, has been reported in sclerosing cholangitis. P-ANCA also often occurs in autoimmune hepatitis, also with unknown antigens. Some patients with RA have ANCA and in particular those with Felty's syndrome.The IIF pattern used to be called GS-ANA but today it is called P-ANCA or atypical ANCA.

BPI-ANCA often occurs in cystic fibrosis and especially in patients with Pseudomonas infection. There is an inverse correlation between the BPI-ANCA level and poor lung function in patients with Pseudomonas infection. P-ANCA with specificities for MPO and elastase etc occurs occasionally in association with drug reactions to, for instance, hydralazine and propyl-thiouracil. It has been reported that antibodies against elastase are particularly common in cocaine abuse.

Rheumatoid arthritis – the anti-CCP test

Rheumatoid arthritis (RA) is a common systemic autoimmune disease which is found in about 1% of people over the world. There are many causes of this disease but it is characterized by synovitis, i.e. inflammation of synovial membranes. It is more common in women than men, which suggests that hormones may influence the onset.

Early signs are pain and swelling in the hand and finger joints. With new successful methods of treatment it is important with early diagnosis and treatment to avoid tissue damage. This requires, however, specific and sensitive serological markers that can detect patients who respond to treatment.



Schematic description of normal joint and joint affected by rheumatoid arthritis.

The diagnosis of RA is primarily based on clinical, radiological and immunological parameters. A standard serological parameter is rheumatoid factor (RF) and IgM RF occurs in 60-80% of the patients. The analysis has a good sensitivity but the specificity is low since RF occur also in healthy people, in patients with other autoimmune diseases (e.g. Sjögren's) and in chronic infections. Even though the specificity is bad, a positive RF test is considered to be an important prognostic factor and the presence of RF is one of the tests that are included in the ACR criteria for RA.

Many laboratories around the world have for many years been looking for a more specific marker for RA and it has long been known that the anti-perinuclear factor (APF) and anti-keratin (AKA) are very specific for RA. The methods have been based on indirect immunofluorescence techniques, where epithelial cells from the mucus membranes of the mouth or rat esophagus are used but although they have been specific, it has been difficult to standardize the analyses and the tests have therefore been of limited use in clinical practice.

We know today that the APF antigen is the citrullinated filaggrin molecule. Filaggrin is an intermediate filament protein in epidermal cells and derives from profilaggrin that is split into filaggrin subunits during cell differentiation. The protein is dephosphorylized and some arginine is transformed into citrulline. This has made it possible to synthesize peptides which contain the antigen and develop simple reproducible ELISA methods. The latest development has been to produce a cyclic citrullinated peptide (CCP2), which has as high a sensitivity as RF but a much higher specificity >95%. In a comparative study of the six most common ELISA panels, it is shown that the panels that use the CCP2 peptide have the highest specificity and sensitivity and that the panels that have citrullinated vimentin, filaggrin or alternative peptides have a lower specificity. Further studies have shown that among 131 potential biomarkers for RA, anti-CCP was the best one and that the combination with additional markers lowered the specificity. The only combination that increased the sensitivity was a combination with IL-6 analysis, which gave a 7% increased sensitivity in a panel of primary care patients with a reduction in the specificity of 2%.

Studies have shown that anti-CCP occurs in about 75% of patients with RA with a specificity of 96%. The antibodies are rare in healthy people and also rare in other inflammatory diseases. Most of the antibodies against CCP are of high-affinity IgG-class. The antibodies appear several years before the first symptoms. The correlation between anti-CCP and early RA is good but not between anti-CCP and age or sex. Anti-CCP seems to have a prognostic value and has a good ability to distinguish between erosive and non-erosive RA. The presence of anti-CCP is related to HLA-DRB1 and today it is thought that there are two subpopulations of RA: one with anti-CCP and HLA-DRB1, which constitutes about 75%, and one anti-CCP negative, which has no correlation with DRB1. It is also discussed if various environmental factors may trigger the presence of anti-CCP, e.g. smoking and bacterial infections. (46-51).

Connective tissue disease – ANA testing

A thorough anamnesis and an objective investigation, aiming at making a preliminary diagnosis and evaluating the disease activity, are necessary to order and interpret the results of an ANA investigation. High disease activity evaluated as new manifestations or deterioration of symptoms together with laboratory findings, like SR, immunoglobulins in serum or acute phase reactants increase the chance of finding the autoantibodies, characteristic of the disease. Thus, serology represents only a last check and verification of the diagnosis.

- ANA should be investigated on suspicion of systemic lupus erythematosus (SLE), where ANA occurs in about 95% of the patients. ANA is in SLE specifically directed against double-stranded DNA (dsDNA) but may also be directed against other nuclear antigens, like the ribonuclear proteins Sm/RNP, SSA/SSB but also histones etc.

- On clinical suspicion of Sjögren's syndrome, which is characterized by chronic dryness of the eyes, mouth, and other mucous membranes and skin, fatigue and some rheumatic symptoms, ANA should also be investigated. In this disease you find ANA, which is especially directed against ribonuclear proteins SSA/SSB.

- On clinical suspicion of scleroderma it is particularly interesting to investigate ANA. The reason is that the autoantibodies are directed against centromeres in the limited form of scleroderma, whereas the antibodies are directed against DNA topoisomerase I (Scl-70) or against nucleolar antigens in the progressive systemic form of scleroderma.

- When the disease mixed connective tissue disease (MCTD) was originally described, it was defined by clinical manifestations but also by the patient having ANA directed against the RNP part of Sm/RNP particles.

- ANA directed against histones occurs in >90% of patients with drug induced lupus syndrome.

- Juvenile rheumatoid arthritis of the oligoarticular type has ANA in up to 70%, whereas ANA as a rule is lacking in reactive forms of arthritis. In adult rheumatoid arthritis ANA occurs in particular against neutrophils, so called granulocyte-specific ANA (GS-ANA). It occurs in 75% of active RF positive RA but also in 50% of RF negative patients.

- In polymyositis ANA sometimes occurs directed against nucleoles but more often antibodies are observed against a number of cytoplasmic enzymes, e.g. histidyl-tRNA synthetase (Jo-1).

Disease	Antibody	ANA pattern	Sensitivity %	Specificity %
SLE	positive ANA	7 or 8	95	low
	anti-ds-DNA		70	>90
	anti-Sm		15	>95
	anti-rRNP		10	>95
	anti-PCNA		3	>95
	anti-histones		70	low
	anti-nRNP		30	low
	anti-SSA(Ro)		30	low
	anti-SSB(La)		20	low
Drug-induced lupus	positive ANA	7	>90	low
	anti-histones		80	medium
	MPO-ANCA		80	medium
MCTD	positive ANA	7	>95	low
	anti-rRNP		>90	>95
Sjögren's syndrome	positive ANA	8	80	low
	anti-SSA		50	medium
	anti-SSB		40	medium
	IgA-RF		70	medium
	IgM-RF		70	low
Scleroderma	positive ANA	1	90	low
	anti-centromere		40	>90
	anti-Scl70		15	>90
	anti-nucleoles		15	>85
Polymyositis/ dermatomyositis	positive ANA	2 or cytoplasm	40	low
	positive cytoplasm		20	medium
	anti-Jo-1		25	high
	anti-nRNP		40	low
Antiphospholipid syndrome	anticardiolipin		100	medium
	anti-ß2 glycoprotein 1		70	high
RA	positive ANA	7	50	low
	IgA-RF		75	low
	IgM-RF		70	medium
	anti-CCP		75	high
	anti-histones		40	low
Juvenile RA	positive ANA	7	70	medium
	anti-histones		60	medium
	IgM-RF		15	medium
	GS-ANA		15	medium

The clinical value of the autoantibody analysis is given in the table.

Complement deficiencies

The complement system is one of the main defense mechanisms of innate immunity. Deficiencies of complement components are generally associated with an increased susceptibility to a wide range of infections. In addition, deficiencies of certain complement components predispose individuals to immune complex disease, such as vasculitis and glomerulonephritis. Recent studies link deficiencies of the complement system to a diminished acquired immune response and to a deficient clearance of apoptotic cells, and subsequently with autoimmunity (Table 1). In addition, recent studies suggest that some complement deficiencies contribute to development of atherosclerosis.

Complement deficiency	Disease
C1q, C1r, C1s	Immune complex disease, e.g. glomerulonephritis, vasculitis and SLE
	Infections
C4, C2	Immune complex disease, e.g. glomerulonephritis, vasculitis and SLE
	Systemic infections with encapsulated bacteria, pyogenic infections
C3	Systemic infections with encapsulated bacteria, pyogenic infections
	Mesangiocapillary or membranoproliferative glomerulonephritis
	SLE
Factor B	Neisserial infections
Properdin, Factor D	Systemic Neisserial infections (mainly N. meningitis)
MBL	Recurrent infections in early childhood
	Aggraviation of susceptibility of infections in primary and secondary immunodeficiencies
	Increased progression of autoimmune diseases
MASP-2	Infections and inflammatory disease
C5, C6, C7, C8, C9	Recurrent systemic Neisserial infections
Factor H	Secondary C3 deficiency and similar disease presentation as in primary C3 deficiency
	Hemolytic uremic syndrom (HUS)
	Membranoproliferative glomerulonephritis
Factor I	Secondary C3 deficiency and similar disease presentation as in primary C3 deficiency
C1-inhibitor	Hereditarty angioedema (HAE)

Table 1. Complement deficiencies and disease.

The complement system is composed of at least 30 proteins that are present in the circulation or in various body fluids in a non-activated (pro-enzymatic) form (Fig. 1). To initiate biologic activity from the complement system, there are three pathways of activation. The best known of these is the classical pathway. This pathway is initiated by the interaction of C1q, the recognition molecule of the classical pathway, with e.g. antigen-antibody complexes, mostly containing IgG or IgM antibodies, and negatively charged antigens. C1q binding leads to activation of its

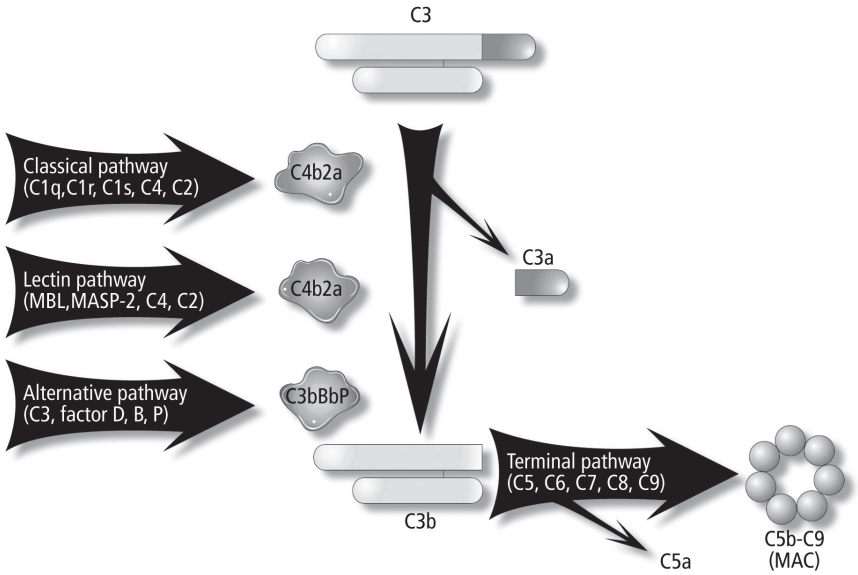


Figure 1. The three pathways of complement activation. Cleavage of C3, which is the central event in the complement process, can be achieved by C3 convertases generated via the three different activation pathways as indicated. This leads to the subsequent activation of the terminal complement pathway and formation of the C5b-C9. The lectin pathway of the complement can be activated by MBL binding to carbohydrates. Recent data indicate that also members of the ficolin family (L-ficolin, H-ficolin) can activate the lectin pathway via MASP-2 and formation of Cab2a.

associated serine proteases C1r and C1s, followed by subsequent activation of C4 and C2, resulting in formation of the classical pathway C3 convertase C4b2a. This enzyme cleaves C3 into C3b and C3a. C3b can attach itself covalently to the activator and following amplification of C3 cleavage can recruit the terminal sequence of complement. Subsequently the highly biologically active anaphylatoxin C5a is released and the terminal complex of C5b-C9, also called the membrane attack

complex, MAC, is formed and can cause e.g. bacteriolysis, cytolysis, apoptosis, and activation of the attacked cell.

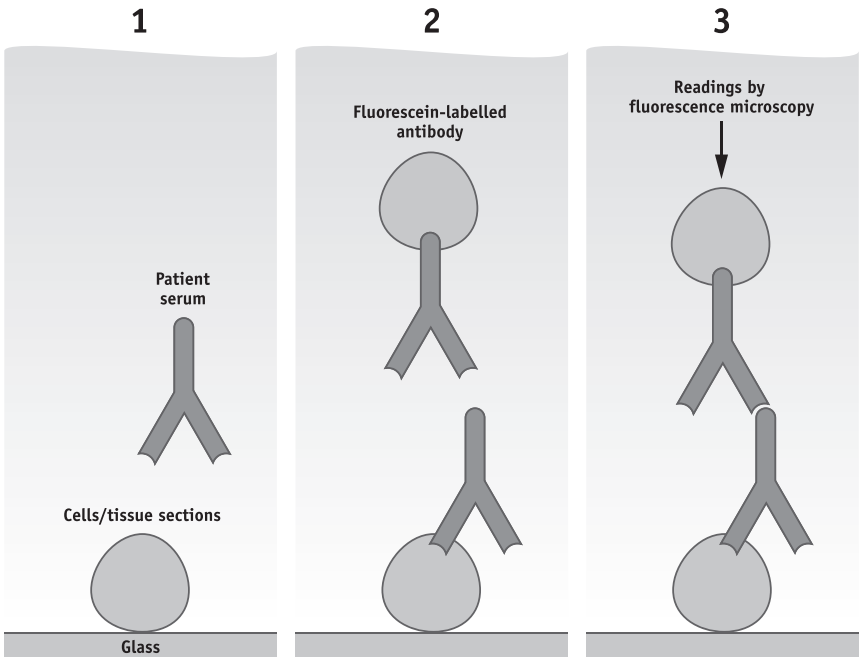
In addition to the classical pathway, activation of complement can also occur via the alternative pathway. This pathway is activated mainly by bacteria and yeast, but also immune complexes composed of IgA or IgG can initiate this pathway, in which the complement components B, D and properdin together with C3 lead to the generation of an alternative pathway C3 convertase C3BbP and subsequent cleavage of C3 and recruitment of the effector pathway C5b-C9.

Finally, a third pathway, which is mainly antibody independent, is initiated by the recognition of specific patterns of carbohydrate moieties by a serum protein, mannose binding lectin (MBL). MBL is associated with a number of proteases called MBL associated serine proteases (MASPs), which upon binding of MBL to its activator are converted from a proenzymatic state to an activated form. Activated MASP-2 then cleaves C4 and C2, again leading to the formation of C4b2a, a C3 convertase identical to the one generated following activation of C1 by the classical pathway.

Activation of C3, independently of which pathway of complement is activated, is one of the most important steps in complement biology. Deposition of C3b on activator surfaces allows phagocytic cells and other immune cells to recognize complement activators (i.e. antigens, bacteria, yeast and viruses) in a more efficient fashion, and therefore it is responsible for an effective clearance of unwanted material. This readily explains why deficiencies in any of the activator pathways or C3 itself may predispose to various types of infections, to an inefficient clearance of immune complexes, or to an aberrant immune response (table 1).

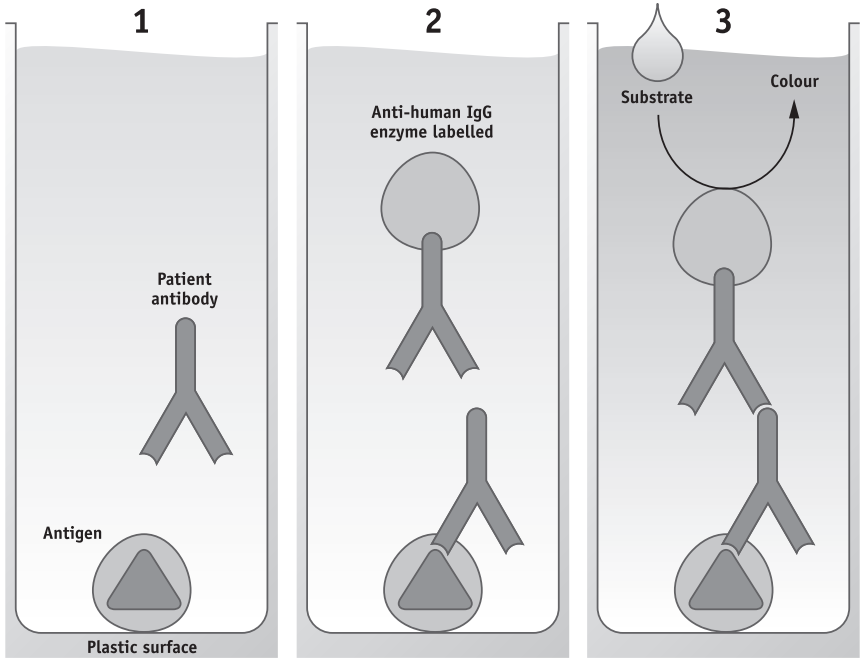
Methods

Indirect Immunofluorescence



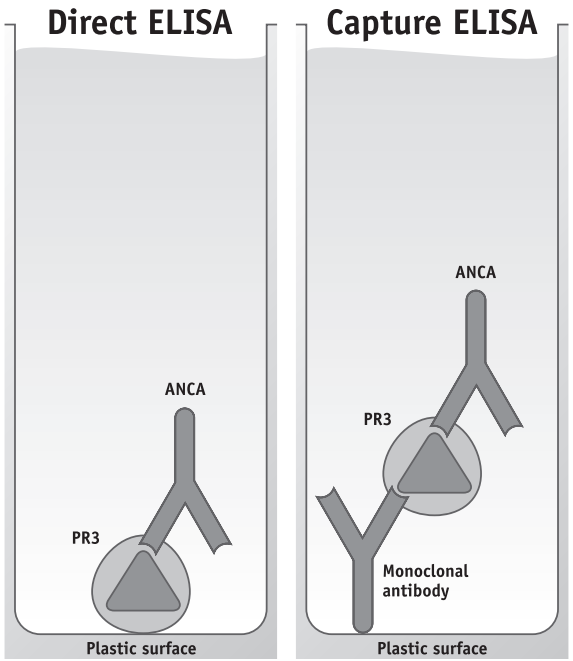
In indirect immunofluorescence investigation a tissue or cells are sectioned and fixed. Patient serum is added and antibodies, if any, react with their antigens. Bound antibodies react with fluorescence-labelled anti-human IgG antibodies. The fluorescence can then be seen in the microscope and where there is fluorescence, there are antibodies from the patient. Antibodies against various antigens give different fluorescent patterns in the cells.

Direct ELISA



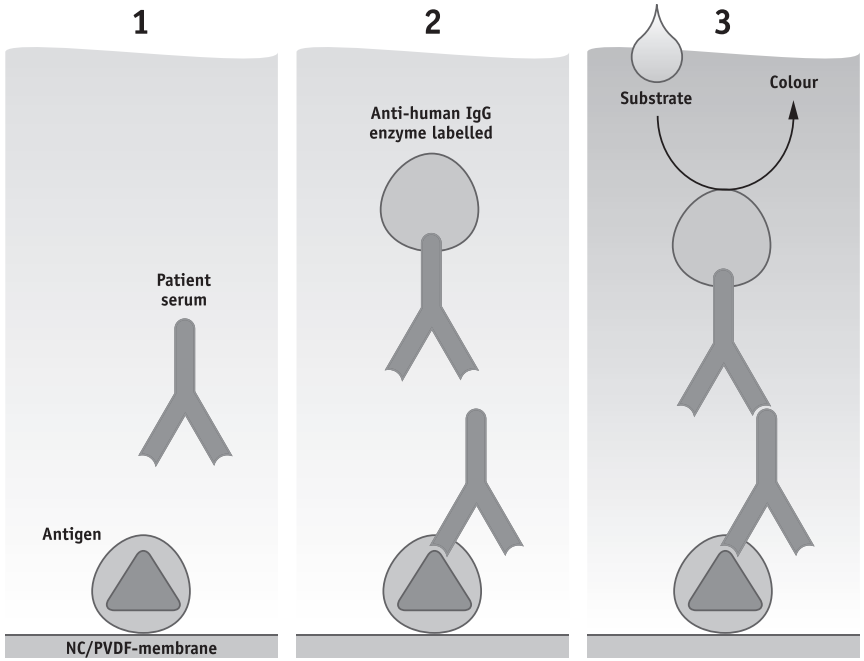
An ELISA consists of several different steps. To start with, the antigen is fixed on a plastic surface and the patient sample is added. If the sample contains specific antibodies they will bind to the antigen. An enzyme labelled antibody against human-IgG is added in the next step. In our case we mostly use alkaline phosphatase. The enzyme labelled antibody can therefore only bind if an antibody against the antigen is present in the patient sample. Then a substrate is added to the enzyme, a substance that changes colour when it is chemically converted by the enzyme. The colour can be quantitated in a spectrophotometer and is a measure of the level of antibodies in the patient.

Capture ELISA



The Capture method is an alternative ELISA, where a monoclonal antibody is used to attach the antigen, i.e. PR3 or MPO, to the plastic. The advantage is that the plastic surface does not block or denature the antigen. In the PR3-ANCA analysis this gives a higher sensitivity, as epitopes are more efficiently exposed. In the MPO-ANCA method the monoclonal antibody has been chosen in order to block an epitope that does not seem to occur in vasculitis and therefore the specificity of vasculitis has increased.

Immunoblot



The immunoblot technique consists of much the same steps as an ELISA but the antigen is here attached to a membrane on which the patient sample is applied. If there are specific antibodies in the sample, they will bind to the antigen. In the next step an enzyme labelled antibody is added and this antibody is directed against human IgG. The enzyme labelled antibody can only bind, if there is an antibody against the antigen in the patient sample. Then a substrate is added to the enzyme. The enzyme converts the substrate chemically and the substrate changes colour. Staining in the form of dark bands indicates that the sample contains antibodies with certain antigen specificity.

Quick Guide

The quick guide is divided into three tables: “Systemic diseases and rheumatology”, “Gastroenterology and endocrinology” and “Neurology and other diseases”. In each table we have listed indications and analyses. For each indication you will find suggestions of first hand analysis, second hand analysis and analyses for special cases.

Systemic diseases and rheumatology

1 Primary analysis
2 Secondary analysis
S In special cases

Autoantibodies	Antiphospholipid syndrome	Churg Strauss' syndrome	Congenital heart block	Connective tissue disease – undifferentiated	CREST syndrome	Drug induced lupus	Glomerulonephritis	Goodpasture's syndrome	IgA nephritis	Juvenile rheumatoid arthritis	Microscopic polyangitis	Mixed connective tissue disease	Polymyositis/Dermatomyositis	Recurrent infections	Renopulmonary syndrome	Rheumatoid arthritis	Scleroderma	Sjögren's syndrome	Systemic lupus erythematosus	Systemic vasculitis	Tubulointerstitial nephritis	Wegener's granulomatosis
ANA screen (HEp-2 cells)	1			1	1	1	1			1		1	1			1	1	1	1			
ANCA expanded analysis (Azu, BPI, Cat G, El, LF, and Lys)		S	2	2		2					2								2			2
ANCA – IIF		S	2	2		1					1								1	1		1
Beta 2-glycoprotein I	1																		1			
Cardiolipin	1					2													1			
CCP				1						1						1						
Centromere				1	1									1			1					
Complement analysis				1			1								1				1	1		
dsDNA	1						1					1			1				1	1		
EJ												1	2		2		2	1	1			
ENA screen (Sm, snRNP, SSA, SSB, Scl-70, Jo-1)	2		1	1			2					1	1		2	2	2	1	1			
Entaktin							2										1					
Fibrillarin															1		1					
GBM, abs against the Goodpasture ag							1	1							1					1		
Histone						1										2			2			
IgA fibronectin complex							1	S	1		S				S					S		S
Jo-1								S					2	2								
Mi-2													2	2								
Mitochondria (type M2)							1	1			1		2		1					1		1
MPO-ANCA		1				2	1				1				1					1		
nRNP (U1-RNP, snRNP)												1	2									
PiZ analysis of alpha 1 antitrypsin		S					S	S			S				S					S		S
PL-7 and PL-12													2	2								
PM/Scl (P100+P75)													2	2			1	2				
PR3-ANCA		1				2	1	1			1				1					1		1
Rheumatoid factor IgA																1	1		2	2		
Rheumatoid factor IgM				1						1						1	1		2	1		
Ribosomal RNP (rRNP, P-protein)																						
RNA polymerase I,II,III																	1	1				
Scl-70 (100)																	1					
Sm																			1			
SRP													2	2								
SSA/Ro52			1										2	2								
SSA/Ro60																		1				
SSA p200			1	1																		
SSB/La			1	1														1				
Streptolysin O							1															
TBM, tubular basement membrane																					1	
Th/To																	2					
tRNA syntetase (Jo-1,PL-7,PL-12,EJ)													1									

Gastroenterology and endocrinology

1 Primary analysis
2 Secondary analysis

Autoantibodies	Addison's disease	Atrophic gastritis	Autoimmune hepatitis	Celiac disease	Crohn's disease	Cystic fibrosis	Dermatitis herpetiformis	Diabetes	Graves' disease	Hashimoto´s thyroiditis	Inflammatory bowel disease	Pemphigoid	Pemphigus	Pernicious anemia	Primary biliary cirrhosis	Sclerosing cholangitis	Thyroid disease	Ulcerative colitis
Adrenal cortex	1								2	2							2	
ANA screen (HEp-2 cells)			1		2						2				1	1		2
ANCA – IIF			2		1						1					2		1
ANCA expanded analysis (Azu, BPI, Cat G, El, LF, and Lys)			2		2						2					2		2
ASCA ,anti-Saccharomyces cerevisiae					1						1							1
BP180 and anti-BP230												1	2					
BPI-ANCA						1												
Collagen type VII												2	2					
Desmoglein 1 and 3												2	1					
Desmoplakin I and II												2	1					
Endomysium				1			1											
GAD-65								1										
Gliadin				1			1											
IA-2								1										
IgA quantitation				1			1											
Insulin								1										
Intrinsic factor (INF)		1												2				
Islet cells								1										
Lactoferrin-ANCA			2		2							1	2			2		2
Laminin 5												1	2					
Liver cytosol antigen 1 (LC-1)			2															
Liver/kidney microsomes (LKM)			1															
Liver/kidney microsomes (LKM-1, Cytokrom P450)			2											2	1			
Mitochondria (AMA)			1												1			
Mitochondria (type M2)			2						2					1			2	
Parietal cells		1							2	2							2	
Skin (intercellular substance, basement membranes)												1	1					
SLA/LP			1															
Smooth muscle (SMA)			1															
Steroid 21-hydroxylase (S21HY)	1																	
Thyroid peroxidase (TPO)	2								1	1							1	1
Thyroglobulin	2								1	1							1	1
Transglutaminase				1			1											
TSH receptor									1	1							1	

Neurology and others

1 Primary analysis

Autoantibodies	Guillain-Barré syndrome	Miller-Fisher syndrome	Multiple sclerosis	Myasthenia gravis	Neuromyelitis optica	Neuropathy – unclear	Paraneoplastic neurological diseases	Stiff person syndrome	Sudden deafness	Thymoma
Yo (Purkinje cells, PCA-1)						1	1			
VGKC potassium channel (serum/liqvor)						1	1			1
VGCC calcium channel						1	1			
Titin				1						1
Ta/Ma 2							1			
Striated muscle				1						1
SGPG	1					1				
Ri/Nova 1						1	1			
Purkinje cells (Tr)						1	1			1
Purkinje cells (PCA 2)						1	1			
Myelin oligodendrocyt glycoprotein (MOG)			1							
Myelin basic protein (MBP)			1							
Myelin associated glycoprotein (MAG)	1		1			1				
MuSK				1						
Hu						1	1			
Hsp 70									1	
Gangliosides (AsalioGM1, GM1, GQ1b, GD1a, GD1b)	1	1				1				
GAD-65								1		
CV2							1			
Cardiolipin									1	
Aquaporin 4					1					
ANCA – IIF									1	
ANA screen (HEp-2 cells)									1	
Amphiphysin 1							1	1		
Acetylcholine receptor				1						

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